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A protective role of γδ T cells in primary infection with *Listeria monocytogenes* in autoimmune non-obese diabetic mice

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SUMMARY

We investigated the host defense mechanism in primary infection with *Listeria monocytogenes* in non-obese diabetic (NOD) mice at pre-diabetic stage showing an impaired responsiveness of the αβ T cells to T-cell receptor (TCR) triggering. The NOD mice showed a deteriorated resistance at the late stage after an intraperitoneal infection with *L. monocytogenes* compared with BALB/c and C57BL/6 mice as assessed by bacterial growth in organs. Consistent with our previous findings, a prominent increase in number of γδ T cells was evident at the early stage after infection, while generation of *Listeria*-specific αβ T cells was impaired in these mice. *In vivo* administration of anti-TCR γδ monoclonal antibody (mAb) allowed *L. monocytogenes* to grow exaggeratedly in the NOD mice. These results imply that γδ T cells may be mainly involved in protection against primary infection with *L. monocytogenes* in NOD mice.

INTRODUCTION

There have been several lines of evidence for dominant γδ T-cell response to infections with various microbial pathogens including *Mycobacteria*, *Staphylococcus*, influenza virus, herpes simplex virus, measles virus, Epstein–Barr virus, *Trypanosoma cruzi* and *Plasmodium falciparum*.[1] We have also reported that the γδ T cells increase significantly in the inflamed sites after primary infections with *Listeria monocytogenes*,[2,3] *Mycobacterium bovis*,[4] *Escherichia coli*,[5] *Salmonella choleraesuis*[6] and Sendai virus.[7] An accelerated bacterial multiplication was evident at the early stage of listerial infection in mice treated with anti-T-cell receptor (anti-TCR) γδ monoclonal antibody (mAb), indicating that γδ T cells play important roles in protection at the early stage after listerial infection.[3] This view was strengthened by the findings of a recent report using transgenic mice carrying a mutant TCR δ gene.[8]

The non-obese diabetic (NOD) mouse.[9] an inbred strain that spontaneously develops diabetes, has been studied as a model for human insulin-dependent diabetes mellitus (IDDM). The pancreatic islet β-cell autoantigens, which are major targets of T cells and antibodies associated with the development of IDDM, have been identified as glutamic acid decarboxylase (GAD),[10,11] 65 000 MW heat-shock protein (HSP),[12,13] peripherin,[14] and carboxypeptidase H.[15] Recent observations have revealed that the environmental influence of microbial agents on IDDM can be associated with disease development.[16] Prevention of diabetes in NOD mice has been reported following immunization with complete Freund's adjuvant (CFA),[17,18] the bacillus Calmette–Guérin vaccine (BCG) of live *Mycobacterium bovis*[19,20] and 65 000 MW HSP.[12,13] Thus, T cells stimulated with the bacterial products may play important roles in immunoregulation of development of diabetes. However, the primary roles of these T cells in host defense against bacterial infection in NOD mice remain unknown.

In this study, we examined the host-defense mechanism against primary infection with *L. monocytogenes* in pre-diabetic NOD mice. We found that αβ T cells showed an impaired responsiveness to listerial infection but γδ T cells normally responded to the infection and played a protective role in primary infection with *L. monocytogenes* in the NOD mice.

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Abbreviations: FCS, fetal calf serum; FITC, fluorescein isothiacyanate; HBSS, Hanks' balanced salt solution; HKL, heat-killed *Listeria*; HSP, heat-shock protein; i.p., intraperitoneally; mAb, monoclonal antibody; NOD, non-obese diabetic; PE, phycoerythrin; TCR, T-cell receptor.

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MATERIALS AND METHODS

Mice

NOD Shi/Jic mice obtained from CLEA (Osaka, Japan) were maintained under specific pathogen-free conditions in the facilities of Nagoya University. Pre-diabetic female mice between 7 and 10 weeks of age were used for this study.

Age- and sex-matched BALB/c or C57BL/6 mice obtained from Japan SLC (Shizuoka, Japan) were used as controls.

Microorganisms

L. monocytogenes strain EGD was used in these experiments. Bacterial virulence was maintained by serial passage in BALB/c mice.[21] Fresh isolates were obtained from infected spleens, grown in tryptic soy broth (Difco Laboratories, Detroit, MI), washed repeatedly, resuspended in phosphate-buffered saline (PBS), and stored at −70° in small aliquots. Heat-killed *Listeria* (HKL) were prepared by incubating viable *L. monocytogenes* at 74° for 120 min.

Cell preparation

Hepatic lymphocytes were prepared as described previously[22] with a slight modification. Briefly, liver was pressed through a 100-gauge stainless steel mesh after perfusion with 20 ml of sterile Hanks' balanced salt solution (HBSS) to eliminate blood. The cell suspension was centrifuged through a 44–67·5% Percoll (Sigma Chemical Co., St Louis, MO) gradient. Cells at the interface were washed twice and used. Spleen cells were obtained from mice by the conventional method.

Proliferation assay

Hamster anti-TCR-$\alpha\beta$ mAb (IgG, H57-597; a gift from Dr R. Kubo, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO) and hamster anti-TCR-$\gamma\delta$ mAb (IgG, UC7-13D5; a gift from Dr J. A. Bluestone, University of Chicago, Chicago, IL) were obtained by growing hybridoma cells in serum-free medium (101 :Nissui Pharmaceutical Co. Ltd, Tokyo, Japan) and collecting the supernatant. Antibodies were then concentrated and purifying by 50% ammonium sulfate precipitation. The purity of the preparations was confirmed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and concentrations of antibodies were determined by the Lowry method. The purified mAb was diluted to the optimal concentrations in PBS and 30 μl/well was added to 96-well flat-bottomed microtitre plates (Becton Dickinson Co., NJ). Plates were incubated for 2 hr at 37° and then washed three times with PBS before use. Freshly isolated hepatic lymphocytes (3×10^5/well) were added into the mAb-coated plates. For antigen-specific stimulation, hepatic lymphocytes were obtained from mice inoculated with 3×10^3 *L. monocytogenes* 10 days previously. The hepatic lymphocytes (3×10^5/well) and syngeneic splenocytes (3×10^5/well) treated with mitomycin C (MMC) (Sigma Chemical Co., St Louis, MO) were added to each well in 96-well flat-bottomed microtitre plates. Heat-killed *Listeria* (corresponding to 1×10^8/ml viable *L. monocytogenes*) were added as antigens. After incubation for 40 hr at 37° wth RPMI containing 10% fetal calf serum (FCS), the cultures were pulsed with 1 μCi of [^{3}H]thymidine ([^{3}H]TdR) per well and harvested 8 hr later. [^{3}H]TdR incorporated in the cells was measured with a liquid scintillation counter.

Bacterial growth

Primary infection with *L. monocytogenes* was performed by intraperitoneal inoculation with a sublethal dose of 8×10^2 or 3×10^3 viable bacteria in a volume of 0·2 ml of PBS on day 0. Mice were anaesthetised with ether and killed by cutting the cervical artery at intervals after intraperitoneal infection. Bacterial growth in the liver or spleen was determined by plating 10-fold serial dilutions of organ homogenates on tryptic soy agar (Nissui Laboratories). The detection limit of this procedure was 10^2 *L. monocytogenes* per organ. The number of colonies were counted after 24 hr of incubation at 37°.

Antibodies and flow cytometric analysis

The following mAb were all purchased. Phycoerythrin (PE)-conjugated anti-TCR-$\alpha\beta$ mAb, PE-conjugated anti-TCR-$\gamma\delta$ mAbs, fluorescein isothiacyanate (FITC)-conjugated anti-TCR-$\gamma\delta$ mAb, FITC-conjugated anti-CD44 mAb, FITC-conjugated anti-CD25 mAb and FITC-conjugated LECAM-1 mAb were from PharMingen (San Diego, CA). Biotin-conjugated anti-CD8 mAb (anti-Lyz), PE-conjugated anti-CD4 mAb (anti-L3T4), and FITC-conjugated anti-Thy-1.2 mAb were from Becton Dickinson & Co. (Oxnard, CA). RED613-conjugated streptavidin was from Gibco BRL (Gaithersburg, MD). The surface antigen of cells were identified by using various mAb in conjunction with the two- to three-colour staining. The stained cells were analysed by FACScan (Becton Dickinson) using the Lysis II program.

In vivo *pretreatment with anti-TCR-$\gamma\delta$ mAb (UC7-13D5)*

Mice were treated with intraperitoneal injection of 200 μg of anti-TCR-$\gamma\delta$ mAb diluted to a final volume of 0·2 ml in PBS, and control mice were injected with 0·2 ml of isotype-control mAb 3 days before primary infection (on day −3). Control mAb was hamster anti-2,4,6-trinitrophenyl (TNP) mAb [IgG, American Type Culture Collection (Rockville, MD) CRL-1968],[23] which was obtained by growing hybridoma cells in serum-free medium and prepared as described previously. Mice were killed 6 days (on day 6) after intraperitoneal inoculation of 8×10^2 viable bacteria in a volume of 0·2 ml of PBS on day 0 and bacterial growth in the liver was determined.

Statistical analysis

The statistical significance of the data was determined by Student's *t*-test, Cochran–Cox test, or χ^2 test. A P value of less than 0·05 was taken as significant.

RESULTS

Susceptibility of pre-diabetic NOD mice against infection with *L. monocytogenes*

We first examined the susceptibility of 7-week-old NOD mice

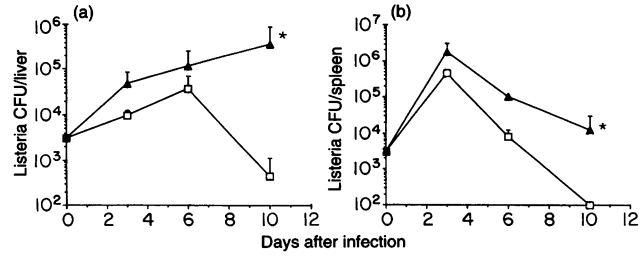

Figure 1. Growth of *L. monocytogenes* in the liver (a) and spleen (b) of mice after an intraperitoneal inoculation with 3×10^3 viable cells. Results were obtained from three different experiments and are presented as the means and SDs for nine mice at each time point. *$P < 0·05$, significantly different from the value for BALB/c control mice by the Cochran–Cox test. (□) BALB/c mice; (▲) NOD mice.

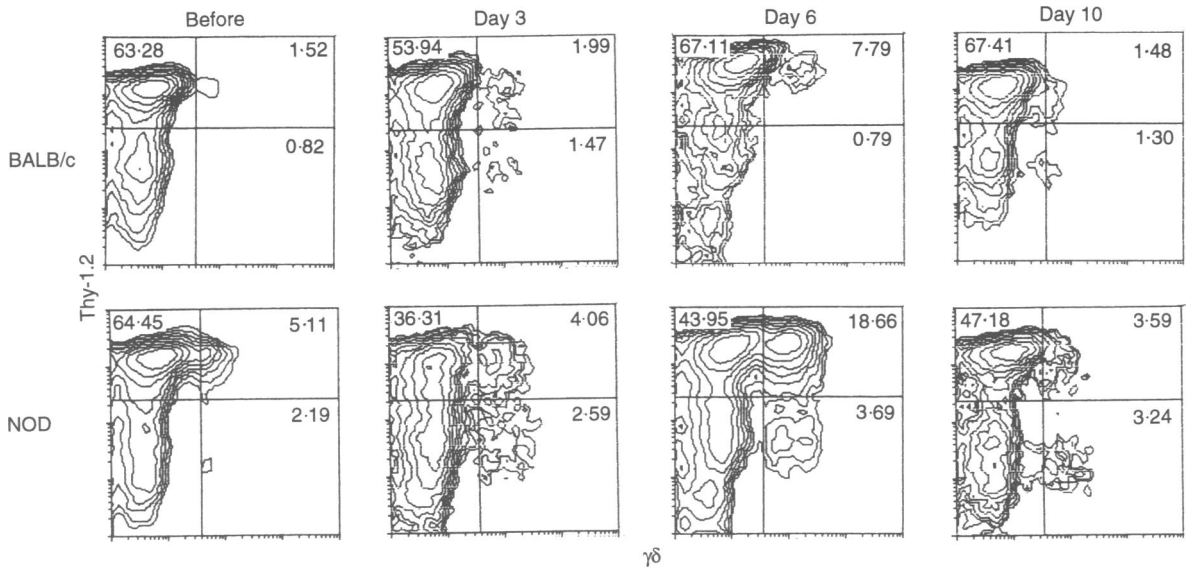

Figure 2. Kinetics of γδ T cells in the liver after an intraperitoneal challenge with *L. monocytogenes*. Mice were inoculated i.p. with 3×10^3 *L. monocytogenes*. Hepatic lymphocytes were stained with fluorescein isothiocyanate (FITC)-conjugated anti-Thy-1.2 mAb and phycoerythrin (PE)-conjugated anti-TCR-γδ mAb. The percentage of each subpopulation was calculated after integration of selected area in contour profile. Representative profiles from eight mice at each time point are shown.

against primary infection with *L. monocytogenes*. Survival rate of NOD mice was examined after an intraperitoneal inoculation with 1×10^3 or 3×10^3 *L. monocytogenes* corresponding to approximately 1/30 or $1/10LD_{50}$ to BALB/c mice inoculated intraperitoneally (i.p.). All of 20 NOD mice survived after infection with 1×10^3 of *L. monocytogenes*, while three of 24 NOD mice died within 8 days after infection with 3×10^3 *L. monocytogenes*. On the other hand, all age- and sex-matched 24 BALB/c mice and 26 C57BL/6 mice survived after infection with 3×10^3 *L. monocytogenes*. Although we could not detect statistically significant difference in survival rate among three strains of mice (χ^2 test), the pre-diabetic NOD mice appear to be somewhat susceptible to listerial infection.

We next examined the kinetics of the bacterial growth in the spleen and liver of NOD mice on days 3, 6, and 10 after an intraperitoneal inoculation with 3×10^3 *L. monocytogenes*. As shown in Fig. 1, the number of bacteria increased to reach a maximal level on day 3 or day 6 in spleen or liver respectively, and thereafter decreased to an undetectable level in both organs by day 10 after infection in BALB/c mice. In C57BL/6 mice, the bacteria were completely eliminated by day 6 after infection with the same dose of *L. monocytogenes* (data not shown). On the other hand, the numbers of bacteria were much the same on day 3 and on day 6 in the NOD mice as those in BALB/c mice at these stages but the number on day 10 in the NOD mice was significantly larger than that in BALB/c mice ($P < 0{\cdot}05$, as

Table 1. Kinetics of γδ T cells and αβ T cells in the liver after intraperitoneal challenge with *L. monocytogenes*

Strain	Before	Day 3	Day 6	Day 10
	Absolute number of γδ T cells in total hepatic lymphocytes $\times 10^5$ (%)			
BALB/c	1·0 ± 0·4 (2·6)	2·2 ± 0·7 (3·5)	6·6 ± 1·3 (6·2)	3·2 ± 1·0 (3·7)
NOD	2·2 ± 1·6 (6·2)	3·9 ± 1·4 (6·9)	**11·5 ± 3·2 (13·2)	*7·3 ± 3·4 (7·7)
	Absolute number of αβ T cells in total hepatic lymphocytes $\times 10^5$ (%)			
BALB/c	21·5 ± 7·9 (56·6)	25·9 ± 8·9 (40·4)	52·0 ± 10·3 (49·1)	49·1 ± 14·5 (55·8)
NOD	22·6 ± 15·7 (62·7)	19·1 ± 6·7 (33·5)	**30·0 ± 8·3 (34·5)	30·8 ± 14·4 (32·8)

Mice were infected intraperitoneally with 3×10^3 *L. monocytogenes*. FACS analysis for expression of TCR γδ and TCR αβ was carried out on hepatic lymphocytes on days 3, 6, and 10 after listerial infection.

Results were obtained from two different experiments and are presented as the means and SDs of eight mice.

*$P < 0{\cdot}05$, **$P < 0{\cdot}01$, significantly different from values for BALB/c on the corresponding stage after infection by the Cochran–Cox test.

assessed by Cochran–Cox test). Thus, NOD mice showed an impaired resistance at late stage during the course of the disease as compared with BALB/c and C57BL/6 mice.

Kinetics of $\alpha\beta$ T cells and $\gamma\delta$ T cells in NOD mice in the course of listerial infection

We have previously showed that $\gamma\delta$ T cells play a protective role at the early stage (from day 3 to day 6) after listerial infection, while $\alpha\beta$ T cells specific for listerial antigen are essential for complete elimination of bacteria around by day 10 after infection.[3] To seek which T cells contribute to the protection against listerial infection in NOD mice, the kinetics of T cells in the liver of NOD mice was examined on days 3, 6 and 10 after an intraperitoneal infection with 3×10^3 *L. monocytogenes*. A representative data of flow cytometric analysis was shown in Fig. 2 and the results of eight mice of each group were summarized in Table 1. There were no significant differences in absolute number of liver lymphocytes from day 3 to day 10 after infection between NOD and BALB/c control mice. Consistent with our previous report,[3] the number of $\gamma\delta$ T cells significantly increased in the liver on day 6 and then decreased gradually by day 10 after infection in both mice (Fig. 2 and Table 1). The increase of the number of $\gamma\delta$ T cells was more prominent in NOD mice on day 6 after infection than that seen in BALB/c mice ($P < 0{\cdot}01$, as assessed by Cochran–Cox test). The cell-surface characteristics of the $\gamma\delta$ T cells by two- or three-colour flow cytometric analysis revealed that approximately half of the $\gamma\delta$ T cells expressed CD8 molecules and the rest were $CD4^-$ $CD8^-$, and most $\gamma\delta$ T cells in the liver of the NOD mice were of $CD44^+$, IL-$2R\alpha^-\beta^+$ L-selectin$^-$ phenotype (data not shown). On the other hand, the number of $\alpha\beta$ T cells in NOD mice was smaller than that in BALB/c mice after infection (on day 6 and day 10, Table 1). Thus, $\gamma\delta$ T cells increased remarkably during listeriosis but $\alpha\beta$ T cells may not notably respond to listerial infection in the NOD mice.

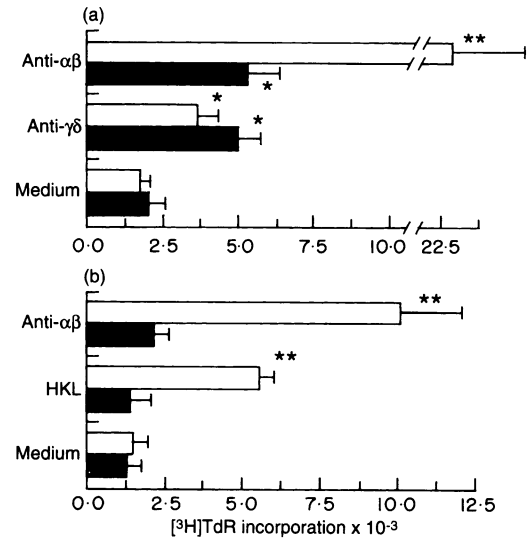

Figure 3. *In vitro* proliferation of T cells in the liver of BALB/c or NOD mice. (a) Freshly isolated hepatic lymphocytes were obtained from naive 7-week-old BALB/c and NOD mice. Hepatic lymphocytes (3×10^5/well) were cultured in anti-TCR-$\alpha\beta$ (20 μg/ml)- or anti-TCR-$\gamma\delta$ (50 μg/ml)-coated plates for 48 hr. The c.p.m. for [^{3}H]thymidine incorporation for 8 hr were measured in triplicated cultures. Results are presented as the means and SDs. $*P < 0{\cdot}01$, $**P < 0{\cdot}001$, significantly different from values for medium only control by Student's *t*-test. (b) Hepatic lymphocytes were obtained from BALB/c or NOD mice inoculated with 3×10^3 *L. monocytogenes* 10 days previously. *Listeria*-specific proliferation was assayed by 8-hr [^{3}H]thymidine incorporation in cells (3×10^5/well) cultured with mitomycin C (MMC)-treated syngeneic spleen cells (3×10^5/well) and HKL (1×10^8/ml) for 48 hr. The c.p.m. for [^{3}H]thymidine incorporation was measured in triplicate cultures. Results are presented as the means and SDs. $**P < 0{\cdot}001$, significantly different from values for medium-only control by Student's *t*-test. These experiments were repeated twice with comparable results.

Impaired *Listeria*-specific cell-mediated immunity in NOD mice

T cells in the thymus, spleen and LN of NOD mice have been reported to be unresponsive after TCR cross-linking even in pre-diabetic stage.[24,25] Therefore, we first examined the *in vitro* proliferative responses of $\gamma\delta$ T cells and $\alpha\beta$ T cells in the liver of the pre-diabetic NOD mice against various doses of immobilized anti-TCR mAb before listerial infection. Maximal responses of $\alpha\beta$ T cells and $\gamma\delta$ T cells were obtained at mAb concentrations of 20 μg/ml or 50 μg/ml, respectively both in BALB/c or NOD mice (data not shown, reference 26). As shown in Fig. 3(a), the $\alpha\beta$ T cells in the liver of the naive NOD mice showed limited blastogenesis after stimulation with TCR-$\alpha\beta$ cross-linking, while the $\gamma\delta$ T cells significantly proliferated in response to TCR-$\gamma\delta$ cross-linking, which was comparable to those in BALB/c mice. These results suggest that $\gamma\delta$ T cells may remain functionally intact in sharp contrast to $\alpha\beta$ T cells in the liver of the NOD mice, which are unresponsive to TCR triggering, similar to thymic and LN T cells in NOD mice as described previously.[24,25]

Next, to examine whether *Listeria*-specific cell-mediated immunity can develop in the NOD mice after listeria infection, T-cell proliferation in response to listerial antigen was investigated in the liver lymphocytes of NOD mice inoculated i.p. with 3×10^3 *L. monocytogenes* 10 days previously. As shown in Fig. 3(b), the liver lymphocytes of BALB/c mice significantly proliferated in response to heat-killed *Listeria* (HKL), while the liver T cells in NOD mice showed limited blastogenesis after stimulation with HKL. Taken together, these results suggest that *Listeria*-specific immunity mediated by $\alpha\beta$ T cells may be severely impaired in NOD mice.

Effects of *in vivo* pretreatment with anti-TCR-$\gamma\delta$ mAb on the eradication of bacteria in NOD mice infected with *L. monocytogenes*

Since $\alpha\beta$ T cells in the NOD mice are unresponsive against listerial infection, it can be speculated that other cells including $\gamma\delta$ T cells than $\alpha\beta$ T cells may contribute to protection against listerial infection in the NOD mice. To investigate the protective role of $\gamma\delta$ T cells in listerial infection in NOD mice, $\gamma\delta$ T-cell-depleted mice were prepared by *in vivo* administration of anti-TCR-$\gamma\delta$ mAb (200 μg/mouse) according to the method described previously.[3] A relatively lower dose (8×10^2) of viable *Listeria* were injected i.p. in mice 3 days after treatment with anti-TCR-$\gamma\delta$ mAb (on day 0), and the bacterial growth in the liver was examined on day 6 after infection. As shown in Fig.

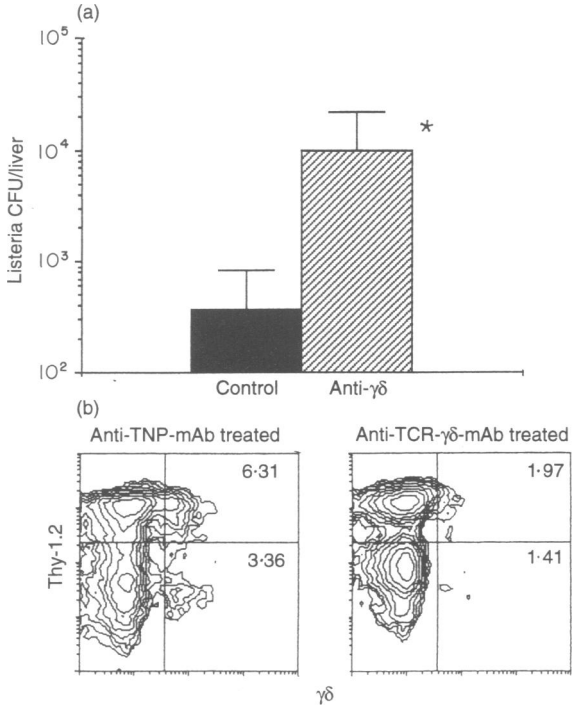

Figure 4. Effects of *in vivo* administration of anti-TCR-γδ mAb (UC7-13D5) on the eradication of bacteria from the liver in NOD mice after infection with *L. monocytogenes*. NOD mice were injected intraperitoneally with 200 μg of anti-TCR-γδ mAb or anti-TNP mAb on day −3 and then inoculated with 8×10^2 *L. monocytogenes* on day 0. (a) The numbers of *Listeria* in the liver on day 6 were determined. Results were obtained from four mice and are presented as the means and SDs. $*P < 0{\cdot}05$, significantly different from value for anti-TNP mAb-treated control mice by the Cochran–Cox test. (▨) anti-γδ mAb-treated NOD mice; (■) control mAb-treated NOD mice. (b) Flow cytometric analysis for expression of TCR γδ on hepatic lymphocytes from NOD mice on day 10 after infection with 8×10^2 *L. monocytogenes*. Hepatic lymphocytes were stained with FITC-conjugated anti-Thy-1.2 mAb and PE-conjugated anti-TCR-γδ mAb. The percentage of each subpopulation was calculated after integration of selected area in contour profile.

4(a), control mAb-treated NOD mice eliminated the bacteria almost completely on day 6 after infection with 8×10^2 *L. monocytogenes*, while γδ T-cell-depleted NOD mice allowed the bacteria to reach up to more than 10^4 colony-forming units (CFU) ($P < 0{\cdot}05$). Flow cytometric analysis showed that TCR γδ on T cells were downmodulated in the liver 3 days after an intraperitoneal administration of 200 μg of anti-TCR-γδ mAb (data not shown), and remained at an undetectable level until 10 days after listerial infection (Fig. 4b). These results suggested that the γδ T cells mainly participate in the host defense at the early stage after primary infection in NOD mice as seen in normal mice.[3]

DISCUSSION

We have reported previously that pretreatment with anti-TCR-γδ mAb significantly inhibited the protection against *L. monocytogenes* at the early stage but not at the late stage after listerial infection.[3] In contrast, pretreatment with anti-αβ mAb inhibited the protection mainly at the late stage after infection.[3] These observations suggest that γδ T cells play an important role at the early stage, while αβ T cells play protective roles at the late stage after listerial infection. In the present study, we have obtained the evidence that the resistance against primary infection with *L. monocytogenes* at the early stage was not impaired in the prediabetic NOD mice and the γδ T cells increased normally in the NOD mice after *L. monocytogenes* infection. On the other hand, *in vivo* responsiveness of αβ T cells appeared to be severely impaired against listerial infection, allowing exaggerated bacterial growth at the late stage after listerial infection. Experiments with NOD mice pretreated with anti-TCR-γδ mAb confirm that γδ T cells play a protective role at the early stage against listerial infection in the NOD mice. However, they are not capable of overall control of infection in compensation for αβ T cells, resulting in an impaired protection against listerial infection in NOD mice.

One of the notable findings in the present study is that γδ T cells increased remarkably at the early stage during the course of listeria infection in the NOD mice. It is possible that γδ T cells may increase in compensation of αβ T cells being rendered unresponsive in NOD mice. However, the studies with mice carrying mutant gene for TCR α or TCR β reveal that the development of γδ T cells does not depend on αβ T cells.[27,28] The accumulation and expansion of γδ T cells after listerial infection may not be affected by that of αβ T cells, as in case of T-cell development. We have previously reported that *Ity/Bcg/Lsh* gene coding probably for a nitric oxide (NO) transporter[29] influences the γδ T-cell response during Salmonellosis which is closely associated with HSP expression in macrophages.[6] BALB/c and C57BL/6 mice are known to be *Ity* sensitive,[30] while NOD mice are *Ity*-resistant mice.[31] Therefore, *Ity* status may affect the γδ T-cell response in the NOD mice after listerial infection. However, protection against listerial infection is not influenced by the *Ity* gene but is controlled by several genes including Hc encoding C5.[32] So far, we can not find any correlation between γδ T-cell response and these genes in case of listerial infection. Interestingly, the development of diabetes in NOD mice is reported to map to a region in *Ity/Bcg/Lsh* gene on chromosome 1,[33,34] raising the possibility that the γδ T-cell response may be somewhat associated with the pathogenesis of diabetes in NOD mice. Further study is needed to clarify the role of γδ T cells in development of diabetes.

αβ T cells in the thymus and the peripheral lymphoid tissues of NOD mice have been reported to be unresponsive to anti-TCR-αβ-mAb-mediated cross-linkage.[25] Rapoport *et al.* reported that the anergy of αβ T cells can be explained by deficient TCR regulation of the pathway of PKC/p21^{ras} activation.[35] Our findings suggest that the αβ T cells in the liver of NOD mice are rendered unresponsive to TCR triggering, similar to the αβ T cells in the thymus and the peripheral lymphoid tissues of these animals. On the other hand, the γδ T cells in NOD mice may not be affected and remain functionally intact in terms of responsiveness to TCR triggering. We cannot at present elucidate the exact causes of the difference in the *in vitro* or *in vivo* reactivity to TCR triggering or listerial infection between αβ and γδ T cells. Although γδ T cells share numerous characteristics with αβ T cells, there have been several reports concerning differences in the activation pathway between γδ T cells and αβ T cells. A subset of γδ T cells, especially epithelia-associated γδ T cells in

the intestine and skin, have recently been found to use the γ chain of the high-affinity receptor for IgE instead of ζ chain of the TCR complex.[36] Ramanujam *et al.* have shown that $\gamma\delta$ T cells expanding selectively in response to a high dose of ionomycin may be able to tolerate high concentrations of free cytoplasmic calcium.[37] Spaner *et al.* have reported that $\gamma\delta$ T cells rapidly proliferate into blasts and the major of the blast die after exposure to antigen.[38] Taken together, it would thus appear that at least a significant fraction of $\gamma\delta$ T cells may be activated via a different pathway from $\alpha\beta$ T cells. Further studies with NOD mice for biochemical mechanisms may provide insight to elucidate the nature of the different pathway of $\gamma\delta$ T-cell activation.

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