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CD95 (Fas) May Control the Expansion of Activated T Cells after Elimination of Bacteria in Murine Listeriosis

Y. FUSE,[1,2] H. NISHIMURA,[2] K. MAEDA,[1] AND Y. YOSHIKAI[2]*

Department of Internal Medicine, Nagoya University Branch Hospital,[1] and Laboratory of Host Defense and Germfree Life, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine,[2] Nagoya, Japan

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CD95 (Fas) is known to mediate activation-induced T-cell death by apoptosis. To understand the role of CD95 during the course of bacterial infection, we examined the kinetics of αβ and γδ T cells in the peritoneal cavities and livers of 5-week-old CD95-defective MRL/*lpr* mice after an intraperitoneal infection with *Listeria monocytogenes*. The number of bacteria in the spleen decreased to an undetectable level by day 10 after infection with 7×10^3 *Listeria* cells similar to the number in MRL/+/+ mice. The number of αβ T cells expressing CD44 and CD95 reached a maximum in the peritoneal cavity on day 6 after listerial infection and thereafter decreased gradually in MRL/+/+ mice, whereas $CD44^+$ αβ T cells without CD95 expression continued to increase throughout the course of listerial infection in MRL/*lpr* mice. Freshly isolated T cells from MRL/+/+ mice infected with *L. monocytogenes* 10 days previously showed DNA fragmentation with apoptosis, whereas such fragmentation was not prominent in T cells from infected MRL/*lpr* mice. In correlation with the increased number of $CD44^+$ αβ T cells, *Listeria*-specific T-cell proliferation of peritoneal exudate cells was significantly greater in MRL/*lpr* mice than in MRL/+/+ mice on day 10 after listerial infection. In contrast to αβ T cells, γδ T cells increased in number only transiently in the peritoneal cavity and liver after listerial infection in both MRL/*lpr* mice and MRL/+/+ mice. These results suggest that CD95-mediated cell death with apoptosis may be involved in termination of the αβ-T-cell-mediated immune response after the battle against *L. monocytogenes* has been won, whereas γδ T cells may undergo apoptosis independently of CD95 during the course of listerial infection.

CD95 (Fas) is a cell surface antigen that delivers an apoptotic signal, and it belongs to a family of molecules that includes tumor necrosis factor receptor, nerve growth factor receptor, and CD40 (8, 17, 33, 49, 53). This molecule is involved in the cytotoxicity for CD95-positive target cells of T cells expressing Fas-L (33, 50, 51, 54) and also in the induction of apoptosis in mature T cells during the process of peripheral deletion, which is thought to be important in establishing peripheral tolerance (7, 22, 52). CD95 is highly expressed on activated T cells (23), and autonomous Fas/Fas-L interaction is involved in elimination of antigen (Ag)-stimulated peripheral T cells for terminating an immune response and for limiting inflammation (4, 18, 34, 44, 56).

Mice homozygous for the mutation *lpr* develop massive lymphoproliferation (48) and an autoimmune syndrome similar to systemic lupus erythematosus and rheumatoid arthritis accompanied by the marked accumulation of an abnormal $B220^+$ double-negative $CD4^-$ $CD8^-$ T-cell population in the lymph nodes and spleen (4, 32, 37). *lpr* mutation is identified as mutant forms of the CD95 genes (53). Homozygosity at the *lpr* locus results in aberrant CD95 transcription and markedly diminished cell surface expression of CD95 (1). Various studies with *lpr* mice have reported on the role of CD95 in the deletion of activated T cells in the periphery (4, 23, 29, 33, 39, 47). When MRL/+/+ mice are injected intraperitoneally (i.p.) with the superantigen staphylococcal enterotoxin B (SEB), mature SEB-reactive Vβ8 T cells initially expand and then decrease by apoptosis, whereas the latter process is severely retarded or cannot be seen in *lpr* mice (29, 46). However, conflicting results have been reported (24, 31, 40). A defect in CD95-mediated apoptosis is thought to cause massive lymphoproliferative disorder in MRL/*lpr* mice (34). Thus, MRL/*lpr*

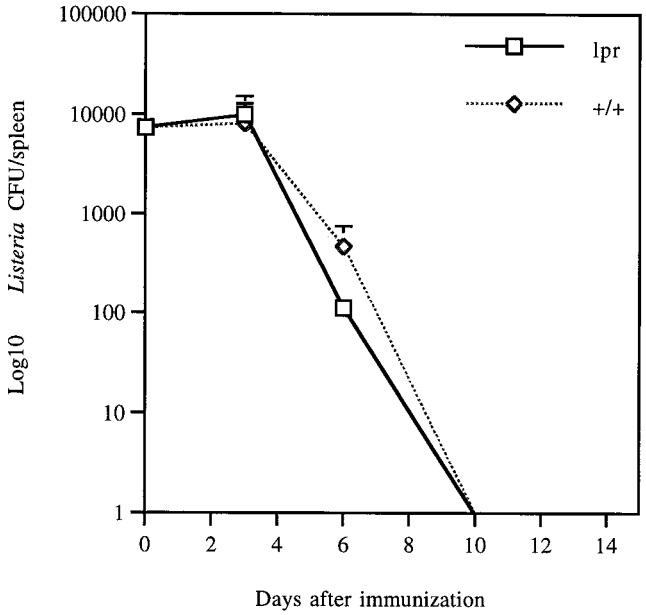

FIG. 1. Kinetics of bacterial growth in the spleen after i.p. injection of *L. monocytogenes*. MRL/*lpr* mice and MRL/+/+ mice were inoculated i.p. with 7×10^3 *L. monocytogenes* cells on day 0. The numbers of *L. monocytogenes* organisms from spleens of infected mice on days 3, 6, 10, and 14 were determined by colony formation assay on tryptic soy agar. Each point and vertical bar are the mean ± standard deviation for five mice.

* Corresponding author. Mailing address: Laboratory of Host Defense and Germfree Life, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, 65 Turumai-cho, Showa-ku, Nagoya 466, Japan. Phone: 052 (744) 2446. Fax: 052 (744) 2449.

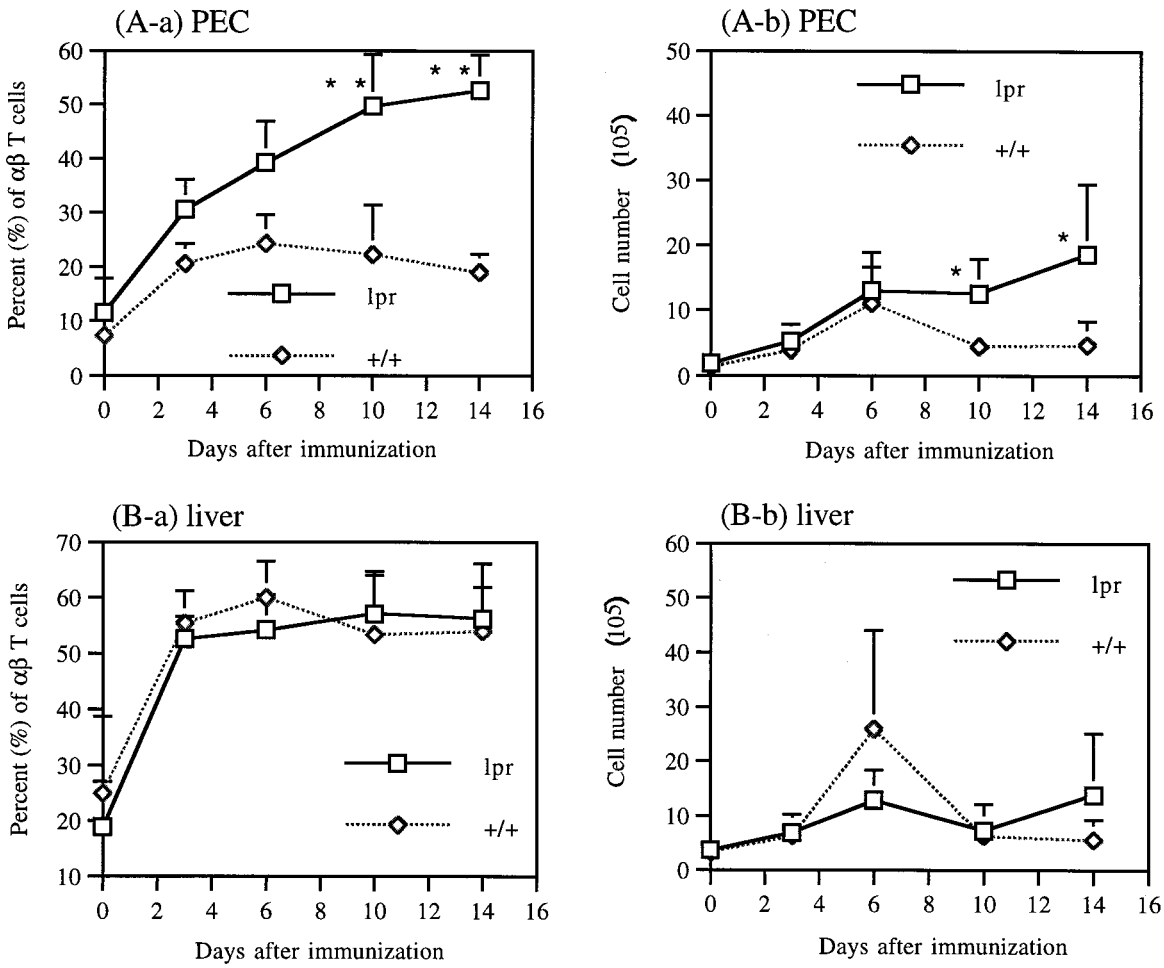

FIG. 2. Kinetics of the percentage of $CD3^+$ $\alpha\beta^+$ T cells in nonadherent cells (a) and the number of $CD3^+$ $\alpha\beta^+$ T cells (b) in the peritoneal cavity (A) and liver (B) after i.p. challenge with *L. monocytogenes*. Nonadherent cells from the peritoneal cavities or livers of MRL/*lpr* mice or MRL/+/+ mice inoculated i.p. with 7×10^3 *L. monocytogenes* cells on day 0 were stained with FITC–anti-CD3 MAb and PE–anti-αβ MAb. Each point and vertical bar are the mean ± standard deviation for five mice. Significant differences from the values for MRL/+/+ mice: *, $P < 0.05$; **, $P < 0.01$.

can be used for analysis of the biological significance of CD95-mediated activation-induced cell death of activated T cells during immune responses.

Listeria monocytogenes, an intracellular pathogen, has been widely used for analyzing cell-mediated immune responses (12). Resistance to *L. monocytogenes* at an early stage after infection is mediated by polymorphonuclear leukocytes, which destroy infected liver cells, followed by natural killer cells, which activate macrophages by means of gamma interferon (IFN-γ) (6). A specific immune response by T cells which leads to sterile eradication of the microbe then develops (12, 21, 30). The γδ T cells play a role in covering the gap between innate and acquired immunity in host defense against listerial infection (10, 15, 36). *Listeria*-specific T cells are known to accumulate preferentially in inflamed sites and to induce granulomatous lesions at the sites of bacterial multiplication. In primary listeriosis, αβ T cells are crucial for the development of granulomatous lesions, whereas γδ T cells are not sufficient for the generation of such organoid lesions (30). Besides αβ CD4 Th1 cells producing IFN-γ, CD8 cytotoxic lymphocytes are reported to contribute to protection against listerial infection (3, 13, 28) through cytotoxicity for infected macrophages and hepatocytes, although the overreaction may result in liver injury in *Listeria*-infected mice (11). It is known that CD95 is abundantly expressed in the liver and that injection of anti-CD95 antibody can induce hepatitis (33, 35, 54). Recent evidence indicated that *L. monocytogenes* induces apoptosis of infected hepatocytes (43). However, it remains elusive whether CD95-mediated apoptosis is involved in protection against and pathogenesis of listerial infection.

In the present study, in order to understand the role of CD95 during the course of listerial infection, we examined the bacterial growth, kinetics of T-cell accumulation, and histological changes in CD95-defective *lpr* mice after an i.p. injection with *L. monocytogenes*. Although there was no difference in resistance to listerial infection in *lpr* mice, deletion of αβ T cells expressing CD44 was severely retarded in *lpr* mice. The implications for the role of CD95 in termination of an immune response to *L. monocytogenes* are discussed.

MATERIALS AND METHODS

Animals. Female 5-week-old MRL/MpJ-*lpr*/*lpr* (MRL/*lpr*) and MRL/MpJ-+/+ (MRL/+/+) mice were purchased from CLEA Japan, Inc. (Tokyo, Japan), and fed under pathogen-free conditions.

Microorganism. *L. monocytogenes* EGD was used for the experiments. Bacterial virulence was maintained by serial passages in BALB/c mice. Fresh isolates

were obtained from infected spleens, grown in tryptic soy broth (Difco Laboratories, Detroit, Mich.), washed repeatedly, suspended in phosphate-buffered saline (PBS), and stored at −70°C in small aliquots. Heat-killed *L. monocytogenes* was prepared by incubating viable *L. monocytogenes* at 74°C for 120 min.

Cell preparation. Mice were inoculated intraperitoneally with viable *L. monocytogenes* in 0.1 ml of PBS on day 0. Peritoneal exudate cells (PEC) were harvested at intervals before and after infection (on days 3, 6, 10, and 14) by peritoneal lavage with ice-cold Hanks' balanced salt solution (HBSS). The cells were collected by centrifuging them at 110 × *g* for 5 min and washed twice with HBSS. Nonadherent cells were harvested after 1 h of culture at 37°C on a plastic dish and suspended at the optimal concentrations.

Liver was perfused with 5 ml of sterile HBSS to eliminate blood, meshed through 100-gauge stainless-steel mesh, and suspended in RPMI 1640 containing 10% fetal calf serum. After being washed with the medium, the cell pellet was suspended in 15 ml of the medium, and cell suspensions were centrifuged at 50 × *g* for 1 min. The suspension was harvested, spun down, suspended in 8 ml of 44% Percoll (Sigma Chemical Co., St. Louis, Mo.), and layered on 5 ml of 67.5% Percoll in a 15-ml tube. The gradient was spun down at 600 × *g* at 20°C for 20 min. Lymphocytes at the interface were harvested, washed twice, and suspended in HBSS.

Bacterial growth. Primary infection with *L. monocytogenes* was performed by an i.p. injection of 7×10^3 viable bacteria in 0.1 ml of PBS on day 0. Mice were anesthetized with ether and killed by cutting the cervical artery at intervals after the i.p. injection (on days 3, 6, 10, and 14). Bacterial growth in spleens was determined by plating 10-fold serial dilutions of organ homogenates on tryptic soy agar. The detection limit of this procedure was 10^2 *L. monocytogenes* per spleen. The colonies were counted after 24 h of incubation at 37°C.

Antibodies. Fluorescein isothiocyanate (FITC)-conjugated anti-CD3 monoclonal antibody (MAb), FITC-conjugated anti-T-cell receptor αβ (TCRαβ) MAb, FITC-conjugated anti-TCR γδ MAb, FITC-conjugated anti-CD44 (pgp-1) MAb, FITC-conjugated anti-CD4 MAb, phycoerythrin (PE)-conjugated anti-TCRαβ MAb, PE-conjugated anti-TCRγδ MAb, PE-conjugated anti-CD95 MAb, PE-conjugated anti-CD8α MAb, PE-conjugated anti-B220 MAb, biotin-conjugated anti-TCRαβ MAb, and biotin-conjugated anti-TCRγδ MAb were purchased from Phar Mingen (San Diego, Calif.). RED613-conjugated streptavidin was purchased from Gibco BRL (Gaithersburg, Md.).

FCM analysis. For two-color flow cytometric (FCM) analysis, cells were stained with FITC-conjugated MAb and PE-conjugated MAb. For three-color FCM analysis, cells were stained with FITC-conjugated MAb, PE-conjugated MAb, and biotinylated MAb followed by streptavidin RED613. Cells were stained at 4°C for 40 min and washed two times between each step with phenol red-free HBSS. Lymphocytes were analyzed with a FACScan (Becton Dickinson). Gates were set for viable cells on forward versus side scatter plots.

Proliferation assay. To obtain peritoneal T cells, peritoneal cells were harvested 3 days after injection of 10% Proteose Peptone (1 ml; Difco) on day 7 after listerial infection (namely, on day 10) and then passed two times through a nylon wool column. The purity of T cells was more than 95% as assessed by FCM analysis with anti-CD3 MAb. The non-nylon-wool-adherent cells (1.5×10^4/well) were incubated with syngeneic splenocytes (1.5×10^4/well) treated with mitomycin (Sigma) and heat-killed *L. monocytogenes* (corresponding to 10^6 viable *L. monocytogenes* per ml) in 96-well plates for 36 h. Then the culture was pulsed with 1 μCi of [^{3}H]thymidine per well and harvested 12 h later. Thymidine incorporated into the cells was measured with a liquid scintillation counter.

DNA fragmentation. Cells were freshly isolated from PEC and liver lymphocytes on day 10 after *Listeria* inoculation and passed through a nylon wool column. Then non-nylon-wool-adherent cells were harvested and treated with 20 mg of RNase per ml for 1 h at 37°C and 20 mg of proteinase K per ml for 1 h at 37°C. The lysate was separated by electrophoresis on a 2.0% agarose gel containing ethidium bromide (0.05 μg/ml). Gels were illuminated with UV light for visualization of DNA fragmentation.

Histology. Formalin-fixed tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin for general morphology.

Statistics. The statistical significance of the data was determined by Student's *t* test. A *P* of less than 0.05 was taken as significant.

RESULTS

Kinetics of bacterial growth after i.p. injection with *L. monocytogenes*. We examined the bacterial growth in peritoneal cavities, livers, and spleens of 5- and 16-week-old *lpr* mice and age- and sex-matched +/+ mice after i.p. injection of a sublethal dose (7×10^3 CFU/mouse) of viable *L. monocytogenes*. As shown in Fig. 1, the number of bacteria in the spleen increased to the maximal level on day 3 and thereafter decreased to an undetectable level by day 10 in both *lpr* and +/+ mice at 5 weeks of age. Similar kinetics was observed in peritoneal cavities and livers (data not shown). There was no difference in the number of bacteria at any stage after infection in 5-week-old *lpr* and +/+ mice.

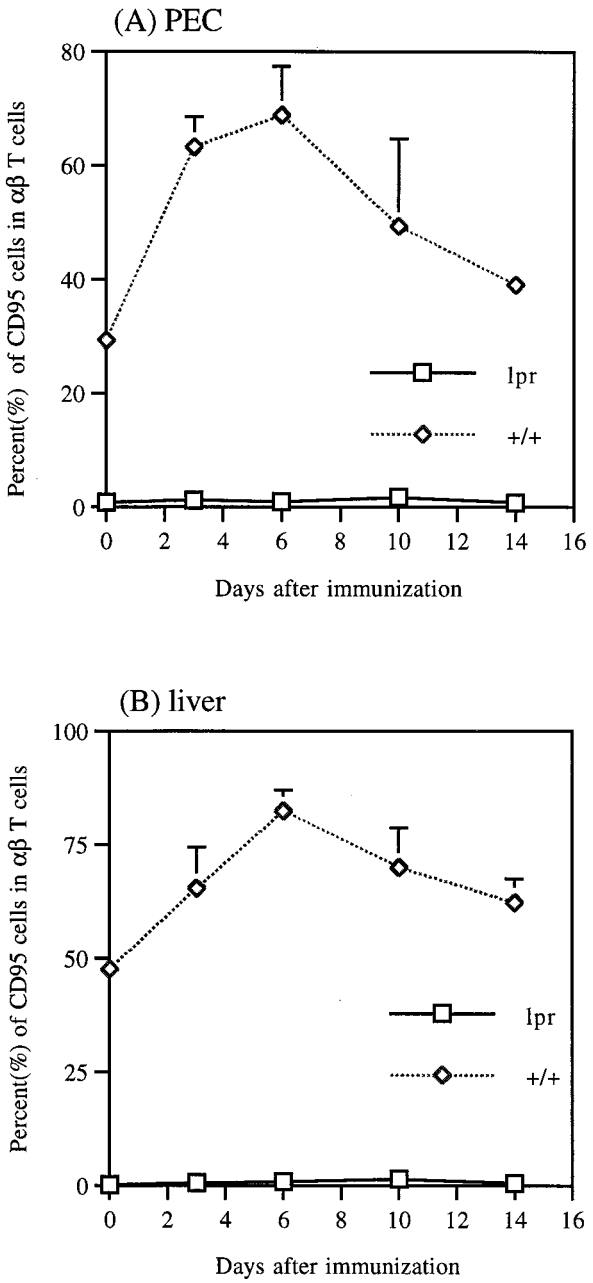

FIG. 3. Kinetics of CD95$^+$ cells in αβ$^+$ T cells from the peritoneal cavities (A) or livers (B) of MRL/*lpr* or MRL/+/+ mice inoculated i.p. with 7×10^3 *L. monocytogenes* cells on day 0. Cells were stained with FITC–anti-αβ MAb and PE-anti-CD95 MAb. Each point and vertical bar are the mean ± standard deviation for five mice.

On the other hand, we found far more bacteria in spleens of 16-week-old *lpr* mice on day 6 after listerial infection than in those of age-matched MRL/+/+ mice (data not shown). However, the bacteria were eliminated by day 10 after listerial infection in *lpr* mice. These results suggest that CD95 may not be involved in protection against listerial infection as assessed by bacterial growth, especially in young *lpr* mice. Considering that an autoimmune disease initially shows up in 8- to 10-week-old MRL/*lpr* mice (29, 37, 46), we used 5-week-old mice throughout the experiments to avoid the influence of the autoimmune disease on the course of listerial infection.

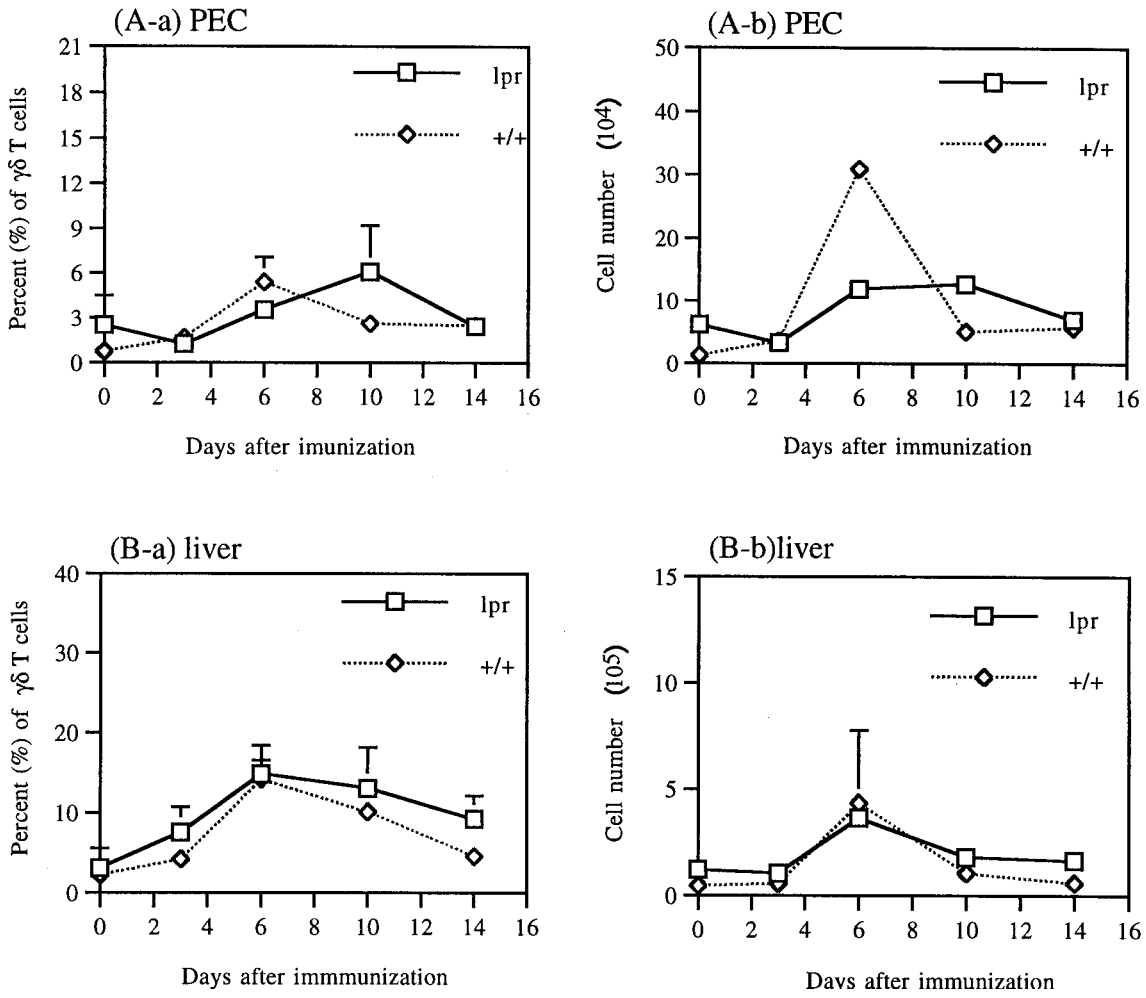

FIG. 4. Kinetics of the percentage (a) and the absolute number (b) of $CD3^+$ $\gamma\delta^+$ T cells from PEC (A) or livers (B) of MRL/*lpr* or MRL/+/+ mice after *Listeria* infection. Each mouse was inoculated i.p. with 7×10^3 *L. monocytogenes* cells on day 0. Cells were stained with FITC–anti-CD3 MAb and PE–anti-γδ MAb. Each point and vertical bar are the mean ± standard deviation for four mice.

Kinetics of αβ T cells and their CD95 expression in peritoneal cavities and livers in MRL/*lpr* or MRL/+/+ mice after i.p. injection with *L. monocytogenes*. The kinetics of T cells in PEC and livers of MRL/*lpr* and MRL/+/+ mice were examined after i.p. injection of 7×10^3 CFU of *L. monocytogenes* per mouse. The percentage of αβ T cells was analyzed by two-color FCM with anti-CD3 and anti-TCRαβ MAbs, and the absolute number was calculated by multiplying the percentage by the number of the lymphocytes. As shown in Fig. 2A-a and A-b, the percentage and absolute number of αβ T cells in PEC or MRL/+/+ mice increased with number of days after infection, reached a peak on day 6, and then decreased. On the other hand, αβ T cells in PEC of MRL/*lpr* mice continued to increase from day 3 to day 14 after infection with *L. monocytogenes*. These tendencies were also obvious in the number of αβ T cells in livers of MRL/*lpr* mice, although these numbers were not significant compared with those seen in PEC (Fig. 2B-a and B-b). We next used two-color FCM analysis with anti-CD95 and TCRαβ MAbs to examine the CD95 expression of αβ T cells accumulating in peritoneal cavities and livers after i.p. injection with *L. monocytogenes*. In MRL/+/+ mice, the proportion of CD95-positive αβ T cells in PEC and livers increased to a peak (69 and 82%, respectively) on day 6 after infection and then decreased in accordance with the decrease in the absolute number of αβ T cells. On the other hand, CD95 expression on αβ T cells in PEC and livers could hardly be found in MRL/*lpr* mice throughout the observation period after listerial infection (Fig. 3).

MRL/*lpr* mice develop massive lymphoadenopathy characterized by the accumulation of an unusual population of T cells, which are $TCR\alpha\beta^+$ $CD4^-$ $CD8^-$ $B220^+$ (4, 37). Therefore, we examined the expression of B220, CD4, or CD8 on αβ T cells accumulating in PEC and liver after listerial infection. However, no appreciable levels of $CD4^-$ $CD8^-$ αβ T cells and $B220^+$ αβ T cells were found throughout the observation period (data not shown).

Kinetics of γδ T cells and their CD95 expression in peritoneal cavities and livers of MRL/*lpr* or MRL/+/+ mice after i.p. injection with *L. monocytogenes*. Kinetics of γδ T cells and their CD95 expression in PEC and livers of MRL/*lpr* and MRL/+/+ mice were examined on days 3, 6, 10, and 14 after i.p. inoculation with 7×10^3 CFU of *L. monocytogenes* per mouse by FCM analysis with anti-CD3 MAb and anti-TCRγδ MAb. As shown in Fig. 4, the percentage and absolute number of γδ T cells in PEC and livers of MRL/+/+ mice peaked on day 6 after listerial infection and then decreased. On the other hand,

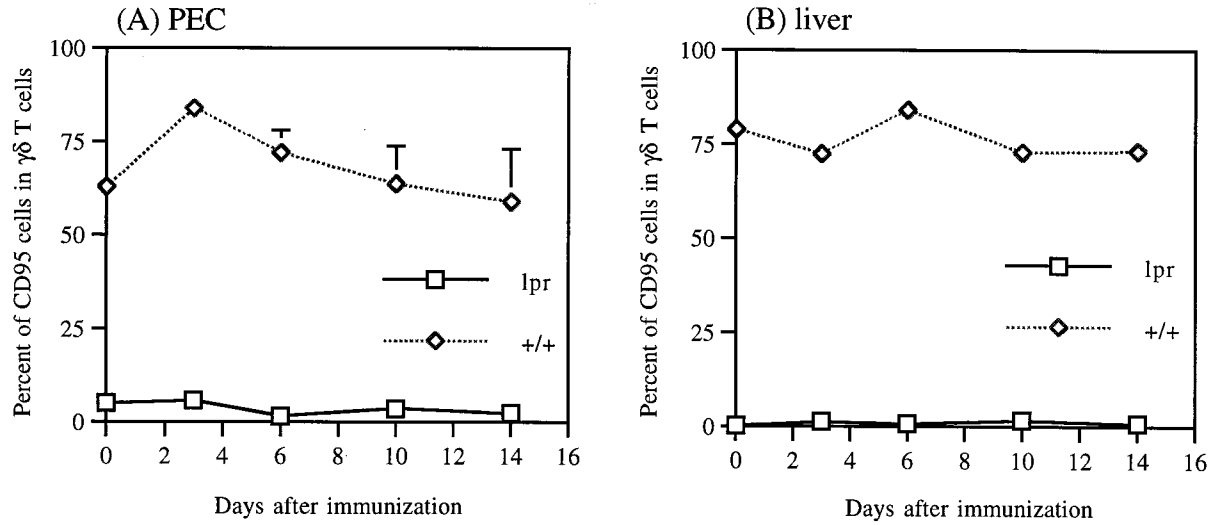

FIG. 5. CD95$^+$ cells in γδ$^+$ T cells from PEC (A) or livers (B) of MRL/*lpr* or MRL/+/+ mice after *Listeria* infection. Each mouse was inoculated i.p. with 7×10^3 *L. monocytogenes* cells on day 0. Cells were stained with FITC–anti-γδ MAb and PE–anti-CD95 MAb. Each point and vertical bar are the mean ± standard deviation for four mice.

numbers of γδ T cells increased slowly in PEC of MRL/*lpr* mice and reached a peak on day 10 after listerial infection. In contrast with αβ T cells, the number of γδ T cells decreased by day 14 after infection in PEC and livers of both MRL/+/+ and MRL/*lpr* mice.

We next examined CD95 expression on γδ T cells by FCM analysis with anti-TCRγδ MAb and anti-CD95 MAb. Numerous γδ T cells in the inflamed sites expressed CD95 in MRL/+/+ mice, whereas CD95 expression was virtually undetected on γδ T cells in MRL/*lpr* mice (Fig. 5). Thus, the decrease in γδ T cells occurred after their peaks, irrespective of their CD95 expression. These results suggest that activated γδ T cells may be deleted independently of the CD95 system.

DNA fragmentation with apoptosis in livers of MRL/*lpr* or MRL/+/+ mice after listerial infection. To determine whether apoptosis is responsible for the decrease in the number of T cells in peritoneal cavity and liver after listerial infection, we examined DNA fragmentation in the freshly isolated T cells from PEC and livers of MRL/*lpr* or MRL/+/+ mice infected with *L. monocytogenes* 10 days previously. As shown in Fig. 6, freshly isolated T cells from PEC or livers of normal MRL/+/+ or MRL/*lpr* mice on day 0 showed no signs of DNA fragmentation, whereas T cells from MRL/+/+ on day 10 after listerial infection showed visible bands of nucleosomal laddering. Such DNA fragmentation was hardly detected in T cells of livers from *Listeria*-infected MRL/*lpr* mice. These results suggest that apoptosis of T cells mediated by CD95 may occur in the inflamed sites during listerial infection.

Accumulation of activated T cells specific for *Listeria* in PEC and livers of MRL/*lpr* mice after listerial infection. We previously reported that T cells expressing CD44, a marker for activation, increase in the infected site during listerial infection in close correlation with an increase in *Listeria*-specific T-cell proliferation (16, 26). Therefore, we used three-color FCM analysis for expression of TCRαβ and CD44 to examine the kinetics of T cells expressing L-selectin and/or CD44 in PEC and livers of MRL/*lpr* mice after infection with *L. monocytogenes*. As shown in Fig. 7, the percentage and absolute number of activated T cells expressing CD44 rapidly increased from day 0 to day 3 and remained at an elevated level until day 14 after listerial infection in both MRL/+/+ and MRL/*lpr* mice.

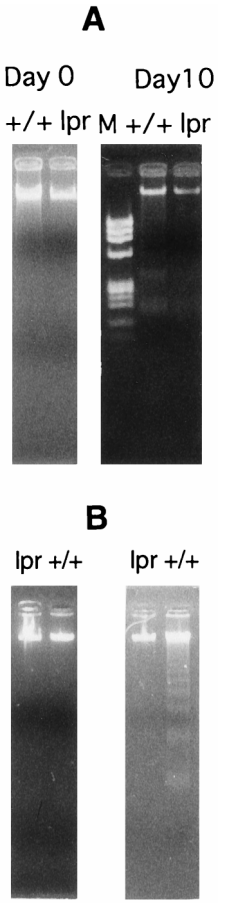

FIG. 6. DNA fragmentation assay. Cells were freshly isolated from peritoneal cavities (A) or livers (B) of naive *lpr* and +/+ mice on day 0 or of *lpr* and +/+ mice on day 10 after *Listeria* immunization and passed through a nylon wool column. Next, nonadherent cells were harvested, and DNA was analyzed for nucleosomal ladders by gel electrophoresis as described in Materials and Methods. Lane M, marker.

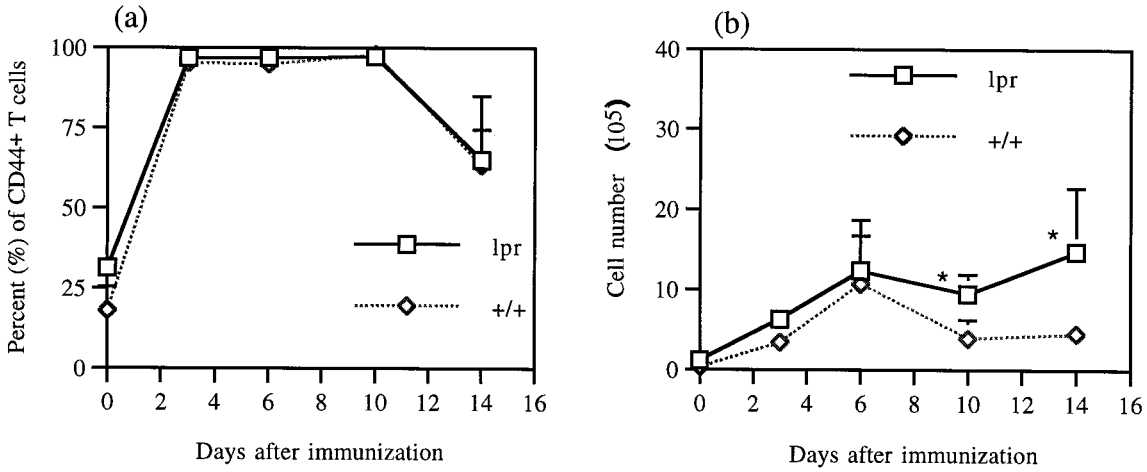

FIG. 7. Kinetics of percentage (a) and absolute number (b) of CD44-expressing cells in $\alpha\beta^+$ T cells from the peritoneal cavities of MRL/*lpr* or MRL/+/+ mice. Each mouse was injected i.p. with 7×10^3 *L. monocytogenes* cells on day 0. Cells were stained with FITC–anti-CD44 MAb and biotin–anti-TCR$\alpha\beta$ MAb. Each point and vertical bar are the mean ± standard deviation for four mice. Significant difference from the values for MRL/+/+ mice: *, $P < 0.05$.

The absolute number of CD44 TCR$\alpha\beta$ T cells was significantly larger in the PEC of MRL/*lpr* mice than in those of MRL/+/+ mice on day 10 after listerial infection.

We next compared the *Listeria*-specific proliferative responses of T cells from PEC of MRL/*lpr* mice and MRL/+/+ mice on day 10 after listerial infection. As shown in Fig. 8, the degree of proliferative response of T cells from PEC of infected *lpr* mice was significantly higher than that of MRL/+/+ mice ($P < 0.05$). These results suggest that MRL/*lpr* mice have a larger number of *Listeria*-specific activated T cells accumulating in the peritoneal cavity at the late stage of infection.

To further confirm that MRL/*lpr* mice showed acquired cellular resistance to listerial infection, we injected a lethal dose of *L. monocytogenes* (10^6 cells) into MRL/*lpr* mice infected with *L. monocytogenes* 14 days previously. Three days later, bacterial counts in spleens were determined. *Listeria*-immune MRL/*lpr* mice showed a strong resistance to secondary listerial infection, as did MRL/+/+ mice (data not shown).

Histological changes in liver lesions in MRL/*lpr* mice after infection with *L. monocytogenes*. To determine the effects of excessive accumulation of activated T cells on inflammation after listerial infection, we compared histological changes in the livers of MRL/*lpr* and MRL/+/+ mice on day 10 after listerial infection. As shown in Fig. 9, granulomatous lesions containing mononuclear cells were scattered in the liver parenchyma of both MRL/+/+ and MRL/*lpr* mice on day 10 after listerial infection, and there was no difference in the numbers of granulomas and kinds of infiltrating cells. These results suggest that granulomatous responses may not be affected by a defect in CD95 in MRL/*lpr* mice after listerial infection.

DISCUSSION

Antigen-primed T cells are known to express CD44 and to accumulate preferentially in the inflamed sites where antigen first invaded (5). PEC have often been used as an enriched source of *Listeria*-primed immune T cells from mice infected i.p. with *L. monocytogenes* (9). We recently showed that *Listeria*-specific T cells expressing CD44 appear preferentially in peritoneal cavity and liver after an i.p. injection of *L. monocytogenes* (16, 26). Consistent with this, our present study revealed that most of the $\alpha\beta$ T cells in the peritoneal cavities and livers of both MRL/+/+ and MRL/*lpr* mice expressed CD44 after listerial infection. We have therefore been able to monitor the fate of activated T cells in vivo after bacterial infection by examining the kinetics of CD44 T cells in inflamed sites such as the peritoneal cavity and liver after listerial infection. The number of CD44 $\alpha\beta$ T cells in the peritoneal cavity and liver increased and then decreased after primary listerial infection in normal mice. Notably, in CD95-defective *lpr* mice, the number of $\alpha\beta$ T cells continued to increase in the infected sites during the course of primary infection with *L. monocytogenes*, and DNA gel analysis revealed that the freshly isolated T cells

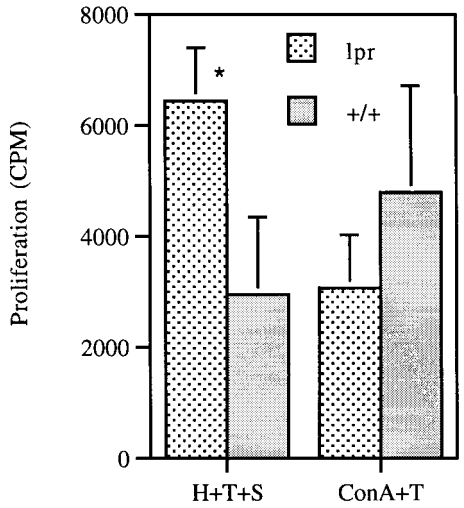

FIG. 8. Proliferative response of nylon wool-passed PEC on day 10 after *Listeria* immunization. T cells were passed through a nylon wool column with PEC of MRL/*lpr* or MRL/+/+ mice on day 10 after i.p. injection with 7×10^3 *L. monocytogenes* cells. T cells (1.5×10^4/well) were incubated with mitomycin-treated syngeneic splenocytes (1.5×10^4/well) and HKL (10^6/well). After 18 h of culture, cells were pulsed with [^{3}H]thymidine for 12 h before harvest, and then thymidine incorporation was measured in triplicate cultures. The data are a representative of three independent experiments, and the results are means ± standard deviations of triplicate cultures. H, heat-killed *L. monocytogenes*; T, nylon-wool-passed PEC; S, syngeneic spleen cells treated with mitomycin; ConA, concanavalin A. Significant difference from the values of MRL/+/+ mice: *, $P < 0.05$.

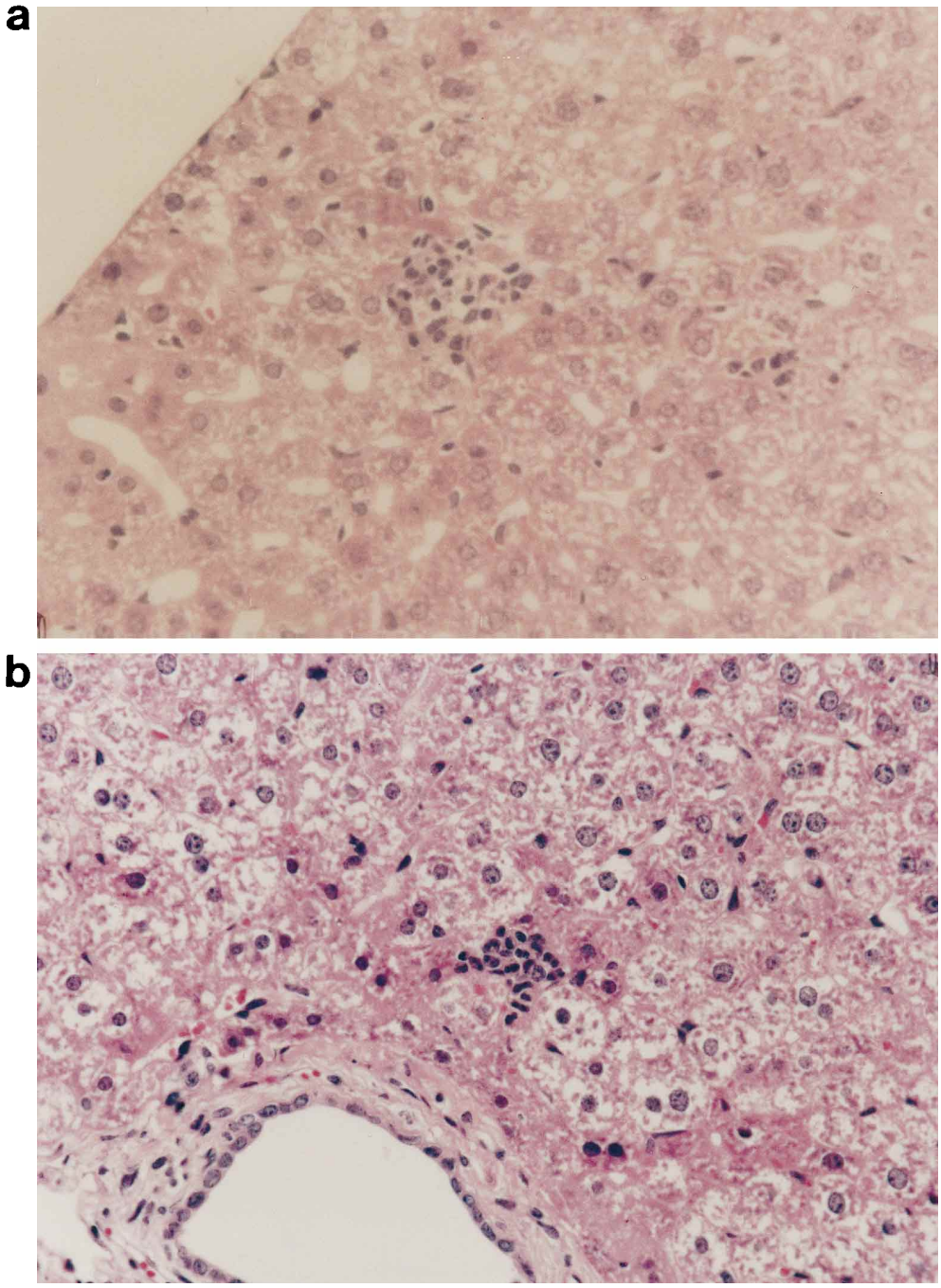

FIG. 9. Liver sections of MRL/*lpr* (a) and MRL/+/+ (b) mice on day 10 after i.p. injection with 7×10^3 *L. monocytogenes* cells. Sections were stained with hematoxylin and eosin. Magnification, ×400.

from infected *lpr* mice showed no DNA fragmentation. A *Listeria*-specific T-cell proliferation assay suggested that *Listeria*-specific T cells are more abundant in the peritoneal cavities of MRL/*lpr* mice than in MRL/+/+ mice on day 10 after listerial infection. These results imply that the decrease in the number of T cells in the inflamed sites after listerial infection may be at least partly the result of CD95-mediated death of activated T cells specific for *L. monocytogenes*. To our knowledge, this is the first evidence suggesting that CD95 is involved in activation-induced cell death by apoptosis of T cells during infection with *L. monocytogenes*.

T lymphocytes undergo cell death by apoptosis either because of a growth factor deficiency (20, 25, 38) or because of activation-induced cell death as a result of repeated antigen stimulation (18, 23, 29, 55). The involvement of CD95 antigen in activation-induced death of T cells is most clearly documented by analysis with *lpr* mice with mutations in CD95. When superantigen SEB is injected into wild-type mice, ma-

ture T cells expressing the SEB-reactive Vβ8 TCR chain initially expand and then delete by apoptosis, whereas this deletion cannot be seen or is severely retarded in *lpr* mice (29, 46). On the other hand, in vivo administration of such superantigen causes deletion of thymic T cells in both MRL/*lpr* and MRL/+/+ mice (14, 58). Thus, Ag-induced deletion of peripheral T cells occurs in MRL/+/+ but not MRL/*lpr* mice. Recently, van Parijs et al. (41) reported that in TCR transgenic mice with an *lpr* gene background, the activation-induced cell death mediated by CD95 is not prevented by a CD28-mediated signal, interleukin-2, or bcl-x_L. Therefore, it is most likely that the increase in CD44 αβ T cells in the peritoneal cavities of MRL/*lpr* mice at the late stage of listerial infection is due to a defect in activation-induced cell death of *Listeria*-primed T cells in the inflamed sites. MRL/*lpr* increased the numbers of an unusual population expressing $B220^+$ $CD4^-$ $CD8^-$ with advancing age. This raises the alternative possibility that the accumulation of this unusual population is responsible for the increase in CD44 T cells after listerial infection in MRL/*lpr* mice. However, these unusual cells were almost never detected throughout the experiments when we used 5-week-old MRL/*lpr* mice. In contrast to the situation with αβ T cells, the deletion of γδ T cells followed by a transient increase is detected in MRL/*lpr* mice after listerial infection. At present, the deletion mechanism of γδ T cells after listerial infection remains unknown. Zheng et al. (57) reported that tumor necrosis factor mediates mature T-cell apoptosis independently of CD95. Therefore, it may be responsible for the deletion of γδ T cells.

It was recently shown that Fas/Fas-L-mediated apoptosis is involved in the cytotoxicity mediated by NK cells, CD4 Th1 cells, CD4 NK cells, and some CD8 cytotoxic T cells (2, 19, 27). Besides CD4 Th1 cells capable of producing IFN-γ, cytotoxic T-lymphocytes (CTL) are known to contribute to protection against listerial infection (13). Induction of apoptosis in infected cells is thought to be an important step in protecting against infection by intracellular parasites. Therefore, it is conceivable that CD95 is involved in protection against listerial infection. The present study revealed, however, that resistance to listerial infection in young MRL/*lpr* mice did not differ from that in MRL/+/+ mice as assessed by bacterial growth. Perforin and grandzyme, a family of serine proteases, are known to be other effector molecules in CTL (42). In MRL/*lpr* mice, these molecules may be responsible for the CTL-mediated host defense against *Listeria* infection.

Activation-induced cell death plays an important role in homeostasis in the immune system through termination of immune response. Hepatocytes express abundant levels of CD95 (54), and injection of cytotoxic antibody against CD95 has been shown to induce a phenotype similar to that of fulminant hepatitis (35). Fetal liver injury has been shown to be induced by CTL after infection with a lethal dose of *L. monocytogenes* (45). Taken together, it can be speculated that prolonged Ag-specific T cells expressing Fas-L may induce excessive inflammation and tissue damage and that autonomous Fas/Fas-L-mediated apoptosis in the activated T cells prevents *Listeria*-infected mice from excessive tissue damage by themselves. Our present study showed, however, that there is no difference in the histological changes in MRL/*lpr* and MRL/+/+ mice as assessed by light histological examination. We do not at present find any pathological effects of prolonged Ag-specific αβ T cells on liver damage after listerial infection. γδ T cells are reported to play regulatory roles in inflammatory reactivity during listerial infection (11, 30). γδ T cells may normally control excessive tissue damage in MRL/*lpr* mice after listerial infection. Further studies are required to elucidate the roles of CD95 in pathophysiology during listerial infection.

In conclusion, CD95-mediated cell death with apoptosis may be involved in termination of the αβ T-cell-mediated immune response after the battle against *L. monocytogenes* has been won, whereas γδ T cells may undergo apoptosis independently of CD95 during the course of listerial infection.

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