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Analysis of Cytokine mRNA Expression in *Listeria*-Resistant C57BL/6 and *Listeria*-Susceptible A/J Mice during *Listeria monocytogenes* Infection

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This laboratory previously reported that mRNA expression for many cytokines, as determined by reverse transcription-polymerase chain reaction analysis, is induced rapidly in the spleen during murine listeriosis. In the present study, the patterns of cytokine mRNA expression in spleens and livers of *Listeria*-resistant C57BL/6 and *Listeria*-susceptible A/J mice were compared. In addition, in situ hybridization was performed to evaluate the distributions of cytokine mRNA-expressing cells in these tissues. *Listeria*-resistant C57BL/6 mice demonstrated greater expression of gamma interferon (IFN-γ) and granulocyte-macrophage colony-stimulating factor (GM-CSF) mRNAs in the spleen than *Listeria*-susceptible A/J mice. Greater numbers of cells expressing IFN-γ and GM-CSF mRNAs were observed by in situ hybridization in the spleens of C57BL/6 mice than in those of A/J mice. C57BL/6 and A/J mice did not differ in their expression of IFN-γ mRNA in the liver. Nor did C57BL/6 and A/J mice differ in their expression of tumor necrosis factor alpha, interleukin-1α (IL-1α), IL-2, IL-4, or IL-6 mRNA in the liver or spleen, as determined by reverse transcription-polymerase chain reaction and in situ hybridization. These results indicate that the greater resistance of C57BL/6 mice to *Listeria monocytogenes* infection is associated with greater expression of IFN-γ and GM-CSF mRNAs in the spleen and GM-CSF mRNA in the liver.

Genetically determined anti-*Listeria* resistance is associated with an enhanced ability to mobilize inflammatory neutrophils and mononuclear phagocytes to sites of infection (4–8, 24). Although cytokines play an important role in the development of anti-*Listeria* resistance (2, 9–12, 20, 21, 23), little is known about how cytokine responses differ between *Listeria*-resistant and *Listeria*-susceptible strains of mice, especially in the early stages of *Listeria monocytogenes* infection.

Our laboratory recently reported that gamma interferon (IFN-γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin-6 (IL-6) mRNAs were expressed in the spleens of BDF_1 mice within hours after *L. monocytogenes* challenge (16). We also observed increased numbers of IFN-γ mRNA-expressing cells in the liver 1 day after *L. monocytogenes* challenge (25). These data indicate that some cytokine mRNAs are expressed rapidly during the early stages of the host response to *L. monocytogenes* infection. In the present study, we examined the possibility that cytokine mRNA expression in the spleen and liver differs between *Listeria*-resistant C57BL/6 and *Listeria*-susceptible A/J mice. Our results show that C57BL/6 mice have higher splenic expression of IFN-γ and GM-CSF mRNAs and higher hepatic expression of GM-CSF mRNA than A/J mice, observations that may be related to their enhanced resistance to *L. monocytogenes* infection.

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MATERIALS AND METHODS

Mice. Male 5-to-6-week-old C57BL/6 and A/J mice were obtained from Jackson Laboratory (Bar Harbor, Maine). These mice were housed under microisolator caps (Lab Products, Frederick, Md.) at the animal care facility of the University of Wisconsin School of Veterinary Medicine (an American Association for Laboratory Animal Care-approved facility) and given Purina Lab Chow (Ralston Purina, St. Louis, Mo.) and water ad libitum. Mice were allowed to acclimate to the animal care facility for at least 1 week before being used in an experiment.

Bacterial infection. *L. monocytogenes* EGD was prepared as described previously (9). Log-phase bacteria were suspended in tryptose phosphate broth (Difco, Detroit, Mich.) containing 20% glycerol and stored as aliquots at −70°C. The bacteria were thawed and diluted in pyrogen-free phosphate-buffered saline (PBS) at working concentrations immediately before injection. Mice were injected intravenously with *L. monocytogenes* in a total volume of 0.2 ml of pyrogen-free saline. At various times after injection, the mice were euthanized by cervical dislocation, and their spleens and livers were removed. One-quarter of each spleen and a lobe of each liver were processed for RNA extraction as described below. An additional one-quarter of the spleen and a portion of the liver were removed to 4% paraformaldehyde in PBS, processed, and embedded in paraffin for in situ hybridization. The remaining portions of spleen and liver were homogenized in sterile water, and serial dilutions of the homogenates were plated on blood agar (Remel, Lenexa, Kans.). The plates were incubated at 37°C for 24 to 48 h, and the colonies were enumerated. The results are presented as the mean $\log_{10}$ viable *L. monocytogenes* cells per spleen or liver ± the standard error of the mean (three mice per time point).

RNA extraction and cDNA preparation. A portion of each

spleen was teased apart in Hanks' balanced salt solution containing 0.02% azide. The spleen cells were washed once, suspended in 500 μl of lysis solution (4 M guanidinium isothiocyanate, 0.5% *N*-lauroylsarcosine, 25 mM sodium citrate, and 100 mM 2-mercaptoethanol) and stored at −70°C until processed further. A 0.5-g sample of liver was homogenized in 6 ml of lysis solution for 15 s with a Tissue Tearor (Biospec Products, Bartlesville, Okla.) and frozen at −70°C until processed further. After thawing, 500 μl of homogenate was mixed with 33 μl of 3 M sodium acetate (pH 3.5), 500 μl of water-saturated phenol, and 100 μl of chloroform. The mixtures were chilled on ice for 15 min and centrifuged at 12,000 × *g* for 10 min. The aqueous phase was removed, and the RNA was precipitated with an equal volume of isopropanol at −20°C for 90 min. The precipitates were washed with 70% ethanol, dried, and resuspended in ribonuclease-free water.

Reverse transcription-polymerase chain reaction analyses (RT-PCR). The entire RNA product from one-quarter of the spleen or 10 μg of total RNA from the liver was combined with 1 μg of oligo(dT)$_{15}$ (Promega, Madison, Wis.) and heated to 65°C for 5 min prior to RT. After cooling, the nucleic acids were combined with 14 μl of the following mixture: 20 mM dithiothreitol, 1 mM (each) dATP, dGTP, dCTP, and dTTP, 35 U of RNasin (Promega), and 525 U of Moloney murine leukemia virus reverse transcriptase (GIBCO BRL Life Technologies, Inc., Gaithersburg, Md.) in the manufacturer's reaction buffer. The RT reaction mixtures were incubated for 2 h at 42°C. The products were stored at −20°C until used for PCR assays.

PCR primers for murine β-actin, tumor necrosis factor alpha (TNF-α), IL-1α, IL-2, IL-4, IL-6, IFN-γ, GM-CSF, and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) were obtained from Clontech (Palo Alto, Calif.). One microliter of each cDNA preparation was amplified with 500 nM concentrations of each 5′ and 3′ primer, 200 μM (each) dATP, dGTP, dCTP, and dTTP, 1.25 U of *Taq* DNA polymerase (Promega), and the manufacturer's buffer in a final volume of 50 μl. The PCR parameters were 30 cycles (primer exhaustion was determined not to occur over 30 cycles by dilution analysis) of 1 min of denaturation at 94°C, 2 min of annealing at 60°C, and 3 min of extension at 72°C. Reaction products were visualized by agarose gel electrophoresis of 8 μl to produce single bands from cDNA (but not genomic DNA) template. The remaining products were denatured for slot blot analysis.

Semiquantitative analysis of IL-1α, IL-2, IL-4, IL-6, IFN-γ, GM-CSF, β-actin, and G3PDH was performed by hybridization of ^{32}P-labelled specific oligonucleotide probes (Clontech) to PCR products immobilized on nitrocellulose membranes. The PCR products were denatured with 0.5 N NaOH–0.15 M NaCl for 30 min at 65°C, neutralized with 1 M ammonium acetate, and blotted onto a nitrocellulose membrane with a slot blot apparatus (Schleicher and Schuell, Keene, N.H.). The membrane was baked at 80°C for 1 h and then prehybridized at 55°C for 1 h in a hybridization solution consisting of 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) with 0.9 M NaCl and 0.09 M sodium citrate (pH 7.0), 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). Hybridization was performed overnight at 55°C in the same solution with 4 × 10^6 cpm of ^{32}P-labelled oligonucleotide probe per ml, which had been labelled previously at the 5′ end with T4 polynucleotide kinase. Posthybridization washes consisted of two room temperature washes for 30 min each in 2× SSC (0.3 M NaCl, 0.03 M sodium citrate [pH 7.0]) with 0.1% SDS and one wash at 55°C for 20 min in 2× SSC with 0.1% SDS. The bound radioactivity was detected and quantified with an Ambis Radioanalytic Imager (Ambis, Inc., San Diego, Calif.). Cytokine mRNA data were normalized relative to the β-actin mRNA signal for the individual mouse. The highest β-actin mRNA signal obtained in an experiment was considered 100%, and the β-actin signal for each mouse was expressed as a relative percentage of this maximal value. This percentage was then used to adjust the count-per-minute data for each cytokine mRNA for that mouse.

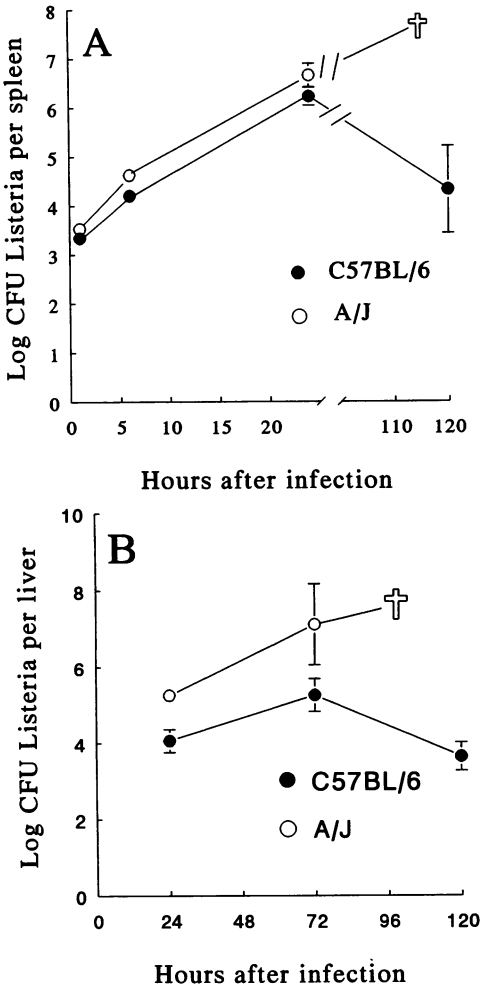

FIG. 1. Kinetics of bacterial counts in spleens (A) and livers (B) of C57BL/6 and A/J mice infected with 5 × 10^4 (for spleen analyses) and 5 × 10^3 (for liver analyses) *L. monocytogenes* EGD. The data are expressed as the mean ± the standard error of the mean $\log_{10}$ CFU of *L. monocytogenes* per organ for three mice per datum point. Crosses indicate the times of death and approximate numbers of *L. monocytogenes* at death for the A/J mice.

In situ hybridization analysis of cells containing cytokine mRNA. The in situ hybridization procedure was a modification of the procedure described by Hemmati-Brivanlou et al. (15). A lobe of each liver was fixed in 4% paraformaldehyde buffered with PBS. The liver was processed in alcohol and embedded in paraffin. Five-micrometer-thick sections were mounted on Probe-On Plus slides (Fisher Scientific, Pittsburgh, Pa.). The sections were deparaffined and successively hydrated with 100, 95, and 70% alcohols. The rehydrated

sections were treated with 100 μg of proteinase K per ml in PBS for 20 min at 37°C and acetylated with 0.25% (vol/vol) acetic anhydride in 0.1 M triethanolamine HCl (pH 8.0) for 10 min at 22°C. The sections were neutralized with 0.1 M Tris buffer (pH 7.5) that contained 0.1 M NaCl, 5 mM $MgCl_2$, and 0.05% Brij 35 (Sigma Chemical Co.). A hybridization cocktail consisting of 0.5 μg of cRNA probe per ml (digoxigenin labelled by in vitro transcription with the Boehringer Mannheim Genius labelling system), 6× SSC, 50% formamide, 5% dextran sulfate, 50 mM dithiothreitol, 0.2 mg of sheared salmon sperm DNA per ml, 0.125 mg of tRNA per ml, 0.02% Ficoll (Sigma Chemical Co.), 0.02% polyvinylpyrrolidone, and 0.1% Triton X-100 (Sigma Chemical Co.) was added to the slides, and the slides were incubated in a humidified chamber, first at 90°C for 15 min and then at 42°C for 2 h. Sense RNA probes were generated from the same plasmid templates as the cRNA probes and used as negative controls. Posthybridization washes consisted of five 1-min washes in 2× SSC with 0.1% SDS and 0.05% Brij 35 followed by two 1-min washes in 0.1× SSC with 0.1% SDS and 0.05% Brij 35 at 22°C. Two high-stringency washes for 5 min at 42°C in the same solution followed by two 1-min washes in 0.1× SSC with 0.1% SDS and 0.05% Brij 35 and two 1-min washes in 2× SSC with 0.1% SDS and 0.05% Brij 35 at 22°C were conducted. Hybridization was detected as described by the manufacturer (Boehringer Mannheim). Briefly, slides were dipped for 1 min in buffer 1 (100 mM Tris [pH 7.5], 150 mM NaCl, 0.05% Brij 35) and incubated for 30 min at 22°C with 2% normal sheep serum and 0.3% Triton X-100 in buffer 1. Antidigoxigenin antibody conjugated with alkaline phosphatase was diluted 1:500 with buffer 1 containing 1% normal sheep serum and 0.3% Triton X-100 and incubated on the tissue sections for 2 h at 22°C. The slides were washed twice with buffer 1 (15 min at 22°C) and then once with buffer 2 (100 mM Tris [pH 9.5], 100 mM NaCl, 50 mM $MgCl_2$, 0.05% Brij 35) for 2 min at 22°C. A color development solution consisting of 30 μg of nitroblue tetrazolium chloride per ml and 225 μg of 5-bromo-4-chloro-3-indolylphosphate in 10 ml of buffer 2 with 2.4 mg of levamisole per ml was applied to the slides. The slides were incubated in a humidified, light-tight box for 18 h. The reactions were stopped with buffer 3 (10 mM Tris, 1 mM EDTA [pH 8.0]), and the sections were counterstained with nuclear fast red dye.

Statistical analysis. The statistical significance of count-per-minute data obtained from cytokine RT-PCR blots was determined by a one-way analysis of variance. If the F value was significant ($P < 0.05$), then relevant comparisons between groups were done with the Bonferroni modification of the t test.

RESULTS

***L. monocytogenes* infection.** In the first experiment, to study splenic cytokine expression, C57BL/6 and A/J mice were inoculated intravenously with 5×10^4 CFU of *L. monocytogenes* EGD. Bacterial counts increased in the spleens of both strains of mice at 6 and 24 h after challenge, with the bacterial counts being significantly higher in A/J mice than in C57BL/6 mice (Fig. 1A). All of the A/J mice died by 3 days after challenge, whereas the C57BL/6 mice all survived; the number of *L. monocytogenes* cells recovered from the spleens of C57BL/6 mice was decreasing by 120 h compared with the bacterial burdens at 24 h after challenge. In the second experiment, to study hepatic cytokine expression, C57BL/6 and A/J mice were inoculated intravenously with 5×10^3 CFU of *L. monocytogenes* EGD. Bacterial counts in the livers of A/J mice were significantly higher than in those of C57BL/6 mice from day 1 until all the A/J mice had died by day 4 (Fig. 1B).

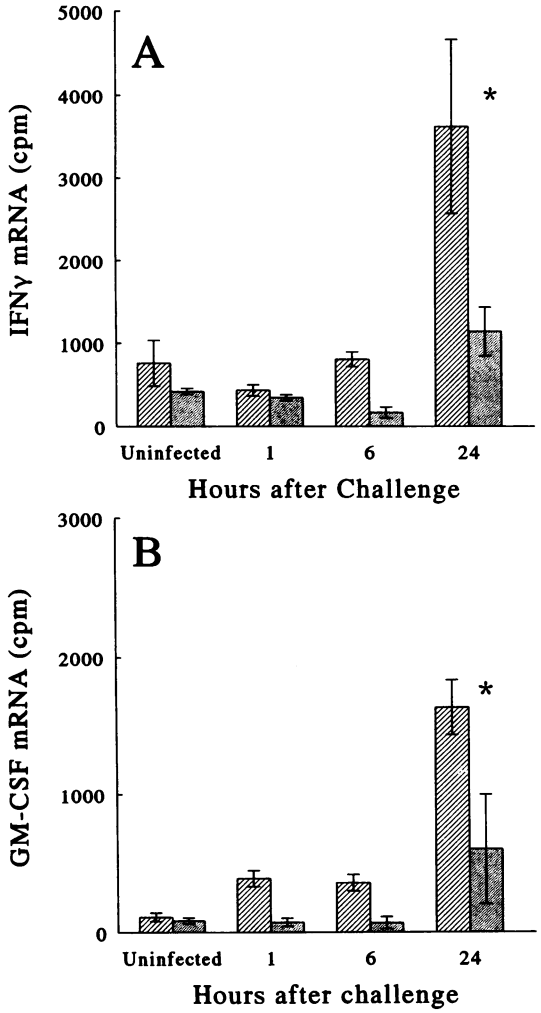

FIG. 2. Comparison of IFN-γ (A) and GM-CSF (B) mRNA expression in spleen cells of C57BL/6 (hatched bars) and A/J (stippled bars) mice infected with *L. monocytogenes* EGD. PCR products obtained as described in Table 1, footnote *a*, were denatured, neutralized, and blotted onto nitrocellulose membranes. The membranes were baked and hybridized with the ^{32}P-labelled probe for each cytokine. Data are expressed as the mean ± the standard error of the mean counts per minute for three mice per datum point. *, $P < 0.05$.

Semiquantitative RT-PCR analysis of splenic cytokine mRNA expression. Visualization of PCR products from spleen RNA samples by agarose gel electrophoresis allowed a qualitative, but not quantitative, analysis of differences between C57BL/6 and A/J cytokine mRNA expression. In a previous study, we showed that IFN-γ and GM-CSF mRNAs were expressed during the early stages of murine listeriosis (16). We, therefore, chose to perform a semiquantitative RT-PCR comparison with ^{32}P-labelled oligonucleotide hybridization probes for IFN-γ and GM-CSF mRNAs. The relative amounts of IFN-γ mRNA did not increase significantly until 24 h after infection, at which time a fourfold increase in signal compared with that for uninfected

TABLE 1. Semiquantitative RT-PCR analysis of liver cytokine mRNA[a]

Cytokine	Cytokine mRNA (mean ± SEM cpm) per mouse[b]					
	Preinfection		1 day postinfection		3 days postinfection	
	C57BL/6	A/J	C57BL/6	A/J	C57BL/6	A/J
GM-CSF	7.1 ± 3.4	10.0 ± 2.0	119.3 ± 38.6[c]	54.3 ± 3.8[c]	83.3 ± 25.4	66.1 ± 10.9
IL-1α	41.5 ± 26.2	44.2 ± 25.9	89.7 ± 22.7	121 ± 29.2	61.4 ± 45.1	17.6 ± 11.8
IFN-γ	43.3 ± 5.2	53.0 ± 6.4	330 ± 202	416.7 ± 49.3	236.7 ± 78.0	246.7 ± 165.8
TNF-α	20.7 ± 0.3	27.7 ± 3.8	171.3 ± 112.2	129.3 ± 3.4	28.0 ± 11.0	41.7 ± 26.2
IL-6	13.3 ± 0.9	15.3 ± 0.6	46.7 ± 16.8	111.3 ± 14.2	66.7 ± 36.4	50.3 ± 36.8
IL-2	37 ± 2.6	36.7 ± 9.2	897.7 ± 103.5	716.3 ± 122.7	1,064 ± 331.6	515 ± 272.6
IL-4	24 ± 2.6	24 ± 2.9	56 ± 14.6	60 ± 11.3	28 ± 9.1	23.7 ± 5.8
G3PDH	675 ± 246	311 ± 26	260 ± 16	1,044 ± 678	1,828 ± 1,134	496 ± 256

[a] Ten microliters of total RNA from the liver of each mouse was reverse transcribed into cDNA. A 1-μl aliquot of each cDNA was used for 30 cycles of PCR with cytokine-specific primers. The PCR products were immobilized on nitrocellulose membranes with a slot blot apparatus. For each cytokine, ^{32}P-labelled oligonucleotide probes were hybridized to the cDNA on the membranes. The radioactivity bound to the membranes was quantified with an Ambis radioanalytic imager, and the data were reported as counts per minute.

[b] The count-per-minute data were averaged from total RNA obtained from the livers of three mice per group.

[c] Significant differences ($P < 0.05$) exist between the results from C57BL/6 mice and those from A/J mice.

mice was measured (Fig. 2). At 24 h after challenge, the *L. monocytogenes*-infected A/J mice had IFN-γ mRNA levels slightly greater than those in the uninfected A/J mice but nearly fourfold less IFN-γ mRNA than the *L. monocytogenes*-infected C57BL/6 mice ($P < 0.05$).

GM-CSF mRNA expression in *Listeria*-infected C57BL/6 mice was increased by 1 h after infection and markedly increased by 24 h after challenge (Fig. 2). This contrasted sharply with the limited ability of A/J mice to significantly increase the expression of this cytokine mRNA during the first 24 h after challenge ($P < 0.05$ versus expression in C57BL/6 mice). The amount of β-actin mRNA present in the spleens of A/J mice did not increase significantly during the 24 h after challenge. However, the results in Fig. 2 are corrected for variations in C57BL/6 mouse β-actin mRNA expression, as described in Materials and Methods. This lack of change was consistent with the grossly enlarged spleens of C57BL/6 mice compared with those of A/J mice at 24 h after challenge.

Semiquantitative RT-PCR analysis of liver cytokine mRNA expression. Influx of inflammatory cells and lymphocytes into the liver of BDF_1 mice occurs within 1 day after *L. monocytogenes* challenge (25). To accommodate this delay in liver inflammation, we decided to evaluate cytokine expression at 1, 3, and 5 days after challenge rather than at the earlier time points used to evaluate the response in the spleen. As summarized in Table 1, levels of expression of IL-1α, IFN-γ, TNF-α, IL-6, IL-2, and IL-4 mRNA in both C57BL/6 and A/J mice were all significantly increased at 24 h after inoculation with 5×10^3 *L. monocytogenes*. Only the values for GM-CSF mRNA at 1 day after inoculation differed significantly for C57BL/6 and A/J mice ($P < 0.05$). By 3 days after challenge, the mRNA levels for GM-CSF, IFN-γ, IL-6, and IL-2 remained elevated for both C57BL/6 and A/J mice, whereas mRNA levels for IL-1α, TNF-α, and IL-4 had largely returned to the baseline levels noted for uninfected mice. At this point, there were no significant differences between C57BL/6 and A/J mice.

In situ hybridization analysis of cytokine-expressing cells. The significant differences in splenic IFN-γ and GM-CSF mRNA expression observed by semiquantitative RT-PCR were visually confirmed by in situ hybridization. The spleens of C57BL/6 mice at 24 h after challenge contained more cells expressing IFN-γ mRNA (Fig. 3A and B) and GM-CSF mRNA (Fig. 3C and D) than did the spleens of A/J mice. Consistent with the semiquantitative RT-PCR data in Table 1, there were no significant differences between the numbers of cytokine mRNA-expressing cells in the livers of C57BL/6 mice and those of A/J mice.

DISCUSSION

In a previous study (16), we showed that the cytokine mRNA response in the spleens of BDF_1 mice occurs quite rapidly during *L. monocytogenes* infection. Significant increases in IL-6, GM-CSF, and IFN-γ mRNAs were detected by RT-PCR within hours of challenge with viable *L. monocytogenes* (16). We also evaluated cytokine production by RT-PCR and in situ hybridization in the livers of *L. monocytogenes*-infected BDF_1 mice and detected changes in the cytokine mRNA response at 1 day after challenge (25). In the present study, we combined these two techniques to compare the cytokine mRNA responses in the spleens and livers of genetically resistant C57BL/6 and genetically susceptible A/J mice during experimental listeriosis. These two strains of mice did not differ in expression of mRNA for TNF-α, IL-1α, IL-2, and IL-4 in either the spleen or liver, as assessed by both RT-PCR analysis and in situ hybridization. Our results are consistent with a report that macrophages from C57BL/6 and A/J mice do not differ in their ability to transcribe IL-1α and IL-1β mRNA in response to lipopolysaccharide stimulation in vitro (1). Semiquantitative analysis with ^{32}P-labelled oligonucleotide probes indicated that the resistant C57BL/6 mice expressed greater amounts of IFN-γ and GM-CSF mRNAs in the spleen than did susceptible A/J mice (Fig. 2). This was confirmed by visual observation of greater numbers of IFN-γ mRNA- and GM-CSF mRNA-positive cells by in situ hybridization in the spleens of *L. monocytogenes*-infected C57BL/6 than in those of A/J mice (Fig. 3). A similar dichotomy between C57BL/6 and A/J mice was not observed for the liver, in which there were no significant differences in the mRNA responses for these cytokines as evaluated by either semiquantitative RT-PCR or in situ hybridization analyses.

Prior studies have provided evidence that anti-*Listeria* resistance in mice correlates with a rapid and vigorous influx of inflammatory neutrophils and mononuclear phagocytes (4, 5, 7, 8, 24). The relatively poor inflammatory cell response of A/J mice results in fewer phagocytic cells, which may have reduced listericidal activity, at sites of bacterial infection (5,

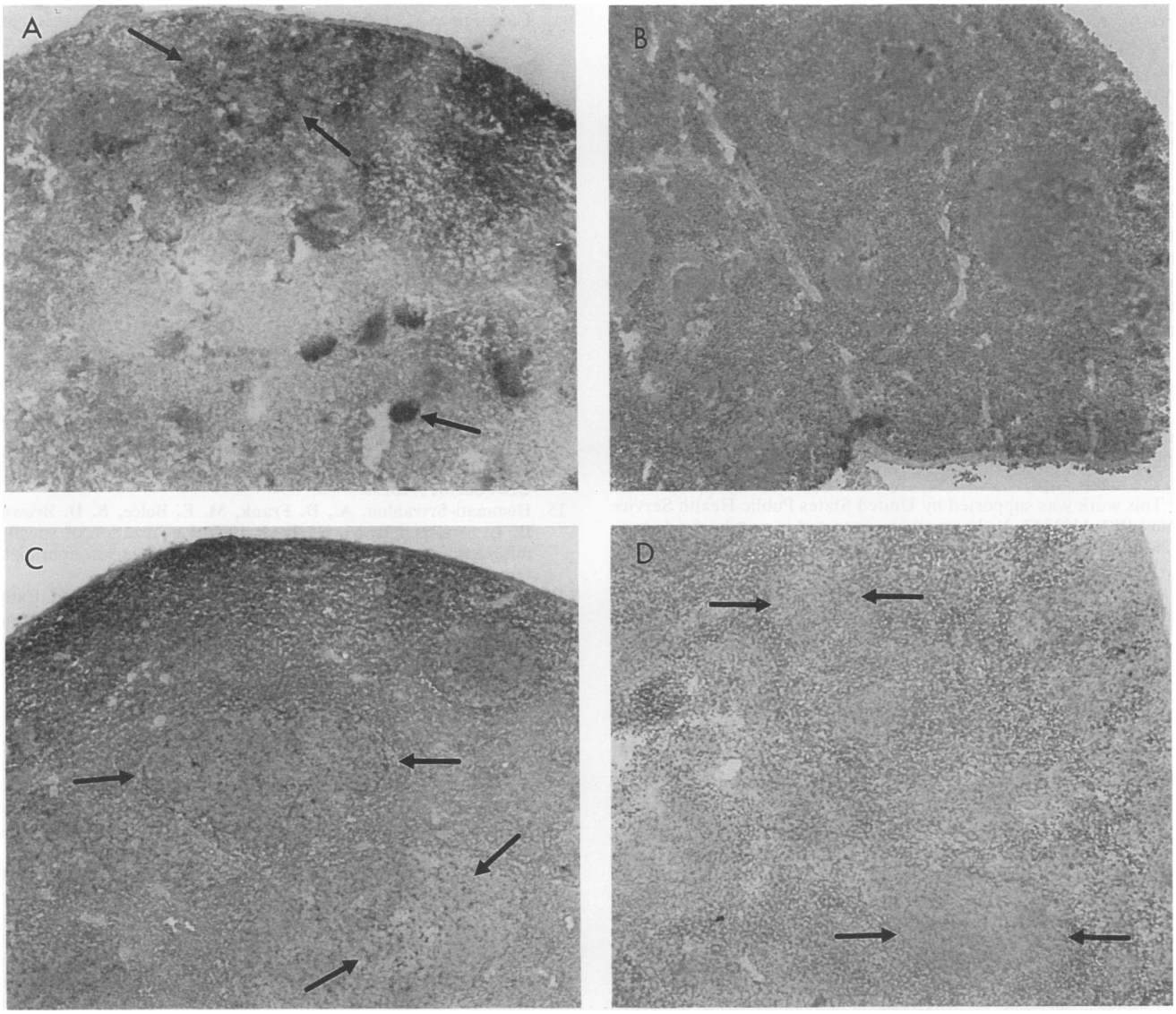

FIG. 3. In situ hybridization analysis of IFN-γ-expressing (A and B) and GM-CSF-expressing (C and D) cells in the spleens of *Listeria*-infected C57BL/6 and A/J mice. Paraffin-embedded spleen sections were subjected to in situ hybridization as described in Materials and Methods. The cells containing specific cytokine mRNA were detected with alkaline phosphatase-conjugated antidigoxigenin antibody and counterstained with nuclear fast red dye. Resistant C57BL/6 mice (A) had accumulations of IFN-γ-expressing cells (some areas are shown by arrows) in their spleens, which were not observed in the spleens of susceptible A/J mice (B). Cells expressing GM-CSF (C) were observed in circular areas (areas bordered by arrows) within the white pulp of resistant C57BL/6 spleens. Analogous areas (D) in the spleens of susceptible A/J mice (areas bordered by arrows) did not contain GM-CSF-expressing cells.

7). This study provides additional information which suggests that the susceptible A/J mice may be deficient in the expression of mRNA for cytokines that are thought to contribute to host defense against *L. monocytogenes*. There is evidence for the beneficial roles of IFN-γ and GM-CSF in protective immunity to listeriosis. Neutralization of endogenous IFN-γ with monoclonal antibodies markedly reduces anti-*Listeria* resistance in mice (2). Conversely, administration of murine recombinant IFN-γ significantly augments resistance to infection (17). In vitro studies have shown that exposure of macrophages to IFN-γ makes them less permissive for intracellular multiplication of *L. monocytogenes* (22). It might be inferred from our data that the increased resistance of C57BL/6 mice results in part from a similar effect of IFN-γ on the multiplication of *L. monocytogenes* in hepatocytes and mononuclear phagocytes in vivo. A role for GM-CSF in anti-*Listeria* immunity has been inferred from its increased production during experimental listeriosis (3, 26) and from evidence that $CD4^+$ cells that produce GM-CSF are capable of transferring resistance to naive recipients (18, 19).

It is interesting that we did not detect substantial differences between C57BL/6 and A/J mice in the production of IL-2 and IL-4 mRNAs in the liver (Table 1) or spleen (data not shown). This contrasts with the genetically determined resistance of mice to *Leishmania major* infection, in which susceptible BALB/c mice produce principally IL-4 and resistant C57BL/6 mice produce a strong IFN-γ response (13, 14). We have reported that administration of anti-IL-4 monoclonal antibody increases the resistance of BDF_1 mice to *L.*

monocytogenes infection without stimulating a proportionally greater production of IFN-γ (9). Although the findings of the present study are consistent with IFN-γ having an important role in the superior anti-*Listeria* resistance of C57BL/6 mice, the data do not suggest that increased production of IL-4 is associated with the greater susceptibility of A/J mice to experimental listeriosis.

In summary, the results of this study provide evidence that resistant C57BL/6 and susceptible A/J mice differ in their abilities to produce IFN-γ mRNA and GM-CSF mRNA in the spleen during experimental *L. monocytogenes* infection. It is possible that the speed and vigor with which the infected animal produces these cytokines may be important in determining the outcome of infection.

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REFERENCES

1. **Brandwein, S. R., and L. Maenz.** 1992. Defective lipopolysaccharide-induced production of both interleukin-1α and interleukin-1β by A/J mouse macrophages is posttranscriptionally regulated. J. Leukocyte Biol. **51:**570–578.
2. **Buchmeier, N. A., and R. D. Schreiber.** 1985. Requirement of endogenous interferon-γ production for resolution of *Listeria monocytogenes* infection. Proc. Natl. Acad. Sci. USA **82:**7404–7408.
3. **Cheers, C., A. M. Haigh, A. Kelso, D. Metcalf, E. R. Stanley, and A. M. Young.** 1988. Production of colony-stimulating factors (CSFs) during infection: separate determinations of macrophage-, granulocyte-, granulocyte- macrophage-, and multi-CSFs. Infect. Immun. **56:**247–251.
4. **Cheers, C., I. F. C. McKenzie, H. Pavlov, C. Waid, and J. York.** 1978. Resistance and susceptibility of mice to bacterial infection: course of listeriosis in resistant or susceptible mice. Infect. Immun. **19:**763–770.
5. **Czuprynski, C. J., and J. F. Brown.** 1985. Genetically determined resistance to listeriosis is associated with increased accumulation of inflammatory neutrophils and macrophages which have enhanced listericidal activity. Immunology **55:**511–518.
6. **Czuprynski, C. J., P. M. Henson, and P. A. Campbell.** 1984. Killing of *Listeria monocytogenes* by inflammatory neutrophils and mononuclear phagocytes from immune and nonimmune mice. J. Leukocyte Biol. **35:**193–208.
7. **Gervais, F., A. Morris-Hooke, T. A. Tran, and E. Skamene.** 1986. Analysis of macrophage bactericidal function in genetically resistant and susceptible mice by using the temperature-sensitive mutant of *Listeria monocytogenes*. Infect. Immun. **54:**315–321.
8. **Gervais, F., M. Stevenson, and E. Skamene.** 1984. Genetic control of resistance to *Listeria monocytogenes*: regulation of leukocyte inflammatory responses by the Hc locus. J. Immunol. **132:**2078–2083.
9. **Haak-Frendscho, M., J. F. Brown, Y. Iizawa, R. D. Wagner, and C. Czuprynski.** 1992. Administration of anti-IL-4 monoclonal antibody 11B11 increases the resistance of mice to *Listeria monocytogenes* infection. J. Immunol. **148:**3978–3985.
10. **Haak-Frendscho, M., K. M. Young, and C. J. Czuprynski.** 1989. Treatment of mice with human recombinant interleukin-2 augments resistance to the facultative intracellular pathogen *Listeria monocytogenes*. Infect. Immun. **57:**3014–3021.
11. **Havell, E. A., L. L. Moldawer, D. Helfgott, P. L. Kilian, and P. B. Sehgal.** 1992. Type 1 IL-1 receptor blockade exacerbates murine listeriosis. J. Immunol. **148:**1486–1492.
12. **Havell, E. A., and P. B. Sehgal.** 1991. Tumor necrosis factor-independent IL-6 production during murine listeriosis. J. Immunol. **146:**756–761.
13. **Heinzel, F. P., M. D. Sadick, B. J. Holaday, R. L. Coffman, and R. M. Locksley.** 1989. Reciprocal expression of interferon or interleukin 4 during the resolution or progression of murine leishmaniasis. Evidence for expansion of distinct helper T cell subsets. J. Exp. Med. **169:**59–72.
14. **Heinzel, F. P., M. D. Sadick, S. S. Mutha, and R. M. Locksley.** 1991. Production of interferon-γ, interleukin 2, interleukin 4, and interleukin 10 by $CD4^+$ lymphocytes *in vivo* during healing and progressive murine leishmaniasis. Proc. Natl. Acad. Sci. USA **88:**7011–7015.
15. **Hemmati-Brivanlou, A., D. Frank, M. E. Bolce, B. D. Brown, H. L. Sive, and R. M. Harland.** 1990. Localization of specific mRNAs in *Xenopus* embryos by whole-mount *in situ* hybridization. Development **110:**325–330.
16. **Iizawa, Y., J. F. Brown, and C. J. Czuprynski.** 1992. Early expression of cytokine mRNA in mice infected with *Listeria monocytogenes*. Infect. Immun. **60:**4068–4073.
17. **Kiderlen, A. F., S. H. E. Kaufmann, and M. L. Lohmann-Matthes.** 1984. Protection of mice against the intracellular bacterium *Listeria monocytogenes* by recombinant immune interferon. Eur. J. Immunol. **14:**964–967.
18. **Magee, D. M., and E. J. Wing.** 1988. Antigen-specific production of colony-stimulating factors by *Listeria monocytogenes*-immune L3T4-positive cells. J. Infect. Dis. **157:**94–99.
19. **Magee, D. M., and E. J. Wing.** 1989. Secretion of colony-stimulating factors by T-cell clones. Role in adoptive protection against *Listeria monocytogenes*. J. Immunol. **143:**2336–2341.
20. **Nakane, A., T. Minagawa, M. Kohanawa, Y. Chen, H. Sato, M. Moriyama, and N. Tsuruoka.** 1989. Interaction between endogenous gamma interferon and tumor necrosis factor in host resistance against primary and secondary *Listeria monocytogenes* infections. Infect. Immun. **57:**3331–3337.
21. **Nakane, A., A. Numata, and T. Minagawa.** 1992. Endogenous tumor necrosis factor, interleukin-6, and gamma interferon levels during *Listeria monocytogenes* infection in mice. Infect. Immun. **60:**523–528.
22. **Portnoy, D. A., R. D. Schreiber, P. Connelly, and L. G. Tilney.** 1989. γ interferon limits access of *Listeria monocytogenes* to the macrophage cytoplasm. J. Exp. Med. **170:**2141–2146.
23. **Poston, R. M., and R. J. Kurlander.** 1991. Analysis of the course of IFN-γ mRNA and protein production during primary murine listeriosis. J. Immunol. **146:**4333–4337.
24. **Stevenson, M. M., P. A. L. Kongshavn, and E. Skamene.** 1981. Genetic linkage resistance to *Listeria monocytogenes* with macrophage inflammatory responses. J. Immunol. **127:**402–407.
25. **Wagner, R. D., and C. J. Czuprynski.** 1993. Cytokine mRNA expression in livers of mice infected with *Listeria monocytogenes*. J. Leukocyte Biol. **53:**525–531.
26. **Young, A. M., and C. Cheers.** 1986. Colony-forming cells and colony-stimulating activity during listeriosis in genetically resistant or susceptible mice. Cell. Immunol. **97:**227–237.