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ROLES OF CD4+ AND CD8+ CELLS, AND THE EFFECT OF ADMINISTRATION OF RECOMBINANT MURINE INTERFERON γ IN LISTERIAL INFECTION

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Resistance to infection by the facultative intracellular bacterium *Listeria monocytogenes* is mediated by T lymphocytes (1, 2). The response of T cells is antigen specific and results in the activation of the microbicidal activity of macrophages (3, 4). IFN-γ and other lymphokines produced by T cells appear to be involved in this activation of macrophages (5).

The acquisition of delayed-type hypersensitivity (DTH)[1] and the focal accumulation of macrophages in infected sites are correlated with resistance to listerial infection (6). Studies on adoptive transfer of T cells from *Listeria*-immune mice indicated that both CD4+ and CD8+ cells were required for DTH (7), macrophage accumulation (8–10), and optimal protection (7–11), but that, when large numbers of cells were transferred, CD8+ cells alone were sufficient for resistance to *Listeria* (11). Recently, Mielke et al. (12) showed directly, by in vivo administration of anti-CD4 and anti-CD8 mAbs, that CD4+ cells were responsible for DTH, whereas CD8+ cells played the predominant role in protection against *Listeria*. Furthermore, they demonstrated that CD8+ cells mediated protection by activating resident macrophages without accumulating blood macrophages at the site of infection (13). Thus, the expression of DTH against *L. monocytogenes* and macrophage accumulation are attributable to CD4+ cells, whereas protective immunity against *Listeria* is mainly due to CD8+ cells (12, 13). However, in similar studies, Czuprynski et al. (14) showed the protective effect of CD4+ cells by demonstrating exacerbation of listeriosis characterized by numerous foci of liver necrosis in mice treated with anti-CD4 mAb. The liver was recently found to be the main target organ of *Listeria* (15).

In this study, we investigated the roles of CD4+ and CD8+ cells in primary sublethal and lethal infection with *L. monocytogenes* by examining the effects of in vivo administration of anti-CD4 and/or anti-CD8 mAb on recovery of bacteria, the levels of transaminases, and mortality. We also examined the protective effect of IFN-γ in lethally infected mice.

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[1] *Abbreviations used in this paper:* DTH, delayed-type hypersensitivity; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; H-E, hematoxylin-eosin; rMuIFN-γ, recombinant murine IFN-γ; TSA, trypticase-soy agar; TSB, trypticase-soy broth.

Materials and Methods

Mice. Female BALB/c, congenitally athymic BALB/c (*nu/nu*) mice and their heterozygous littermates were used at the age of 8–10 wk. These mice were obtained from Shizuoka Laboratory Animal Center (Shizuoka, Japan) and bred in the Laboratory Animal Center for Biomedical Research, Nagasaki University School of Medicine.

Bacteria. L. monocytogenes, strain IID 575, originally obtained from the National Institutes of Health, Bethesda, MD, was provided by Dr. Yoshikawa, Institute of Medical Science, Tokyo University (Tokyo, Japan). It was kept virulent by successive passages in mice and stored frozen at −70°C in trypticase-soy broth (TSB) with 20% glycerol. For each experiment, a sample was thawed and suspended in sterile saline solution for intravenous injection. Recovery of *Listeria* was assayed by homogenizing livers and spleens in saline solution and plating 10-fold serial dilutions of the homogenates on trypticase-soy agar (TSA) with SRBC. Colonies were counted after incubation for 24 h at 37°C (16). The LD_{50} values against BALB/c and BALB/c *nu/nu* mice were $\sim 4 \times 10^3$ and $\sim 3 \times 10^4$ CFU, respectively. BALB/c mice were treated intravenously with $4–16 \times 10^2$ CFU of bacteria for sublethal infection and $7–8 \times 10^3$ CFU of bacteria for lethal infection. BALB/c *nu/nu* mice were treated intravenously with 10^4 CFU of bacteria for sublethal infection.

mAbs. Anti-L3T4 (CD4) mAb, a rat antibody of the IgG2b Ig class produced by hybridoma GK1.5 (17), was kindly provided by Dr. Fitch, University of Chicago (Chicago, IL). The anti-Lyt-2.2 (CD8) mAb used was described previously (18). The titers of anti-L3T4 (CD4) and anti-Lyt-2.2 (CD8) mAbs, determined by antibody-mediated complement-dependent cytotoxicity assay, were both 1:20,000. These antibodies were used in the form of ascites from hybridoma-bearing mice. The concentrations of anti-L3T4 (CD4) and anti-Lyt-2.2 (CD8) mAbs in pooled ascites were 2.8 and 7.1 mg/ml, respectively, as determined by protein assay (Bio-Rad Laboratories, Richmond, CA) and quantitative cellulose acetate electrophoresis.

Complement (C). Preselected rabbit serum was used as complement.

In Vivo Administration of mAb. Mice were anesthetized with ether, and 200 μl of mAb (ascites) diluted 1:8 with MEM was injected intravenously through the retrobulbar venous plexus (18). We found previously by flow cytometric analysis that single injections (25 μl of ascites) of anti-L3T4 (CD4) mAb and anti-Lyt-2.2 (CD8) mAbs resulted in the elimination from peripheral lymphoid tissues of $CD4^+$ cells for ~2 wk and $CD8^+$ cells for ~6 wk, respectively (19). Therefore, we injected anti-L3T4 (CD4) mAb at intervals of 2 wk, and anti-Lyt-2.2 (CD8) mAb at intervals of 4 wk. Experiments lasted no more than 60 d. Blocking of the functions of $CD4^+$ cells and $CD8^+$ cells in vivo by mAb treatment was monitored by skin graft rejection in combinations of $H\text{-}2^b$ mutant strains and C57BL/6 (20).

Recombinant Murine IFN-γ (rMuIFN-γ) and Its Administration to Mice. rMuIFN-γ was produced and purified to >95% by Genentech Laboratory (San Francisco, CA) (21). The lot (No. 4407-47) used was obtained through Drs. Naruse and Satoh (Daiichi Seiyaku Co. Ltd., Tokyo, Japan). The antiviral activity of rMuIFN-γ was 8.6×10^6 U/ml and the protein concentration was 0.98 mg/ml, as determined by the Lowry method. The solution of rMuIFN-γ was diluted with PBS before use. IFN-γ was injected within several hours after inoculation of bacteria.

Pretreatment of Spleen Cells with mAb and C. Spleen cells (5×10^7) were incubated with mAb in a volume of 200 μl for 30 min at 4°C with occasional mixing. They were then pelleted and resuspended in rabbit complement diluted 1:10 with MEM, incubated for 30 min at 37°C, and then washed twice. In adoptive transfer experiments, 3×10^7 cells were injected intravenously.

Measurement of Serum Transaminase. Serum samples were diluted 1:5 with distilled water, and glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were measured with a Clinalyzer RX 40 (Nippon Denshi, Tokyo, Japan).

Histological Examination. Organs removed from mice were fixed in 10% buffered formaldehyde and stained with hematoxylin-eosin (H-E). Gram staining of paraffin sections was used for detection of bacteria in tissues.

Results

Effect of In Vivo Administration of Anti-L3T4 (CD4) mAb and/or Anti-Lyt-2.2 (CD8) mAb on Multiplication and Clearance of Bacteria, and Liver Cell Necrosis in Sublethal Listeriosis. Injection of 10^3 CFU of *L. monocytogenes* into BALB/c mice caused sublethal listeriosis. To determine the roles of T cell subsets in primary sublethal listeriosis, we examined the effect of in vivo administration of anti-L3T4 (CD4) mAb, anti-Lyt-2.2 (CD8) mAb, and a combination of both mAbs on the multiplication and clearance of the bacteria and liver cell necrosis. The injections of anti-L3T4 (CD4) mAb and anti-Lyt-2.2 (CD8) mAb caused selective depletions of $CD4^+$ cells and $CD8^+$ cells, respectively (see Materials and Methods). As shown in Fig. 1, the number of bacteria recovered from the liver and spleen of the infected mice treated with MEM (control) reached a peak of $\sim 10^5$ CFU on days 2–4 and then decreased to an undetectable level by day 10. Treatment of mice with anti-L3T4 (CD4) mAb increased the peak number of the bacteria, whereas treatment with anti-Lyt-2.2 (CD8) mAb or a combination of the two mAbs decreased the peak number.

Treatment of mice with anti-L3T4 (CD4) or anti-Lyt-2.2 (CD8) mAb resulted in clearance of the bacteria, although this was delayed, but treatment with a combination of the two mAbs blocked bacterial clearance. These results indicated that either $CD4^+$ or $CD8^+$ cells were necessary for, and capable of, mediating clearance of the bacteria. As the delay of bacterial clearance was significantly greater after treatment with anti-Lyt-2.2 (CD8) mAb than after treatment with anti-L3T4 (CD4) mAb,

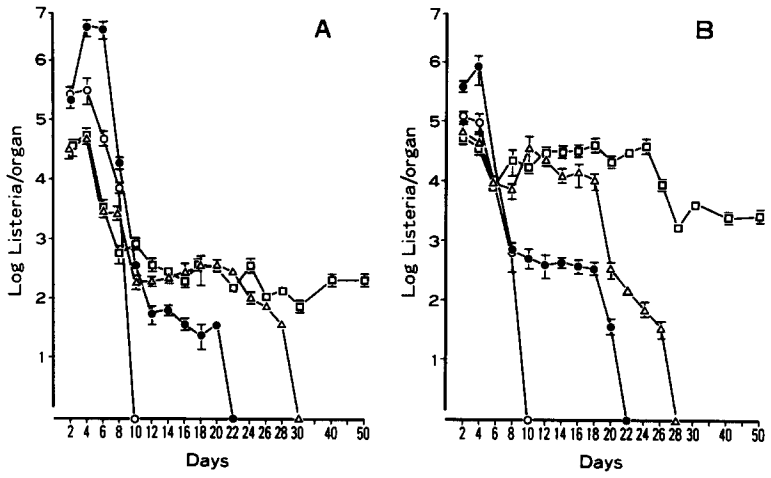

FIGURE 1. Effects of in vivo administration of anti-L3T4 (CD4) mAb (●), anti-Lyt-2.2 (CD8) mAb (Δ), or a combination of both mAbs (□) on recovery of bacteria from the liver (*A*) and spleen (*B*). (○) MEM control. Female BALB/c mice were injected intravenously with 10^3 CFU of *L. monocytogenes* on day 0, anti-L3T4 (CD4) mAb on days −1, 13, 27, and 41, and anti-Lyt-2.2 (CD8) mAb on days −1 and 27. The number of *Listeria* recovered from organs of infected mice on the indicated days was determined by colony formation assay on TSA. Values are means ± SD for groups of three to seven mice and means for groups of two mice. The detection limit was 1 Log *Listeria*.

$CD8^+$ cells may be more efficient than $CD4^+$ cells in mediating clearance of bacteria in sublethal listeriosis.

Serum GOT and GPT are parenchymal cell enzymes commonly used as markers of liver cell necrosis. The serum transaminase levels were slightly elevated (GOT, <200 U/ml; GPT, <100 U/ml) in mice treated with MEM (control) after injection of 10^3 CFU of *Listeria* (Fig. 2). Treatment of mice with anti-L3T4 (CD4) mAb increased the peak levels further, whereas treatment with anti-Lyt-2.2 (CD8) mAb or both mAbs lowered the levels slightly. Histological examination of the liver on day 4 showed multiple necrotic foci in mice treated with MEM (Fig. 3 *A*) and larger and more numerous necrotic foci in mice treated with anti-L3T4 (CD4) mAb.

The increases in the peak number of bacteria recovered from the liver and spleen, and in the levels of serum transaminases in mice treated with anti-L3T4 (CD4) mAb, were studied further using different sized inocula of *Listeria* for infection. As shown in Tables I and II, these increases were even clearer in mice infected with a larger number of bacteria (1.6×10^3 CFU), but were not observed in mice infected with fewer bacteria (4×10^2 CFU).

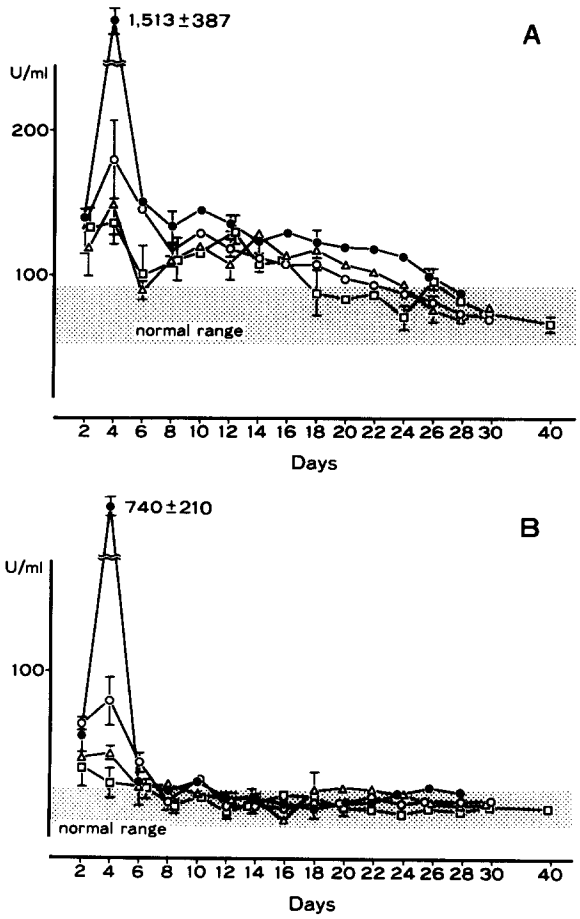

FIGURE 2. Serum GOT (*A*) and GPT (*B*) levels in the mice shown in Fig. 1. Explanations are as for Fig. 1.

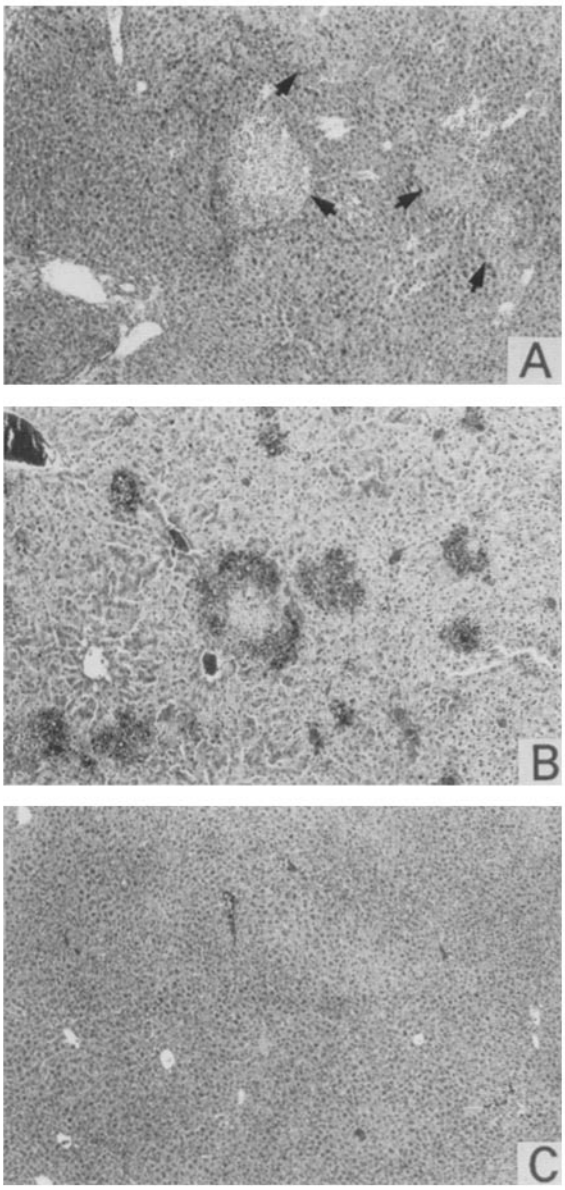

FIGURE 3. Photomicrographs of the liver in listeriosis. Specimens were obtained on day 4 from a mouse inoculated with a sublethal dose (10^3 CFU) (*A*), and on day 3 from a mouse inoculated with a lethal dose (7×10^3 CFU) (*B*) of *Listeria*, and on day 14 from a mouse inoculated with a lethal dose and rMuIFN-γ on day 0 (*C*). H-E, ×40. Multiple necrotic foci (*arrows*) are observed in *A*, multiple small abscesses (*stained dark*) containing bacterial clusters and neutrophils in diffusely necrotic liver are seen in *B*, and no obvious necrotic foci can be seen in *C*.

Effect of In Vivo Administration of Anti-L3T4 (CD4) mAb and/or Anti-Lyt-2.2 (CD8) mAb on Mortality, Bacterial Multiplication, and Liver Cell Necrosis in Lethal Listeriosis. Injection of $>7 \times 10^3$ CFU of *L. monocytogenes* into BALB/c mice caused lethal listeriosis; the mice died after 4–8 d from systemic infection and massive liver necrosis. We investigated the effect of in vivo administration of anti-L3T4 (CD4) mAb and/or anti-Lyt-2.2 (CD8) mAb on survival of these mice with lethal listeriosis (Fig. 4). Treatment with anti-L3T4 (CD4) mAb did not prolong survival significantly, but

TABLE I

Effect of Anti-L3T4 (CD4) mAb on Bacterial Recovery from the Liver and Spleen of Mice with Sublethal Listeriosis

Organ	Treatment	Number of bacteria recovered		
		4.0×10^2*	8.0×10^2	1.6×10^3
		log CFU		
Liver	MEM	3.34 ± 0.22	5.14 ± 0.99	5.56 ± 0.36
	Anti-L3T4 (CD4) mAb	3.20 ± 0.09	6.30 ± 0.04	6.50 ± 0.30
Spleen	MEM	3.20 ± 0.22	4.52 ± 0.52	4.66 ± 0.38
	Anti-L3T4 (CD4) mAb	3.95 ± 0.06	4.41 ± 0.52	5.71 ± 0.34

* Dose of *Listeria* injected (CFU). Bacteria were recovered from the liver and spleen on day 4 after infection.

treatment with anti-Lyt-2.2 (CD8) mAb, or a combination of both mAbs, prolonged survival markedly.

The effects of these mAbs on multiplication of bacteria in lethal listeriosis were then investigated. After treatment of mice with MEM (control) or anti-L3T4 (CD4) mAb, the recovery of bacteria increased exponentially in the liver and spleen resulting in death, whereas after treatment with anti-Lyt-2.2 (CD8) mAb or a combination of both mAbs, the recovery of bacteria increased to a peak of $\sim 10^8$ CFU on day 3 and then decreased (Fig. 5). Treatment of mice with anti-Lyt-2.2 (CD8) mAb resulted in clearance of the bacteria by day 40, whereas after treatment with a combination of the two mAbs, the mice remained persistently infected and $\sim 10^3$–10^4 CFU of bacteria were recovered from the liver and spleen for up to at least 60 d (data not shown).

Increases in the serum levels of transaminases (GOT, >500 U/ml; GPT, >150 U/ml) were observed as early as day 2 in all mice treated with MEM (control) or mAb (Fig. 6). After treatment with MEM (control) or anti-L3T4 (CD4) mAb, the levels of serum transaminases increased continuously until death, whereas after treatment with anti-Lyt-2.2 (CD8) mAb or a combination of the two mAbs, the levels of serum transaminases increased to a peak on day 3, and then decreased to within the normal range by day 14. Histopathological examination of the liver showed massive necrosis on day 3 in mice treated with MEM (Fig. 3 *B*) or anti-L3T4 (CD4) mAb.

TABLE II

Effect of Anti-L3T4 (CD4) mAb on Serum Levels of Transaminases in Mice with Sublethal Listeriosis

Transaminase	Treatment	Serum transaminase		
		4.0×10^2*	8.0×10^2	1.6×10^3
		U/ml		
GOT	MEM	93 ± 4	163 ± 58	175 ± 7
	Anti-L3T4 (CD4) mAb	102 ± 1	254 ± 63	1,387 ± 212
GPT	MEM	42 ± 4	97 ± 57	100 ± 16
	Anti-L3T4 (CD4) mAb	48 ± 2	225 ± 50	847 ± 82

* Dose of *Listeria* injected (CFU). Serum transaminases were measured on day 4 after infection.

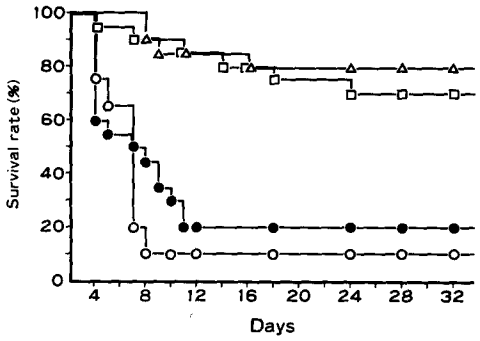

FIGURE 4. Effects of in vivo administration of anti-L3T4 (CD4) mAb (●), anti-Lyt-2.2 (CD8) mAb (Δ), or a combination of both mAbs (□) on mortality in lethal listeriosis; (○) MEM control. Female BALB/c mice were treated intravenously with 7×10^3 *Listeria* on day 0, anti-L3T4 (CD4) mAb on days −1, 13, and 27, and Lyt-2.2 (CD8) mAb on days −1 and 27. Each group consisted of 20 mice.

In mice treated with anti-Lyt-2.2 (CD8) or a combination of the two mAbs, moderate liver necrosis was observed on day 3 followed by recovery by day 14.

These results suggested that $CD8^+$ cells played a role in irreversible multiplication of the bacteria and massive liver necrosis, which causes death in lethal listeriosis. Adoptive transfer experiments with nude mouse recipients were performed to confirm this point. Transfer of spleen cells from normal BALB/c mice pretreated with anti-L3T4 (CD4) mAb and complement, but not anti-Lyt-2.2 (CD8) mAb and

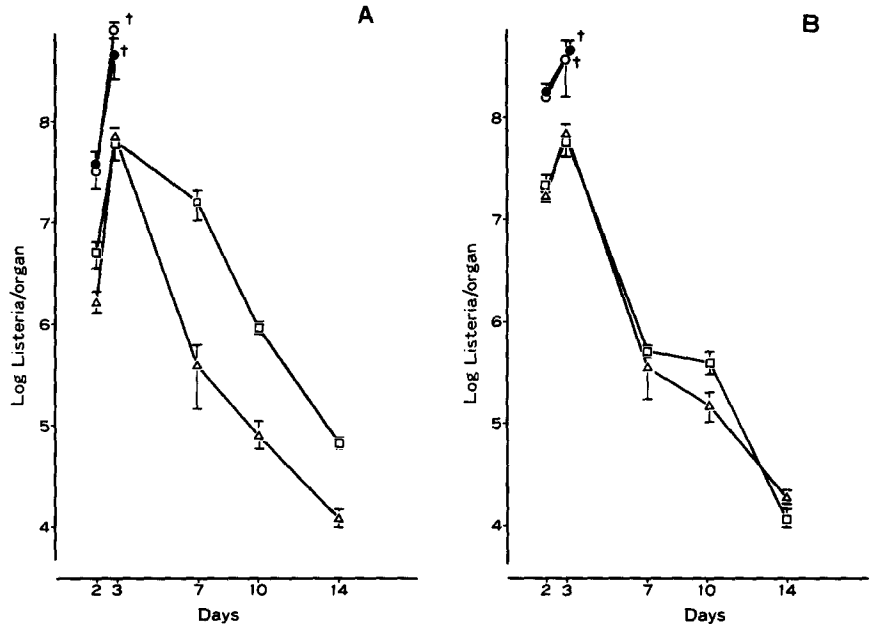

FIGURE 5. Effects of administration of anti-L3T4 (CD4) mAb (●), anti-Lyt-2.2 (CD8) mAb (Δ), or a combination of both mAbs (□) on multiplication of *Listeria* in the liver (*A*) and spleen (*B*) in lethal listeriosis; (○) MEM control. Female BALB/c mice were injected intravenously with 7×10^3 CFU of *Listeria* on day 0, anti-L3T4 (CD4) mAb on days −1, 13, 27, 41, and 55, and anti-Lyt-2.2 (CD8) mAb on days −1, 27, and 55. Each group consisted of four to six mice.

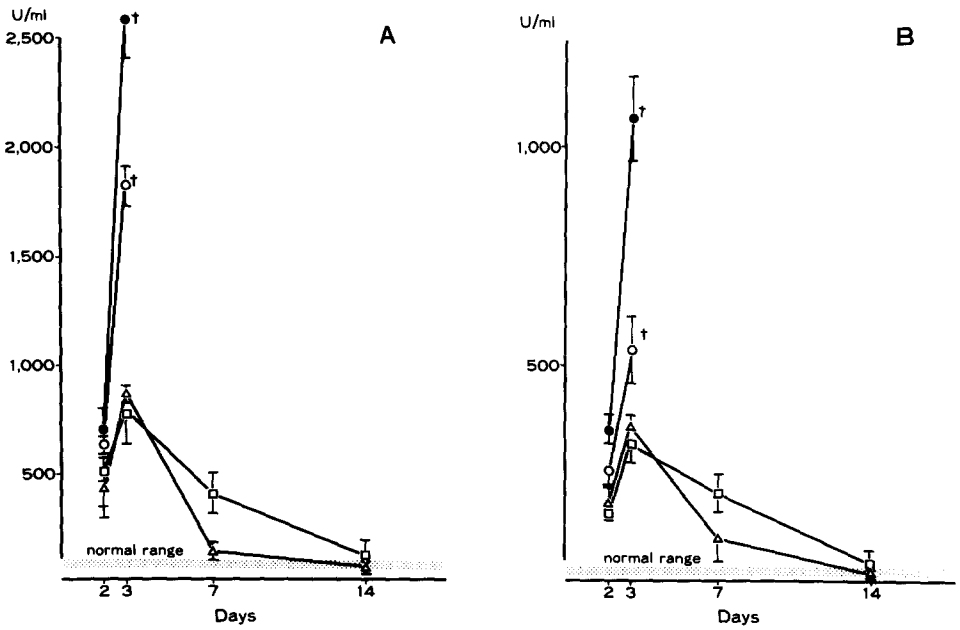

FIGURE 6. Serum GOT (*A*) and GPT (*B*) levels in the mice shown in Fig. 5. Explanations are as for Fig. 5.

complement, killed recipient BALB/c *nu/nu* mice inoculated with 10^4 CFU of bacteria (Fig. 7).

Effect of In Vivo Administration of rMuIFN-γ on Mortality, Bacterial Multiplication, and Liver Cell Necrosis in Lethal Listeriosis. In mice with lethal listeriosis that were untreated or had been pretreated with anti-L3T4 (CD4) mAb, injection of rMuIFN-γ (3×10^4 U/mouse, i.v.) resulted in marked prolongation of survival (Table III and Fig. 8), decrease in multiplication of bacteria (Fig. 9), and restoration of transaminase levels to normal (Fig. 10). Histological examination showed recovery from necrosis in the liver of mice on day 7 after treatment with either MEM (Fig. 3 *C*) or anti-L3T4 (CD4) mAb and rMuIFN-γ.

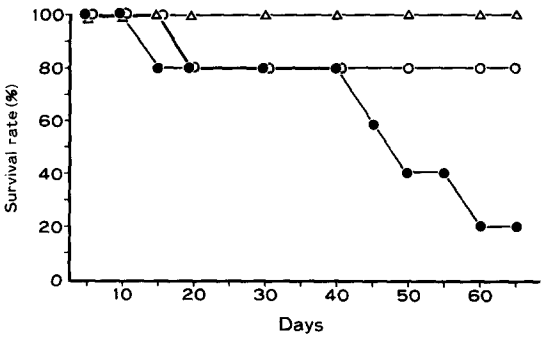

FIGURE 7. Adoptive transfer experiments. BALB/c *nu/nu* mice were injected intravenously on day −7 with BALB/c *nu/+* spleen cells that had been pretreated with anti-L3T4 (CD4) mAb (●) or anti-Lyt-2.2 (CD8) mAb (Δ) and complement; (○) untreated BALB/c *nu/nu* mice. Recipient nude mice were injected with 10^4 CFU of *Listeria* on day 0. The LD_{50} against BALB/c *nu/nu* mice was ~3×10^4 CFU. Each group consisted of five mice.

TABLE III

Effect of Administration of rMuIFN-γ on Mortality in Lethal Listeriosis

Number of mice surviving/number challenged			
MEM alone	3×10^{2}*	3×10^{3}	3×10^{4}
0/5	0/5	3/5	5/5

* Dose of murine rMuIFN-γ injected (U). Female BALB/c mice were injected intravenously with 7×10^{3} CFU of *Listeria* and rIFN-γ in MEM on day 0. Survival was determined on day 20.

Discussion

In this study, we found that in mice with sublethal listeriosis, $CD8^+$ cells augmented the peak recovery of bacteria and the extent of liver cell necrosis, and $CD4^+$ cells suppressed this $CD8^+$ cell-mediated augmentation. Treatment of mice with anti-CD4 mAb increased the peak recovery of bacteria and the levels of serum transaminases, whereas treatment with anti-CD8 mAb or a combination of the two mAbs had the reverse effects. These effects were evident in mice treated with higher doses ($0.8–16 \times 10^{3}$) of bacteria. In mice treated with a combination of the two mAbs, the liver and spleen remained persistently infected ($10^{2}–10^{4}$ CFU/organ) after the initial decrease in the bacterial recovery. Since persistent infection was also observed in BALB/c *nu/nu* mice inoculated with a sublethal dose of *Listeria* (unpublished results and reference 16), the mechanism(s) of resistance to *Listeria* multiplication in the persistent infection appears to be T cell independent. In fact, macrophage-mediated protection against *Listeria* was shown to be effective to a significant extent in euthymic mice as well as in nude mice (16). For complete clearance of bacteria, however, T cells are definitely required (1, 2, 16, and this paper). We demonstrated that either $CD4^+$ or $CD8^+$ cells were necessary for, and capable of, mediating clearance of the bacteria. $CD8^+$ cells were more efficient than $CD4^+$ cells, but for optimal clearance both were necessary.

It has been controversial whether $CD4^+$ cells or $CD8^+$ cells are responsible for protection against *Listeria*. Previous studies on adoptive transfer of lymphocytes from immune mice to naive recipients showed that both $CD4^+$ and $CD8^+$ cells were required for resistance to *Listeria* (7–11). However, when larger numbers of lymphocytes were transferred, $CD8^+$ cells were sufficient for protection (11). Recently,

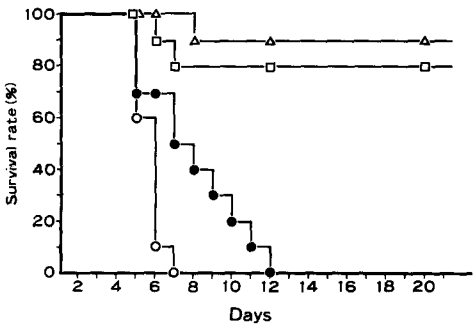

FIGURE 8. Effect of administration of rMuIFN-γ on mortality in lethal listeriosis. Female BALB/c mice were pretreated with either MEM (Δ) or anti-L3T4 (CD4) mAb (□) on day −1, and inoculated with 7×10^{3} CFU of *Listeria* and rIFN-γ (10^{4} U) on day 0. (○) MEM alone; (●) anti-L3T4 (CD4) mAb alone. Each group consisted of 10 mice.

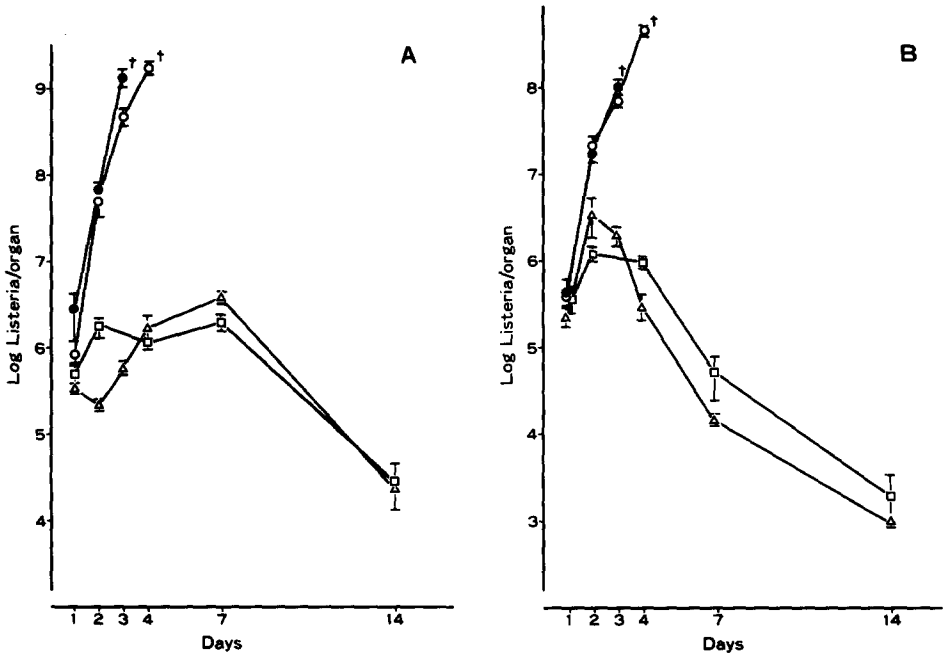

FIGURE 9. Effect of administration of rMuIFN-γ on multiplication of *Listeria* in the liver (*A*) and spleen (*B*) in lethal listeriosis. Female BALB/c mice were pretreated with either MEM (△) or anti-L3T4 (CD4) mAb (□) on day −1 and then injected intravenously with 7×10^3 CFU of *Listeria* and rIFN-γ (10^4 U) on day 0. (○) MEM alone; (●) anti-L3T4 (CD4) mAb alone. Each group consisted of four to six mice.

Mielke et al. (12) demonstrated directly, by in vivo administration of anti-CD4 and anti-CD8 mAb, that $CD8^+$ cells play the main role in protection in mice: administration of anti-Lyt-2 (CD8) but not anti-L3T4 (CD4) mAb resulted in inhibition of bacterial clearance determined at a later stage after infection. On the other hand, Czuprynski et al. (14) showed that in vivo administration of anti-L3T4 (CD4) mAb to mice resulted in increased recovery of bacteria and exacerbation of liver necrosis early (day 3) after inoculation of a sublethal dose of *Listeria*. Thus, they concluded that $CD4^+$ cells were essential for protection. The present findings in kinetic analyses of bacterial recovery and the levels of serum transaminases were consistent with all these results and revealed that these findings are not conflicting, but rather reflected different phases during the course of infection.

We also demonstrated that in lethal listeriosis, treatment of mice with anti-CD8 mAb, or a combination of anti-CD4 and anti-CD8 mAbs, but not anti-CD4 mAb alone, markedly prolonged survival, and resulted in decrease in recovery of bacteria after a peak of $\sim 10^8$ CFU and in a decrease in the levels of serum transaminases. These findings indicated that $CD8^+$ cells were responsible for irreversible multiplication of the bacteria and severe liver necrosis causing death in lethal listeriosis. In this regard, it is noteworthy that 95% of an intravenous inoculum of *Listeria* is cleared by the liver and that hepatocytes, rather than macrophages, are the main targets of *Listeria* in the liver (15). Hepatocytes are incapable of inactivating the bacteria

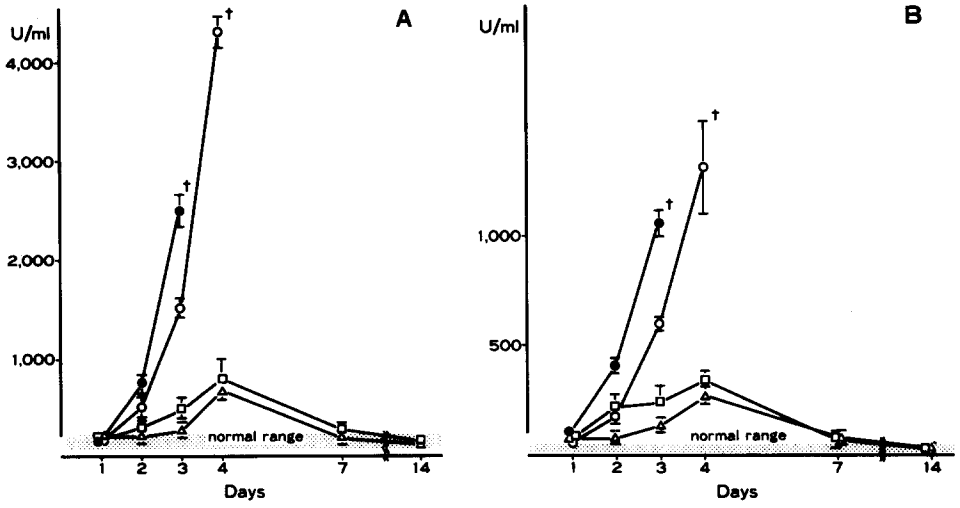

FIGURE 10. Serum GOT (*A*) and GPT (*B*) levels in the mice shown in Fig. 9. Explanations are as for Fig. 9.

intracellularly (15). Our results are consistent with the findings that *Listeria*-specific $CD8^+$ CTL are generated from spleen cells derived from infected mice (22). During the course of viral infection, $CD8^+$ CTL are generated against virally infected target cells, and these CTL have been shown to be important for antiviral defense (23). However, it is unknown whether the liver damage by $CD8^+$ cells observed in sublethal and lethal listerial infection represents a relevant mechanism of antibacterial resistance. The effects of administration of anti-CD4 and anti-CD8 mAbs were evident as early as day 2 after primary sublethal or lethal infection with *Listeria*. These findings indicated early involvement of T cells in host defense against primary listeriosis and are consistent with the findings previously described (2).

We found that administration of rMuIFN-γ to mice with lethal listeriosis markedly prolonged survival, and resulted in a decrease in the multiplication of bacteria and in recovery from liver necrosis in mice treated with either MEM or anti-CD4 mAb. These findings are consistent with previous reports of decreased bacterial recovery after administration of rMuIFN-γ (24) and the requirement of endogenous IFN-γ for recovery from *Listeria* infection (25, 26). The mechanism(s) by which IFN-γ protects mice against lethal listeriosis is unknown, but several possibilities may be considered. First, exogenously administered rIFN-γ may decrease the multiplication of bacteria by activating macrophages, subsequently resulting in recovery from liver cell necrosis. This possibility is consistent with reports that IFN-γ activates macrophages to promote antimicrobial activity in vitro (27, 28) and in vivo (24), and that macrophages ingest and inactivate *Listeria* (15, 29), rather than supporting its growth, as has been generally assumed (15). Second, IFN-γ may exert its effect by binding to surface receptors and inducing intracellular changes that are unfavorable for development of *Listeria*. This possibility is supported by reports that receptors for IFN-γ are present on various cells, including hepatocytes (30), and that IFN-γ induces phenotypic and functional alterations (31). Indeed, this mechanism has re-

cently been shown to operate in hepatocytes with malaria parasites (30). These two mechanisms are not mutually exclusive and could occur simultaneously. Third, IFN-γ may inhibit $CD8^+$ cell-mediated cytotoxicity. However, since IFN-γ has so far been regarded as an essential lymphokine for the proliferation and differentiation of cytotoxic T lymphocytes (32, 33), this possibility seems unlikely.

Summary

Studies were made on the effects of in vivo administration of anti-CD4 mAb, anti-CD8 mAb, or a combination of both mAbs on multiplication of bacteria, the levels of serum transaminases, and mortality in mice infected with *Listeria monocytogenes*. Results showed that in sublethal infection, $CD8^+$ cells enhanced the peak of bacterial multiplication and liver cell necrosis, and $CD4^+$ cells suppressed $CD8^+$ cell-mediated enhancement. Results also showed that either $CD4^+$ or $CD8^+$ cells were necessary for, and capable of, mediating clearance of the bacteria. $CD8^+$ cells were more efficient than $CD4^+$ cells, but for optimal clearance both were necessary.

In lethal listeriosis, treatment of mice with anti-CD8 mAb or a combination of both anti-CD4 and anti-CD8 mAbs, but not anti-CD4 mAb only, protected mice from death by decreasing multiplication of bacteria in the liver and spleen after a peak of $\sim 10^8$ CFU, and lowering the elevated serum levels of transaminases. These findings indicated that $CD8^+$ cells were responsible for causing irreversible systemic *Listeria* infection and severe liver necrosis.

In lethal listeriosis, administration of rMuIFN-γ markedly prolonged survival by decreasing multiplication of bacteria and promoting recovery from liver necrosis.

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