Protective Roles of γδ T Cells and Interleukin-15 in Escherichia coli Infection in Mice

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The number of γδ T cells in the peritoneal cavity was increased after an intraperitoneal (i.p.) injection with Escherichia coli in lipopolysaccharide (LPS)-responsive C3H/HeN mice but not in LPS-hyporesponsive C3H/HeJ mice. The γδ T cells preferentially expressed invariant Vγ6 and Vδ1 chains and proliferated to produce a large amount of gamma interferon in the presence of LPS. Mice depleted of γδ T cells by T-cell receptor δ gene mutation showed impaired resistance against E. coli as assessed by bacterial growth. Macrophages from C3H/HeN mice infected with E. coli expressed higher levels of interleukin-15 (IL-15) mRNA than those from the infected C3H/HeJ mice. Administration of anti-IL-15 monoclonal antibody inhibited, albeit partially, the appearance of γδ T cells in C3H/HeN mice after E. coli infection and diminished the host defense against the infection. These results suggest that LPS-stimulated γδ T cells play an important role in the host defense against E. coli infection and that IL-15 may be partly involved in the protection via an increase in the γδ T cells.

T-cell receptor (TCR)-γδ T cells are present in only small numbers in peripheral lymphoid tissues but respond against infection by intracellular bacteria such as Mycobacterium tuberculosis (24), Listeria monocytogenes (43), Salmonella cholerae-suis (9), and other pathogens (16, 26). The contribution of the γδ T cells to protection against infection with intracellular bacteria has been tested in mice depleted of the cells. We have previously reported that pretreatment with anti-TCR-γδ monoclonal antibody (MAb) impaired the host defense early after infection with L. monocytogenes (17). Mice rendered deficient in γδ T cells by homologous recombination of the TCR-δ chain gene showed an impaired host defense against M. tuberculosis (29). Thus, γδ T cells may play important roles in the host defense against infection by intracellular parasites. On the other hand, TCR-δ-deficient mice showed exaggerated intestinal damage after oral infection with Eimeria vermiciformis, suggesting that some γδ T cells, such as intraepithelial γδ T cells, play a role in resolution of the inflammatory process (45). γδ T cells may be heterogeneous in function during the course of infectious diseases.

γδ T cells are reported to respond to various bacterial products, such as tetanus toxoid (28), staphylococcal enterotoxin A (46), heat shock protein 65 (HSP65) (4), and isopentenyl pyrophosphate (34, 56), through a TCR-dependent mechanism. On the other hand, it has been reported that a significant fraction of the γδ T-cell population is stimulated by lipopolysaccharide (LPS) (30, 41, 51), a cell wall component of gram-negative bacteria, through an apparently TCR-independent mechanism. We have recently found by using TCR-δ-deficient mice that γδ T cells play an important role in the priming of macrophages for tumor necrosis factor alpha (TNF-α) production in response to LPS (38). Takada et al. have reported prominent increases in γδ T cells in the peritoneal cavities of some strains of mice infected with Escherichia coli, a gram-negative extracellular bacterium (55). Protection against extracellular bacteria is thought to depend mainly on neutrophils and antibody (Ab) (58). Therefore, it is of interest to elucidate whether LPS-stimulated γδ T cells are involved in the host defense against infection with E. coli.

Interleukin-15 (IL-15) is a novel cytokine that uses β and γ chains of IL-2 receptor for signal transduction and shares many properties with, despite having no sequence homology to, IL-2 (14, 15). IL-2 is produced mainly by activated T cells, whereas IL-15 is produced by a wide variety of tissues, including placenta, skeletal muscle, kidney, and macrophages, upon stimulation with LPS (15, 54). IL-15 has stimulatory activities for natural killer (NK) cells, γδ T cells, and B cells (1, 5, 6, 15). We have recently reported that γδ T cells appearing after Salmonella infection or in intestinal intraepithelial lymphocytes can proliferate in response to exogenous IL-15 or IL-15 derived from infected macrophages (18, 39). A significant number of γδ T cells, which emerge at the early stage of infection well before the appearance of IL-2-producing γδ T cells, may preferentially use IL-15 from stimulated macrophages as a growth factor. However, the role of IL-15 in the host defense against bacterial infection remains to be elucidated.

In the present study, to elucidate the roles of γδ T cells and IL-15 in protection against infection, we examined the host defense against E. coli infection in mice depleted of γδ T cells or IL-15. Mice depleted of γδ T cells by TCR-δ gene targeting showed exaggerated bacterial growth after E. coli infection. Administration of an anti-IL-15 MAb inhibited the appearance of γδ T cells after infection and impaired the host defense against E. coli. The implications of these findings for the roles of γδ T cells and IL-15 in the host defense against E. coli infection are discussed.

MATERIALS AND METHODS

Mice, C3H/HeJ, C3H/HeN, and C57BL/6 mice were purchased from Japan SLC (Shizuoka, Japan). Eight- to 10-week-old female mice were used for the experiments. TCR-δ−− mice, which lack the TCR-δ gene, have previously been described (23). Briefly, chimeric mice were produced by injecting ES clones into C57BL/6 mice. A homogeneous TCR-δ−− population was established by backcrossing.
Crossing δ homogenotes to C57BL/6 mice more than five times. The resultant homogenotes (TCR-δ−/−) were bred to obtain the TCR-δ−/− homogenotes. Mice were housed under specific-pathogen-free conditions and offered food and water ad libitum.

Microorganisms and reagents. E. coli (ATCC 26: American Type Culture Collection, Rockville, Md.) grown in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) was washed repeatedly, resuspended in phosphate-buffered saline (PBS), and stored at −80°C in small aliquots until used. LPS derived from E. coli O26:B6 or Salmonella typhimurium was purchased from Sigma Chemical Co. (St. Louis, Mo.). Mitomycin C (MMC) was purchased from Kyowa Hakko Kogyo Co. (Tokyo, Japan).

Fluorescein isothiocyanate (FITC)-conjugated anti-CD3ε MAb (145-2C11), phycoerythrin (PE)-conjugated anti-CD8α MAb (H57-597), PE-conjugated anti-TCR-γδ (GL-3), and biotin-conjugated anti-CD3ε MAb were purchased from Pharmingen (San Diego, Calif.). Red-613-conjugated streptavidin was obtained from Life Technologies (Gaithersburg, Md.). Anti-IL-15 MAb (G277-3588) was purchased from Pharmingen, and isotype control antibody (rat immunoglobulin G) was from Inter-Cell Technologies, Inc. (Hopewell, N.J.).

Preparation of lymphocytes. Mice were intraperitoneally injected with E. coli at a dose of one-fifth the 50% lethal dose (LD50) (10^8 CFU/mouse) in 1 ml of PBS on days 0, 1, 2, 3, 5, and 7 after inoculation for 110 × for 5 min, washed twice, and resuspended at optimal concentrations in RPMI 1640 medium (GIBCO, Grand Island, N.Y.) supplemented with 10% serum. Smear specimens for differentiation of lymphocytes were prepared with Giemsa stain and were stained with a Giemsa-stained mesh. After the coarse pieces were removed by centrifugation at 50 × for 1 min, the cell suspensions were again centrifuged, resuspended in 8 ml of 45% Percoll (Sigma), and layered on 5 ml of 67.5% Percoll. The gradients were centrifuged at 600 × g at 20°C for 20 min. Lymphocytes at the interface were harvested and washed twice with Hank's balanced salt solution.

Bacterial growth. Mice were inoculated ip. with 10^8 CFU of E. coli in 1 ml of PBS. The peritoneal contents were lavaged with 3 ml of PBS and harvested after washing. The samples were serially diluted with PBS. The liver and spleens were removed and separately placed in homogenizers containing 5 ml of PBS. Samples were spread on Tripton-Soya agar (Nissui Pharmaceutical, Tokyo, Japan) plates, and colonies were counted after incubation for 24 h at 37°C.

Flow cytometry. Non-plastic-adherent PEC and liver lymphocytes were incubated with saturating amounts of FITC-, PE-, and biotin-conjugated Abs for 30 min at 4°C. To detect biotin-conjugated MAb, cells were stained with Red-613-conjugated streptavidin after incubation with a primary MAb. Cells were analyzed with a FACScan flow cytometer (Becton Dickinson, San Jose, Calif.). The liver lymphocytes were carefully gated by forward and side light scattering. The data were analyzed with FACScan Research software (Becton Dickinson).

Proliferation assay. The γδ T cells were purified by cell sorting with an EPICS ELITE (Coulter, Hialeah, Fla.) electric cell sorter from the non-plastic-adherent cells and liver lymphocytes on day 3 after E. coli infection. The purity of sorted cells was more than 97% (data not shown). Ninety-six-well tissue culture plates were incubated overnight at 4°C with 100 μl of anti-TCR-γδ (145-2C11) MAb, PE-conjugated anti-TCR-γδ (H57-597), or PBS, and then washed with 100 μl of PBS. On day 2, 0.2 ml of culture medium containing 10% fetal calf serum was added. After 3 days, the number of bacteria in C3H/HeN mice was significantly higher than in C3H/HeJ mice.

Expression of cytokine genes. C3H/HeJ and C3H/HeN mice were killed 3 days after ip. inoculation with E. coli (10^8 CFU/mouse). Expression of total RNA from sorted γδ T cells and cDNA synthesis were performed as described above. PEC were spread in plastic plates and incubated for 1 h in a CO2 incubator at 37°C. After nonadherent cells were washed away with PBS, adherent cells were used in in vitro experiments. Of the adherent cells, >95% were macrophages, as assessed by morphological findings. Extraction of total RNA from macrophages and cdDNA synthesis were performed as described above. Serial dilutions of total RNA were prepared with 20 pmol of random primer in 21 μl reaction mixtures for reverse transcription. Synthesized cdDNAs were amplified by PCR with primers derived from the murine cdDNA. The specific primers were as follows: IL-2 sense, 5'- GGT CTT CAC TCT GAT AAT TCG C-3'; IL-2 antisense, 5'-TGA TGG ACC TAC AGG ACC TCG TGA C-3'; IL-4 sense, 5'-GGC AGC AGC GCA CAG GAT CCG T-3'; IL-4 antisense, 5'-GAC TAT TCC AGT GTC TCT G-3'; IL-6 sense, 5'-TGG CAG TTC AGT CAG ATG GAT-3'; IL-6 antisense, 5'-TGC GAC CAG ATG GAA TGT CAC C-3'; IL-10 sense, 5'-TAC CTG GTA GAA GTG ATC GC-3'; IL-10 antisense, 5'-CAT GTA TGT TTC TAT GC-3'; IL-12 sense, 5'-GGA GAC CCA CCT GCC CAT TGA ACT-3'; IL-12 antisense, 5'-CAA GTG TGC ATC GTA CCA TG-3'; IL-15 sense, 5'-GGA TTC AGT TTC ACC CCA GT-3'; IL-15 antisense, 5'-GCA TCC TGT TTC GAA CCA G-3'; IFN-γ sense, 5'-AGC GTG GTA GAG GAT CAC CGA C-3'; IFN-γ antisense, 5'-GAT ACA GCT TAC GAG G-3'; TNF-α sense, 5'-GAT ACA GCT GAG GAA GTG ATC C-3'; TNF-α antisense, 5'-GAC TAT TCC AGT CCA GTG AAT TCG G-3'; transforming growth factor β (TGF-β) sense, 5'-CTT TAG GAA GCA GCT GTG TT-3'; TGF-β antisense, 5'-CCA GAG CGC ACA ATC AGT TT-3'. The PCR product was subjected to electrophoresis on a 1.5% agarose gel (Nakari Tesque) and transferred to a GeneScreen Plus filter (New England Nuclear), and probes were labeled with [32P]dCTP by using the Megaprim DNA labeling system (Amersham International, Amersham, United Kingdom) according to the manufacturer’s instructions. The β-actin and 18S probes were labeled with [32P]dCTP by using the Megalabel 5' label kit (Takara Shuzo Co., Ltd., Kyoto, Japan) according to the manufacturer’s instructions. After hybridization, the filters were incubated in 1 × Nuclease 3'-10% dextran sulfate–10 μg of heat-denatured salmon sperm DNA per ml for 18 h at 60°C, and then the filters were washed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–1% SDS for 15 min at 60°C. The radioactivity of each band of PCR product was analyzed with a Fujix BAS2000 Bio-image analyzer (Fuji Photo Film Co., Ltd., Tokyo, Japan).

For nucleotide sequencing, reverse transcription-PCR products were resolved in low-melting-point agarose gels, isolated, and cloned into TA vector PCR II (Invitrogen). Purified double-stranded DNAs were sequenced by using the Taq Dye Primer Cycle sequencing kit and an ABI 373A DNA Sequencer (Applied Biosystems, Foster City, Calif.).

RESULTS

Kinetics of bacterial growth in organs after ip. inoculation with E. coli. C3H/HeN and C3H/HeJ mice were inoculated ip. with E. coli at a dose of one-fifth the 50% lethal dose (LD50) (10^8 CFU/mouse). E. coli O26:B6 (one-fifth the LD50) inoculated mice were used to examine the kinetics of bacterial growth in the peritoneal cavity, liver, and spleen were examined. As shown in Fig. 1, the number of bacteria in the peritoneal cavity and spleen had decreased by day 3 of infection in both mouse strains. However, on day 2, the number of bacteria in C3H/HeN mice was sig-
significantly smaller than that in C3H/HeJ mice ($P < 0.05$). There was no difference in the bacterial number in the liver between the strains of mice at any stage after $E. coli$ infection.

**Kinetics of $\gamma \delta$ T cells in the peritoneal cavity and liver after $E. coli$ infection.** To examine the cell influx in the peritoneal cavity and liver after $E. coli$ infection, we analyzed the kinetics of PEC from C3H/HeJ and C3H/HeN mice inoculated i.p. with $10^6$ CFU of $E. coli$. The numbers of polymorphonuclear leukocytes (PMN) and macrophages in the peritoneal cavity were smaller in C3H/HeJ than in C3H/HeN mice on day 3 after $E. coli$ infection (PMN, $2.5 \pm 0.6 \times 10^6$ versus $32.3 \pm 5.5 \times 10^6$; macrophages, $18.3 \pm 3.6 \times 10^6$ versus $87.5 \pm 16.0 \times 10^6$ [$n = 5$]). The absolute numbers of lymphocytes did not differ significantly for the two strains at any stage after $E. coli$ infection (data not shown). Flow cytometry analysis of the expression of CD3 and TCR-$\gamma \delta$ was carried out with the non-adherent PEC and liver lymphocytes from both strains of mice on days 0, 1, 3, 5, and 7 of infection. A representative result from three independent experiments is shown in Fig. 2A. $\gamma \delta$ T cells in the nonadherent PEC of C3H/HeN mice increased, constituting more than 30% of all cells on day 3 after $E. coli$ inoculation, whereas the percentage of $\gamma \delta$ T cells in the PEC of C3H/HeJ mice was only 4.0% at this stage of infection. No significant difference in the proportions of $\gamma \delta$ T cells in livers of C3H/HeJ and C3H/HeN mice was observed at any stage of infection (data not shown).

**Proliferation and cytokine production of $\gamma \delta$ T cells in the peritoneal cavity induced by $E. coli$ infection.** To investigate the functions of $\gamma \delta$ T cells induced by $E. coli$ infection, we first examined the expression of cytokine genes in freshly isolated $\gamma \delta$ T cells from C3H/HeN and C3H/HeJ mice infected with $E. coli$ 3 days previously by cell sorting with an electric cell sorter. The $\gamma \delta$ T cells in the peritoneal cavities of $E. coli$-infected mice expressed high levels of mRNAs specific for IFN-$\gamma$, TNF-$\alpha$, and TGF-$\beta$ but not IL-2, IL-4, or IL-6 (Fig. 3).

We next examined the proliferative response and cytokine production of the $\gamma \delta$ T cells induced by $E. coli$ infection in the peritoneal cavities and livers of C3H/HeJ mice. $\gamma \delta$ T cells were purified by cell sorting from the nonadherent peritoneal cells on day 3 of $E. coli$ infection. The $\gamma \delta$ T cells were incubated for 48 h on anti-TCR-$\gamma \delta$ MAb-coated dishes with an optimum dose (10 $\mu$g/ml) of LPS in the presence or absence of MMC-treated spleen cells. Figure 4A shows that $E. coli$-induced $\gamma \delta$ T cells in the peritoneal cavity exhibited a strong proliferative response to LPS even in the absence of MMC-treated spleen cells, whereas $\gamma \delta$ T cells from liver showed no proliferation in response to LPS.

To assess whether the $\gamma \delta$ T cells from liver produced IFN-$\gamma$ at the protein level in response to LPS, we examined the production of cytokines with or without LPS. Figure 4B shows that $\gamma \delta$ T cells stimulated with LPS produced a large amount of IFN-$\gamma$, whereas neither IL-2 nor IL-4 was detected in the supernatant. The $\gamma \delta$ T cells from the liver did not produce either IFN-$\gamma$ or IL-4 in the presence of LPS. These results suggest that the peritoneal $\gamma \delta$ T cells induced by $E. coli$ infection produce IFN-$\gamma$ in response to LPS.

**V$\gamma$ and V$\delta$ gene expression in $\gamma \delta$ T cells in the peritoneal cavity and liver in mice infected with $E. coli$.** To examine the V gene expression of the $\gamma \delta$ T cells in the peritoneal cavity and liver in C3H/HeN mice following $E. coli$ infection, total RNA was extracted from $\gamma \delta$ T cells sorted from nonadherent PEC and livers of mice inoculated with $E. coli$ 3 days previously, and V gene expression was analyzed by reverse transcription-PCR. As shown in Fig. 5, the $\gamma \delta$ T cells in PEC expressed the V$\gamma$6 and V$\delta$1 genes preferentially, whereas the $\gamma \delta$ T cells in the livers of C3H/HeN mice expressed V$\gamma$1/2, V$\gamma$4, and a diversity of V$\delta$ genes. We further examined the V gene expression of the $\gamma \delta$ T cells in the peritoneal cavity after stimulation with LPS in vitro. $\gamma \delta$ T cells expressing the V$\gamma$6 and V$\delta$1 genes were enriched after stimulation with LPS (Fig. 5).

To determine the junctional diversity of the V$\gamma$6-Jy1 and V$\delta$1-J$\delta$2 gene rearrangements, we determined the nucleotide sequences of the V$\gamma$6 and V$\delta$1 transcripts of the peritoneal $\gamma \delta$ T cells in $E. coli$-infected mice. All of 20 V$\gamma$6-V$\gamma$1 transcripts...
and 18 of 20 V\beta 1-J\delta2 transcripts showed no junctional diversity and the same junctional joining (data not shown), resulting in in-frame invariant canonical sequences, which are preferentially expressed in fetal thymocytes at the late stage (approximately day 17) of gestation and in the intraepithelial lymphocytes of reproductive organs such as the uterus (20, 22). We have already reported that most \gamma\delta T cells in the peritoneal cavities of naive C3H/He mice express V\gamma 1/2 and V\delta 6 transcripts with junctional diversity (17). Taken together, these results suggest that the V\gamma and V\delta expression of the E. coli-induced \gamma\delta T cells in the peritoneal cavity is different from that of \gamma\delta T cells in the peritoneal cavities of naive mice and in the livers of E. coli-infected mice.

Effects of \gamma\delta T-cell-depletion on the eradication of bacteria in mice infected with E. coli. To confirm a protective role for \gamma\delta T cells in E. coli infection, TCR-\delta-/- mice with a C57BL/6 background were infected with E. coli (10^8 CFU/mouse) and sacrificed on day 3. Control (TCR-\delta+/-) mice showed an increase in \gamma\delta T cells on day 3 after E. coli infection (data not shown). As shown in Fig. 6A, a significant increase in the number of E. coli cells was detected in the peritoneal cavities, livers, and spleens of TCR-\delta-/- mice compared with control mice. The numbers of PMN, macrophages, and lymphocytes in

![Graph showing kinetics of peritoneal \gamma\delta T cells after i.p. E. coli inoculation.](image-url)
The peritoneal cavities of TCR-δ2/mice were smaller than those for control mice (Fig. 6B). These results suggest that an increase of gd T cells in the peritoneal cavity is one of the factors responsible for the eradication of E. coli.

Involvement of IL-15 in the appearance of gd T cells after infection with E. coli. The gd T cells that appear during the course of infection with E. coli produce IFN-γ, but not IL-2, after LPS stimulation. We have previously shown that IL-15 produced by the macrophages is involved in the stimulation of the gd T cells during salmonellosis (39). We therefore examined whether IL-15 produced by the infected macrophages is involved in the stimulation of the gd T cells during E. coli infection. To determine whether IL-15 was induced in macrophages after E. coli infection, we tried to detect the IL-15 mRNA in the peritoneal macrophages of C3H/HeJ and C3H/HeN mice 3 days after E. coli infection. The levels of expression of the IL-15 gene in macrophages after E. coli infection are presented in Fig. 7. Consistent with previous reports (11, 36), macrophages of C3H/HeN mice expressed TNF-α and IL-6 more abundantly after E. coli infection than did those of C3H/HeJ mice. The level of expression of IL-15 mRNA, especially the longer message, which is translated most efficiently (40), in macrophages after infection with E. coli was higher in C3H/HeN mice than in C3H/HeJ mice.

To elucidate the role of IL-15 in the defense against E. coli infection, we examined the effect of in vivo administration of anti-IL-15 MAb on the appearance of gd T cells and the eradication of E. coli in C3H/HeN mice after infection. C3H/HeN mice were injected i.p. with anti-IL-15 neutralizing MAb (200 µg) or isotype control Ab at 2 h before E. coli challenge, and 3 days later, the numbers of PEC, gd T cells, and bacteria were determined. The absolute number of peritoneal cells in anti-IL-15 MAb-treated mice was much the same as that in control mice at that stage of E. coli infection (data not shown). A typical three-color profile is shown in Fig. 8A. The appearance of gd T cells was partly inhibited in the peritoneal cavity in anti-IL-15 MAb-treated mice after the infection. Bacterial numbers were significantly increased in the peritoneal cavities, livers, and spleens of anti-IL-15 MAb-treated mice compared with the control group (Fig. 8B).

FIG. 3. Expression of cytokine mRNAs in peritoneal gd T cells sorted from C3H/HeJ and C3H/HeN mice infected with E. coli 3 days previously. gd T cells were sorted from nonadherent PEC pooled from five mice of each strain, and total RNA was reverse transcribed into cDNA and amplified by PCR. The results are representative of those from three independent experiments.

FIG. 4. Proliferative response and cytokine production of gd T cells from the peritoneal cavities or livers of C3H/HeN mice in the presence of LPS. (A) Purified populations of gd T cells were incubated (5 × 10⁴/well) in anti-TCR-δ MAb-coated 96-well plates for 48 h in the presence or absence of MMC-treated spleen cells (3 × 10⁴/ml), with or without 10 µg of LPS per ml. During the last 8 h of incubation, 1.0 µCi of [³H]thymidine per well was added. The cells were then harvested, and the amount of [³H]thymidine incorporated was determined by scintillation counting. The data are representative of those from two separate experiments and are expressed as the means of triplicates ± standard deviations. Asterisks indicate significant differences from the values for the control (P < 0.05). (B) Purified gd T cells (5 × 10⁴ cells) were cultured similarly in the presence or absence of MMC-treated spleen cells with or without LPS for 24 h at 37°C, and the culture supernatant was collected. The cytokine activity in the culture supernatant was tested for the presence of IFN-γ by ELISA. The data are representative of two separate experiments and are expressed as the means of triplicates ± standard deviations. Asterisks indicate significant differences from the values for the control (P < 0.05).
with control mice (Fig. 8B). These results suggested that endogenous IL-15 is at least partly responsible for the γδ T-cell proliferation after *E. coli* infection and plays an important role in host defense against the infection.

**DISCUSSION**

It is generally accepted that the host defense against infection with *E. coli*, an extracellular bacterium, is almost exclusively dependent on neutrophils and Ab (58). We show here the possibility that γδ T cells are also involved in the host defense against *E. coli* infection. γδ T-cell numbers were remarkably increased in the peritoneal cavity after an i.p. infection with *E. coli* in LPS-responsive C3H/HeN mice but not in LPS-hyporesponsive C3H/HeJ mice. The γδ T cells appearing in the peritoneal cavity after i.p. infection with *E. coli* produced a large amount of IFN-γ in the presence of LPS. Mice depleted of γδ T cells by TCR-δ gene mutation showed an impaired host defense against *E. coli*. These results suggest that LPS-stimulated γδ T cells help to protect against *E. coli* infection.

Similar to αβ T cells, γδ T cells secrete various cytokines and express cytolytic functions (27). Most γδ T cells appearing after infection with intracellular bacteria are reported to produce Th1-type cytokines, in particular IFN-γ (2, 11, 12, 33, 57), whereas γδ T cells during infection with a helminth, *Nippostrongylus brasiliensis*, preferentially produced Th2-type cytokines, mostly IL-4 (11). Furthermore, γδ T cells, especially in the epithelium, produce TGF-β for immunoregulation and/or immunoglobulin A production (7, 13, 53). We have previously observed, with mice depleted of γδ T cells by treatment with anti-TCR-γ-δ MAb, that γδ T cells contribute to defense early after *Listeria* infection via IFN-γ production (17). Mice with mutated TCR-δ genes showed impaired TNF-α production in response to LPS (38). Our results reveal that the γδ T cells accumulating in the peritoneal cavity after *E. coli* infection expressed IFN-γ mRNA and produced a significant amount of IFN-γ in the presence of LPS under TCR triggering. We speculate that γδ T cells produce IFN-γ in response to *E. coli* and their cell component LPS and activate macrophages which consequently eliminate the *E. coli*. We have previously reported that γδ T cells appearing during the course of listeriosis produced macrophage chemotactic factor, in addition to IFN-γ.

![FIG. 5. Vγ or Vδ usages of γδ T cells in PEC and liver on day 3 after *E. coli* infection. Total RNA extracted from γδ T cells (5 × 10⁶ cells) sorted from five C3H/HeN mice infected with *E. coli* 3 days previously or from γδ T cells stimulated with LPS as described in the legend to Fig. 4 was reverse transcribed into cDNA and amplified by PCR with primers for Cγ or Cδ and various Vγ or Vδ segments, respectively. The Southern blot of γ PCR products was hybridized with MNG6. The Southern blot of δ PCR products was hybridized with an oligonucleotide probe for Jδ1 or Jδ2. The results are representative of those from three independent experiments.](http://iai.asm.org/)

![FIG. 6. Bacterial growth in the peritoneal cavities and spleens of TCR-δ−/− mice after *E. coli* infection. TCR-δ−/− mice and their littermate control mice were inoculated i.p. with 2 × 10⁸ CFU of *E. coli* on day 0. (A) The numbers of *E. coli* CFU recovered from peritoneal cavities and spleens of infected mice on day 3 were determined by colony formation assay on tryptic soy agar. Values are means ± standard deviations for groups of five mice. Asterisks indicate significant differences from the values for control mice (P < 0.05). (B) Populations of PEC obtained from TCR-δ−/− mice and control mice on day 3 after i.p. inoculation with *E. coli*. PMN, macrophages, and lymphocytes were judged by morphologic characteristics after staining with Giemsa solution. Values are means ± standard deviations for groups of five mice.](http://iai.asm.org/)
cells by LPS was indeed accessory cell independent, excluding the possibility that LPS induced expression of ligands for γδ TCR on accessory cells or production of growth factors from accessory cells. Thus, it appears that LPS may have a costimulatory activity for γδ T-cell stimulation upon TCR triggering. The peritoneal γδ T cells induced by *E. coli* infection expressed the Vγδ6 gene, which is expressed by γδ T cells in the uterus and tongue, together with the V61 gene, rearranged to J62, similar to Vγ5 T cells in the epidermis (22). All Vγ6-Jv1 and V61-J62 mRNAs from the γδ T cells we sequenced have no junctional diversity, similar to those from the γδ T cells in the fetal thymus and uterus. On the other hand, the liver γδ T cells expressed Vγ1/2 and did not respond to LPS. Thus, it would appear that only γδ T cells with particular V genes such as Vγ5 and Vδ1 or Vγ6 and Vδ1 are stimulated with LPS from gram-negative bacteria through a TCR-independent mechanism. Further analysis is required to clarify which receptor of the γδ T cells recognizes LPS.

Although LPS from *E. coli* is apparently involved in γδ T-cell stimulation, the ligand for the γδ TCR during *E. coli* infection is not known. It has been reported that Vγ6 and Vδ1 T cells expand at sites of inflammation in the absence of pathogen-derived antigens in *Listeria* infection and in *Listeria*-induced autoimmune orchitis (35, 44). This suggests that Vγ6 and Vδ1 T cells do not respond to foreign antigens but rather respond to a host-derived antigen that is conserved between the host and bacteria. In mice, a high proportion of γδ T cells have been...
found to respond to unique peptides of mycobacterial and mammalian HSP65 (4). The HSP65-reactive γδ T cells characteristically express Vγ1 and Vδ6 with junctional diversity (42). We have previously reported that the peritoneal γδ T cells appearing during infection with intracellular bacteria such as S. choleraesuis (9), L. monocytogenes (17), and Mycobacterium bovis BCG (19) preferentially expressed Vγ6 and Vδ6 genes. However, the present study revealed that the γδ T cells appearing in E. coli infection preferentially expressed Vγ6 and Vδ6 and thus differ from those capable of responding to HSP65. In fact, Takada et al. have reported that γδ T cells from E. coli-infected mice did not proliferate in response to purified protein derivative or HSP65 derived from M. tuberculosis (55). A number of murine γδ-T-cell clones are reported to recognize major histocompatibility complex molecules or major histocompatibility complex-related gene products such as TL and Qa in a manner quite different from the antigen recognition shown by αβ T cells (3, 21, 31, 48). Similarly, a herpesvirus protein was found to directly stimulate γδ T cells independent of antigen processing and presentation (25, 50). Human γδ T cells are stimulated by apparently nonproteinaceous low-molecular-weight ligands, including isopentenyl pyrophosphate, which represents a ubiquitous metabolite of various vitamins, lipids, and steroids in both prokaryotic and eukaryotic cells (8, 49, 56). Therefore, it is of interest to elucidate whether the γδ T cells induced by E. coli recognize such unique antigens in a manner different from that of αβ T cells.

Another notable finding is that IL-15 was involved in protection against E. coli infection. LPS-hyporesponsive C3H/HeJ mice carry the lps" mutation on chromosome 4, and macrophages and B cells in these mice respond poorly to LPS (31, 36). Consistently, the macrophages induced by E. coli infection in C3H/HeJ mice showed an impaired expression of monokine genes such as those for TNF-α and IL-6 compared with that in C3H/HeN mice. In correlation to the sensitivity of macrophages to LPS, γδ T-cell numbers in the peritoneal cavity were remarkably increased after E. coli infection in LPS-responsive C3H/HeJ mice but not in LPS-hyporesponsive C3H/HeJ mice. IL-15 promoter regions contained binding elements for LPS-inducible transcription factors such as NF-IL-6 and NF-κB (60). Consistent with this finding, C3H/HeN mice infected with E. coli expressed higher levels of IL-15 mRNA, especially the longer transcript, than did C3H/HeJ mice infected with E. coli. We have recently found that IL-15 mRNA containing a longer alternative exon 5 is translated most efficiently among IL-15 transcripts than did C3H/HeJ mice infected with E. coli E. coli infection. LPS-hyporesponsive C3H/HeJ mice were protected, albeit partially, the increase in IL-15 mRNA, especially the longer transcript, than did C3H/HeJ mice infected with E. coli. We have recently found that IL-15 mRNA containing a longer alternative exon 5 is translated most efficiently among IL-15 transcripts derived from LPS-stimulated macrophages may be more important in accumulation of γδ T cells in the peritoneal cavity than in the expansion in vivo after E. coli infection. Anti-IL-15 administration only partially inhibited the appearance of the γδ T cells. Skee and Ziegler reported that peritoneal γδ T cells proliferated in response to IL-1 and IL-7 (52). It has been reported that TNF-α and IL-12 synergistically stimulate human γδ T-cell proliferation (59). Although it cannot be ruled out that the amount of anti-IL-15 Ab was insufficient to cause inhibition in our experiments, it appears that the increase in γδ T cells may be attributable in part to cytokines other than IL-15.

In conclusion, LPS-stimulated γδ T cells play important roles in the host defense against E. coli infection. IL-15 released from LPS-stimulated macrophages may be involved in the accumulation of γδ T cells during E. coli infection.

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