IL-17A Produced by γδ T Cells Plays a Critical Role in Innate Immunity against Listeria monocytogenes Infection in the Liver


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IL-17A is originally identified as a proinflammatory cytokine that induces neutrophils. Although IL-17A production by CD4+ Th17 T cells is well documented, it is not clear whether IL-17A is produced and participates in the innate immune response against infections. In the present report, we demonstrate that IL-17A is expressed in the liver of mice infected with *Listeria monocytogenes* from an early stage of infection. IL-17A is important in protective immunity at an early stage of listerial infection in the liver because IL-17A-deficient mice showed aggravation of the protective response. The major IL-17A-producing cells at the early stage were TCR γδ T cells expressing TCR Vγ4 or Vγ6. Interestingly, TCR γδ T cells expressing both IFN-γ and IL-17A were hardly detected, indicating that the IL-17A-producing TCR γδ T cells are distinct from IFN-γ-producing γδ T cells, similar to the distinction between Th17 and Th1 in CD4+ T cells. All the results suggest that IL-17A is a newly discovered effector molecule produced by TCR γδ T cells, which is important in innate immunity in the liver. The *Journal of Immunology*, 2008, 181: 3456–3463.

**Materials and Methods**

**Animals**

Wild-type C57BL/6 mice were purchased from Japan SLC. The IL-17A-/− mice (21), the TCR Cα−/− mice (22) of C57BL/6 background, and the Vγ4/6−/− mice (23) were back-crossed more than eight times to the *L. monocytogenes* and *Candida sp* (4–6). The participation of IL-17A has also been reported in infection with the intracellular bacteria *Mycobacterium tuberculosis* and *Mycobacterium bovis* BCG (7, 8).

Recently, IL-17A-producing CD4+ T cells, the Th17 cells, have been shown to be distinct from classical Th1 and Th2 cells. Interestingly, the development of Th17 cells was inhibited by IFN-γ and IL-4. In addition, TGF-β and IL-6 were found to be essential in the commitment of naive T cells to Th17 through the induction of IL-23 receptor (9–12).

CD4+ T cells are not the only IL-17A-producing T cell population. We demonstrated that Fas-signaling induced IL-17A-producing TCR γδ T cells (13). IL-17A-producing CD4+ CD8− TCR γδ and TCR αβ T cells were also reported in adhesion molecule deficient mice, and were named Tn for neutrophil-regulatory T cells (14). The majority of the IL-17A-producing T cells in mycobacteria-infected mice were also TCR γδ T cells (7, 8). We therefore speculated that TCR γδ T cells with functions equivalent to Th17 cells exist just as TCRγδ T cells with type 1 (IFN-γ) (15, 16), type 2 (IL-4, IL-10) (17, 18), or type 3 (TGF-β) cytokine profiles exist (19, 20), and that the IL-17A-producing TCR γδ T cells participate in protective immunity before appearance of Th17.

In this study, we demonstrate that TCR γδ T cells with restricted Vγ usage develop into IL-17A-producing T cells and participate in the innate immune response against the intracellular bacterial pathogen *L. monocytogenes* at an early stage of infection. The IL-17A produced by the TCR γδ T cells was indispensable in innate immunoprotection against the infection.

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C57BL/6 mice. To generate the bone marrow (BM) \(^3\) chimeras, the IL-17A \(^{−/−}\) recipient mice were irradiated with 9 Gy using an x-ray source, then injected i.v. with 2 × 10 \(^8\) BM cells from wild-type, TCR C \(^{+/+}\), IL-17A \(^{−/−}\) mice, or 1:1 mixture of TCR C \(^{+/+}\) and IL-17A \(^{−/−}\) BM cells. The mice were maintained under conventional conditions for 5 wk until analysis. Experiments were conducted according to the Institutional Ethical Guidelines for Animal Experiments and the Safety Guideline for Living Modified Organism Experiments of the University of the Ryukyus under approval of the Animal Experiments Safety and Ethics Committee and the Living Modified Organism Experiments Safety Committee of the University of the Ryukyus, respectively.

Microorganisms and bacterial infection

L. monocytogenes strain EGD was inoculated in C57BL/6 mice, fresh isolates were obtained from infected spleens, grown in tryptic soy broth (Difco), resuspended in PBS, and stored at −80°C in small aliquots until use. Mice were infected by i.p. inoculation of 5 × 10 \(^8\) CFU of L. monocytogenes. Heat killed L. monocytogenes were obtained by incubating viable L. monocytogenes at 70°C for 2 h.

Cell preparation

Liver mononuclear cells were prepared as described previously (24). Splenocytes were prepared by forcing minced spleens through stainless steel mesh and were used after RBC lysis. To enrich TCR \(^{+}\)T cells, cells were passed through the nylon wool columns. Then the TCR \(^{+}\)T cells were enriched by magnetic cell separation system (autoMACS; Miltenyi Biotec) by using biotin- or FITC-conjugated anti-TCR C \(^{+}\)mAb (GL-3; BD Biosciences) and streptavidin or anti-FITC microbeads (Miltenyi Biotec), respectively.

Gene expression analysis by RT-PCR

Using Trizol reagent (Invitrogen) total RNA was extracted. First strand cDNA was synthesized using reverse transcriptase (Superscript; Invitrogen). The first-strand cDNA was amplified by PCR using Taq polymerase (Takara Shuzo). Real-time PCR amplifications were analyzed using the iCycler IQ and the Real-Time PCR Optical System Software version 3.0 (Bio-Rad). The ΔCt method was used to normalize expression level of transcripts by β-actin expression level as previously reported (7). The specific primers were as follows: IL-17A sense (5′-GAT CAG GAC GCG CAA ACA TG-3′), IL-17A antisense (5′-AGT TTG CTT AGA AAC GTG GG-3′), β-actin sense (5′-TGG AAT CCT GTG GCA TCC ATG AAA C-3′), β-actin antisense (5′-TAA GAC GCA GCT CAG TAA CAG TCC G-3′).

In some experiments cDNA was amplified with TCR V \(^{α}\) or V \(^{β}\) sense primers and C \(^{γ}\) or C \(^{δ}\) antisense primers (25). PCR products were electrophoresed through a 1.8% agarose gel, stained with ethidium bromide, and photographed using the Gel-Documentation system (Bio-Rad).

FACS analysis

To detect intracellular cytokine expression, cells were cultured with 1 μg/ml calcium ionophore, 25 ng/ml PMA, and brefeldin A for 4 h. Cells were first stained for surface Ags and then with Cytofix/Cytoperm (BD Biosciences) according to the manufacturer’s instructions. Intracellular cytokine staining was performed using biotin-conjugated anti-C \(^{α}\) mAb (GL3; BD Biosciences) followed by allophycocyanin-conjugated streptavidin, FITC-conjugated anti-TCR V \(^{γ4}\) (BD Biosciences), FITC-conjugated anti-TCR V \(^{υ1}\) (2.11, provided by Dr. P. Pereira, Institute Pasteur, Paris, France), PE-conjugated anti-IL-17A, and PE-conjugated anti-I \(^{β}\)-actin (BD Biosciences). Nomenclature of V \(^{γ}\) is according to Heilig and Tonegawa (26). In some experiments cells were first stained with FITC-conjugated anti-C \(^{α}\) mAb (GL3; BD Biosciences) and then stained with biotin-conjugated 17DI mAb (27) followed by allophycocyanin-conjugated streptavidin and PE-conjugated anti-IL-17A mAb. The stained cells were analyzed with a flow cytometer FACS Calibur (BD Biosciences).

Bacterial counts in organs

The L. monocytogenes-infected mice were sacrificed on day 1, day 3, and day 5 of the infection, and the livers were removed. The organs were homogenized in saline, and the bacterial number in each organ was determined.

[3] Abbreviations used in this paper: BM, bone marrow; mBD, mouse β-defensin; ALT, alanine aminotransferase; MPI, mean fluorescence intensity.

**FIGURE 1.** Expression and function of IL-17A in the liver of L. monocytogenes-infected mice. A, Wild-type (WT) mice were infected via the intraperitoneal route with viable L. monocytogenes. IL-17A expression of liver mononuclear cells was analyzed by real time PCR method. The data are expressed as the increase in expression level compared with that before infection. The result shown is representative of three independent analyses. *, p < 0.05 compared with day 0. WT or IL-17A \(^{−/−}\) mice were infected with viable L. monocytogenes, and bacterial burden in the liver was determined on day 1 and 5 after the infection (B), or liver mononuclear cells (MNC) were collected, and counted (C, left panel). The cells were stained with mAb against leukocyte surface molecules and analyzed by FCM. The ratio of neutrophils (Gr1 \(^{+}\)CD11b \(^{+}\)) was determined and the absolute number of the cell was calculated (C, right panel). D, WT (open bar) or IL-17A \(^{−/−}\) (filled bar) mice were infected with heat-killed L. monocytogenes, liver MNC were collected on day 5 of infection, and neutrophil number was determined. *, p < 0.05 compared with C57BL/6 mice. The analyses were conducted more than three times, and representative data is shown in this figure.

**Histopathology**

The L. monocytogenes-infected mice were sacrificed on day 5 of the infection. The liver was fixed in buffered formalin and embedded in paraffin, and sections were stained with H&E. The stained sections were examined under a BX41 microscope (Olympus) equipped with ×40/13 or ×20/0.50 objectives. Images were acquired with DP70 digital camera and DP software (Olympus). The liver was also embedded in OCT compound (Sakura) frozen in dry ice-acetone, thin sections were stained with allophtocyanin-conjugated anti-CD3 mAb and Alexa 488-conjugated anti-CD11b mAb, and analyzed under Radiance 2100 confocal laser scanning microscope (Bio-Rad) equipped with ×200/0.70 objective. The images of confocal scanning microscope were acquired with LaserSharp 2000 software (Bio-Rad) and merged using Adobe Photoshop software.

**Serum alanine aminotransferase (ALT) levels**

Serum were prepared from the mice on day 1 and day 5 after L. monocytogenes infection and measured by Transamylase C-2 kit (WAKO) according to the manufacturer’s instructions.
Results

Expression of IL-17A in the liver of L. monocytogenes-infected mice

To determine the involvement of IL-17A in the innate immune response against L. monocytogenes infection, we analyzed the expression of IL-17A in the liver of Listeria-infected mice. We found that IL-17A mRNA expression increased from day 1 and maintained high levels of expression to day 5 in the liver after L. monocytogenes infection (Fig. 1A). These results suggest that L. monocytogenes infection induces IL-17A expression at very early stages in the infected liver.

The importance of IL-17A in the innate immune response and in protection against L. monocytogenes infection

We analyzed the role of IL-17A in protection against L. monocytogenes infection during the innate immune response phase. To determine the contribution of IL-17A in the protective response against the infection, we first analyzed the bacterial burden in the liver of the L. monocytogenes-infected IL-17A−/− mice. As shown in Fig. 1B, bacterial counts in the liver of the IL-17A−/− mice were more than 100 times higher than in wild-type mice on day 5 of the infection. These data demonstrate importance of IL-17A in the protective innate immune response against L. monocytogenes infection before acquired immunity is established.

Because IL-17A is known to induce neutrophils, we next analyzed the infiltrating cells in the liver of the IL-17A−/− mice by FACS after L. monocytogenes infection. The total number of the liver-infiltrating cells in IL-17A−/− mice was significantly higher than wild-type mice on day 5 of the infection, although the number was nearly the same as in wild-type mice on day 1. Unexpectedly, the number of neutrophils (Gr1highCD11b+ cells) was higher in the IL-17A−/− mice than that of wild-type mice, although IL-17A is a known neutrophil-inducing factor (Fig. 1C). It is possible that the increased bacterial burden in the liver of the IL-17A−/− mice (Fig. 1B) elicited higher numbers of neutrophils even in the absence of IL-17A. To address this, we analyzed the number of neutrophils in the liver 5 days after injection of heat killed L. monocytogenes. The data showed the number of neutrophils decreased in the IL-17A−/− mice compared with the wild-type mice (Fig. 1D). This suggests that the potential to induce neutrophils was decreased in the IL-17A−/− mice, but that higher number of neutrophils was induced after L. monocytogenes infection in the mice because of increased bacterial burden.

To analyze the influence of IL-17A on L. monocytogenes-induced early granuloma formation, a histological examination was conducted on the liver of infected IL-17A−/− and wild-type mice on day 5 of the infection. The wild-type mice showed small granulomatous lesions (Fig. 2A, upper panel) consisting of CD11b+ cells, possibly macrophages, surrounded by CD3+ T cells (Fig. 2B, upper panels). In contrast, the liver of the IL-17A−/− mice showed higher numbers of mononuclear cell-infiltrating lesions with larger size, irregular shape, and severe inflammatory cell infiltration (Fig. 2A, lower panels). This finding is consistent with the data in Fig. 1C showing increased numbers of infiltrating cells in the IL-17A−/− mice. The liver lesions of the infected IL-17A−/− mice showed scattered CD11b+ cells without an organized CD3+ T cell lining (Fig. 2B, lower panels), indicating that the formation

Statistics

Data were statistically evaluated by Student’s t test using Statwork Software (Cricket Software). A p-value of <0.05 was considered to indicate statistical significance.

FIGURE 2. Pivotal role of IL-17A in the formation of granulomatous lesions at an early stage of L. monocytogenes infection. Mice were infected with L. monocytogenes and were sacrificed at day 5 of infection for histological examination. A, Sections of the liver of wild-type (WT) (upper panels) or IL-17A−/− (lower panels) mice were examined by H & E staining (original magnification ×40 for left panels and ×200 for right panels). B, Sections of WT (upper panels) or IL-17A−/− (lower panels) were examined by confocal laser scanning microscopy with CD11b mAb (green, left panels) and CD3 mAb (red, middle panels) staining (original magnification ×200). Merged image of CD11b and CD3 staining are shown in the right panels. C, Kinetics of serum ALT levels after L. monocytogenes infection before acquired immunity is established. The data shown are representative of three independent experiments with more than five mice in each group.
of organized granulomatous lesions is impaired in the IL-17A−/− mice. Moreover, the outer perimeter of the lesions was surrounded by necrotic hepatocytes (Fig. 2A, lower panels), suggesting infection-induced liver injury in the IL-17A−/− mice. Increased liver injury was also indicated by an increase in serum ALT levels in the IL-17A−/− mice, but not in wild-type mice on day 5 of the infection (Fig. 2C). These results suggest that IL-17A is required not only in the protective response but also in the regulation of granuloma formation during the early stage of L. monocytogenes infection in the liver.

The identification of IL-17A-producing cells at an early stage of L. monocytogenes infection

To determine the phenotype of IL-17A-producing cells at early stage L. monocytogenes infection, liver mononuclear cells were prepared from the wild-type mice on day 5 of infection, stained for cell surface markers and cytoplasmic IL-17A, and analyzed by FCM. As shown in Fig. 3A, TCR γδ T cells (TCR Cδ+ cells) represented more than 80% of IL-17A-producing cells, whereas TCR αβ T cells (TCR Cβ+ cells), which coexpress CD4, represented only 20% of the IL-17A-producing cells. No IL-17A production was detected in CD8+ T cells and NK1.1+ cells. The ratio of IL-17A-producing cells in the TCR γδ T cells (~20–34%) was higher than that in TCR αβ T cells (less than 1%). Furthermore, the mean fluorescence intensity MFI of IL-17A staining of IL-17A+ TCR γδ T cells (MFI = 32.9) was higher than that of IL-17A+ TCR αβ T cells (MFI = 11.8). Fig. 3B shows kinetics of IL-17-producing T cells in the liver after L. monocytogenes infection. The absolute number of IL-17+ TCRγδ T cells was higher on day 5 compared with day 1. In contrast, the absolute number of IL-17+ TCR αβ T cells showed no significant difference between day 1 and 5 after the infection. This suggests increase of IL-17-producing TCR γδ T cells in the course of the L. monocytogenes infection and the T cells became a major IL-17-producing cells on day 5 of the infection.

To further confirm contribution of the TCR γδ T cells in IL-17 production in the L. monocytogenes-infected liver on day 5 of the infection, we separated TCR αβ+ and TCR γδ+ T cells from the liver mononuclear cells, stimulated them with anti-CD3 mAb, and analyzed IL-17 production by ELISA. As shown in Fig. 3C, a high level of IL-17 production was detected on the stimulated TCR γδ T cells but not on stimulated TCR αβ T cells. All the results demonstrate that TCR γδ T cells are the major source of IL-17A production during the early stage of L. monocytogenes infection.

To analyze IL-17A-producing TCR γδ T cells in the liver in detail, we enriched TCR γδ T cells by magnetic sorting, and analyzed them by FACS. As shown in Fig. 3B, we confirmed that approximately one third of the TCR γδ T cells expressed IL-17A. When the enriched TCR γδ T cells were stained with Abs against IL-17A and IFN-γ, IL-17A-producing cells and IFN-γ-producing cells were segregated and only a minor fraction coexpressed both cytokines (Fig. 3D, right panel). Our data suggest that L. monocytogenes infection induces two TCR γδ T cell subpopulations characterized by their expression of IL-17A or IFN-γ.

We previously reported that IFN-γ-producing TCR γδ T cells are TCR Vγ1+ γδ T cells (15, 25). Because IFN-γ-producing TCR γδ T cells largely did not coproduce IL-17A (Fig. 3B), we reasoned that the IL-17A-producing TCR γδ T cells likely express Vγ region genes other than Vγ1. To examine this, we analyzed the Vγ region repertoire of the IL-17A-producing TCR γδ T cells in the liver of L. monocytogenes-infected mice. We first analyzed Vγ and Vδ repertoire of the TCR γδ T cells induced in the liver of the L. monocytogenes-infected wild-type mice by RT-PCR. Fig. 4A shows expression of Vγ1 or 2, Vγ4 and Vγ6 as Vγ genes, and Vδ1, Vδ4, Vδ5, and Vδ6 as Vδ genes by the TCR γδ T cells. Because mAb against Vγ1, Vγ4, and Vγ5 were available, we next analyzed Vγ usage of the IL-17A-producing TCR γδ T cells by FCM. Fig. 4B shows a representative FACS profile of the Vγ staining of the liver TCR γδ T cells on day 5 of the L. monocytogenes infection. IL-17A-producing TCR γδ T cells could be
hardly detected among Vγ1+ T cells. In contrast, more than 30% of TCR Vγ4+ γδ T cells were IL-17A-producing T cells. TCR Vγ5+ γδ T cells were not detected (data not shown), which is consistent with the data of RT-PCR (Fig. 4A). It has been reported that a mAb 17D1 raised against Vγ5/Vδ1+ TCR γδ T cells also recognizes Vγ6/Vδ1+ TCR γδ T cells when pre stained with anti-TCR Cδ mAb (27). Because the liver TCR γδ T cells contained no Vγ5+ TCR γδ T cells, TCR Cδ+ 17D1+ cells are TCR Vγ6+ γδ T cells in the liver. The TCR Vγ6+ TCR γδ T cells detected with the 17D1 mAb contained 10–15% of IL-17A-expressing cells.

We further analyzed Vγ repertoire and IL-17 expression of each Vγ subpopulation of the liver TCR γδ T cells on day 1 and 5 after L. monocytogenes infection. As summarized in Fig. 4C, nearly 80% of the liver TCR γδ T cells expressed Vγ4 on day 1 of the infection, and the Vγ4+ TCR γδ T cells were the major IL-17-producing TCR γδ T cells. On day 5 of the infection, the ratio of the Vγδ+ γδ T cells increased to nearly the same level as the Vγ4+ TCR γδ T cells, and IL-17+ TCR Vγ6+ γδ T cells became detectable.

To confirm the contribution of the TCR Vγ4+ and TCR Vγ6+ γδ T cells in IL-17A production at an early stage of L. monocytogenes infection, we examined the liver γδ T cells sorted from the TCR Vγ4/6+ mice after L. monocytogenes infection. The TCR γδ T cells from the infected Vγ4/6− mice failed to produce IL-17A (Fig. 4D). The lack of IL-17-producing TCR γδ T cells was not compensated by IL-17-producing TCR αβ T cells. Furthermore, Vγ4/6+ mice also showed large inflammatory lesions in the liver on day 5 of the L. monocytogenes infection (Fig. 4E).

Taking these results together, we concluded that TCR Vγ4+ and Vγ6+ γδ T cells are the two major IL-17A-producing TCR γδ T cell populations in the liver at an early stage of L. monocytogenes infection.

**TCR γδ T cells producing IL-17A are essential for early protection against L. monocytogenes infection**

Although we already demonstrated the importance of IL-17A in the early protection against L. monocytogenes infection (Fig. 3A), this did not prove that IL-17A-producing TCR γδ T cells actually contribute to the IL-17A-mediated early protection. To directly test this, we reconstituted irradiated IL-17A−/− mice with BM cells from wild-type, TCR Cδ−/−, or IL-17A−/− mice, and compared their ability to contain L. monocytogenes in early infection. IL-17A−/− mice reconstituted with IL-17A−/− BM cells lack IL-17A-producing cells, and showed a significantly higher level of bacterial burden in the liver on day 5 of the infection compared with that of the IL-17A−/− mice reconstituted with wild-type BM cells (Fig. 5). The IL-17A−/− mice reconstituted with the BM cells of TCR Cδ−/− mice showed a significantly higher bacterial burden in the liver than did IL-17A−/− mice reconstituted with wild-type BM cells, although IL-17A-producing cells other than TCR γδ T cells can develop in the former mice (Fig. 5). These results suggest importance of TCR γδ T cells in early protection against
L. monocytogenes infection. To further prove the contribution of IL-17A derived from TCR γδ T cells, we reconstituted IL-17A−/− mice with BM cells from both C57BL/10 and IL-17A−/− mice. In the mixed BM chimera mice reconstituted with IL-17A−/− and C57BL/10 BM cells, IL-17A-expressing cells can develop from C57BL/10 BM cells, and TCR γδ T cells can develop from IL-17A−/− BM. Therefore, they lack IL-17A-producing TCR γδ T cells but retain IL-17A-producing TCR αβ T cells and IL-17A-non-producing γδ T cells. The mixed BM chimera mice showed a significantly higher bacterial burden in the liver than did IL-17A−/− mice reconstituted with wild-type BM cells. All the results demonstrate that TCR γδ T cells producing IL-17A play an important role in protection during the early stage of L. monocytogenes infection.

Discussion

In the present study, we examined the role of IL-17A in innate immunity against L. monocytogenes infection in the liver. Our data demonstrate that TCR γδ T cells producing IL-17A play a pivotal role in protection during the early stage of infection. Furthermore, IL-17A also enhances the anti-bacterial activity of L. monocytogenes-infected non-phagocytic cells, which correlated with induction of anti-microbial peptide mouse β-defensin (mBD) gene expression. These results indicate that a newly discovered IL-17A-dependent protective mechanism of TCR γδ T cells acts against intracellular bacterial infection in the liver.

Recently, a new lineage of effector CD4+ T cells, Th17, was reported to produce IL-17A and belong to a unique lineage distinct from classical Th1 and Th2 cells (9–12). The IL-17A-producing TCR γδ T cells in this study share several important characteristics with the Th17 cells. First, the IL-17A-producing TCR γδ T cells and IFN-γ-producing TCR γδ T cells represent distinct subsets (Fig. 3B) just as do Th1 and Th17 cells. The TCR Vγ repertoire of IFN-γ-producing and IL-17A-producing TCR γδ T cells is also different because the former express Vγ1 (15) whereas the latter express Vγ2 or Vγ6 (Fig. 4). Second, IL-23 is required for IL-17A production by Th17 and TCR γδ T cells. The expression of IL-17A in the liver disappeared in the L. monocytogenes-infected IL-12/23 p40-deficient mice (S.H., unpublished observation), suggesting the importance of IL-23 in the induction of IL-17A expression by TCR γδ T cells. Furthermore, stimulation with IL-23 alone induced a low level of IL-17A production by naïve TCR γδ T cells (28). Therefore, the development of the IL-17A-producing TCR γδ T cells may be regulated by molecules such as TGF-β, IL-6, and ROR-γt, which regulate Th17 cells. Analysis of the developmental pathway of the IL-17A-producing TCR γδ T cells is now ongoing using a thymus organ culture system.

The importance of TCR γδ T cells in the early protection against L. monocytogenes has been reported (29–32). TCR γδ T cell-deficient mice showed increased bacterial burden after L. monocytogenes infection. Furthermore, the TCR γδ T cell-deficient mice showed large inflammatory lesions in the liver with necrotic hepatocytes (31, 32), which is indistinguishable from those observed in the IL-17A−/− mice. Therefore, we estimate that characteristic lesions of the L. monocytogenes-infected TCR γδ T cell-deficient mice develop in the absence of IL-17A mainly produced by the TCR γδ T cells. However, the protective role of TCR γδ T cell subsets is controversial. A report showed importance of IFN-γ-producing TCR Vγ1+ γδ T cells in intraperitoneal infection system (15), whereas another showed TCR Vγ1+ Vγ4+ γδ T cells are important in i.v. infection system (33). In the latter report, it was demonstrated that deletion of TCR Vγ1+ γδ T cells from wild-type C57BL/10 mice enhanced bacterial elimination, whereas TCR γδ T cell-depleted mice or TCR γδ T cell-deficient mice showed a significant increase in bacterial numbers in the infected organs. It may be possible to explain this observation by mutual regulation of the IFN-γ-producing TCR Vγ1+ γδ T cells and IL-17A-producing TCR Vγ4+ γδ T cells. In the absence of IFN-γ-producing TCR Vγ1+ γδ T cells, the protective response by the IL-17A-producing TCR Vγ4+ γδ T cells may be enhanced. The possibility is now under investigation.

Although IL-17A is generally accepted as a cytokine of acquired immunity produced by Th17 cells, our data demonstrate that IL-17A is also an important cytokine in innate immunity. IL-17A has been previously reported to participate in the immune response against various infections at an early stage of the response. We demonstrate in this report that the TCR γδ T cells express IL-17A at an early stage of L. monocytogenes infection, and contribute to early protection. We recently reported that M. bovis BCG infection in the lung of mice induced IL-17A-producing TCR γδ T cells within 3 days of the infection (7). Intraperitoneal infection of Escherichia coli also induced IL-17A-producing TCR γδ T cells within 6 h after infection (28). In the K. pneumoniae lung infection of the wild-type mice, IL-17A was induced within 18 h after infection (34), and infection of the IL-17A receptor-deficient mice with K. pneumoniae resulted in decreased survival and increased bacterial burden from day 2 of the infection (35). Candida albicans-infected IL-17A receptor-deficient mice also showed decreased survival and increased fungal burden from day 3 after the infection (6). Furthermore, i.p. inoculation of Bacteroides fragilis induced IL-17A expression within 24 h (36). Although the IL-17A-producing cells in the K. pneumoniae-infected mice were CD4+ and CD8+ T cells (37), these responses must be considered as innate immune responses rather than Ag-specific Th17 responses, because their responses were observed before a conventional Ag-specific T cell response could be established during primary antigenic stimulation. It is possible that IL-17A production at an early stage of infection is induced by the stimulation of IL-23 produced by pathogen-activated macrophages/dendritic cells. Consistent with this hypothesis, IL-23 induced by TLR4-mediated signaling induced IL-17A production by TCR Vβ1+ γδ T cells in the E. coli-infected peritoneal cavity (28). LPS from Salmonella also induced IL-17A expression at an early stage after inoculation (38). Therefore, the IL-17A-producing T cells that respond at an early stage of infection, including TCR γδ T cells, could be activated quickly after invasion of the pathogen, through the IL-23 produced by pathogen-activated macrophages/dendritic cells (39).

IL-17A has been reported to be expressed by several T cell subpopulations, including TCR αβ+ CD4+, CD8+, or CD4+CD8+ T cells and TCR γδ+ T cells. We have identified TCR γδ T cells and CD4+CD8+ TCR αβ T cells as FasL-induced IL-17A-producing cells (13). Stark et al. also identified IL-17A-producing TCR γδ T cells and TCR αβlow CD4+CD8− T cells in
adhesion molecule-deficient CD18<sup>−/−</sup> and selectin<sup>−/−</sup> mice, and proposed to call them Tn cells for “neutrophil regulatory T cells” (14). In the L. monocytogenes-infected mice, we detected IL-17A on not only TCR γ<sup>+</sup> “Tn” cells but TCR αβ<sup>+</sup> CD4<sup>+</sup> T cells at an early stage of infection. However, our analysis of BM chimeric mice suggests that IL-17A-producing TCR γ<sup>+</sup> T cells are more important in anti-listerial protection than the IL-17A-producing CD4<sup>+</sup> T cells. In contrast, IL-17A-producing CD4<sup>+</sup> T cells are reported to be important in the early protection against K. pneumoniae infection. Difference in the localization of the bacteria (intracellular for L. monocytogenes vs extracellular for K. pneumoniae) or in expression of ligands recognized by the innate immune system could be the cause of this difference. Further investigation is required to clarify this issue.

Our data suggest that IL-17A produced at an early stage of L. monocytogenes infection enhances early protection. It was reported that Th17 cells coexpress IL-22, and that IL-17A and IL-22 cooperatively induced expression of anti-microbial peptides including β-defensins and S100A8/9 molecules (40). We also observed expression of IL-22, mBD-2, and mBD-3 in the L. monocytogenes-infected liver, which was markedly diminished in the IL-17A deficient mice (S.H. and G.M., unpublished observation). mBD-3 has been reported to display a broad spectrum of anti-microbial activity against Gram-negative and Gram-positive bacteria and fungi (41, 42), and the human homologue of mBD-3-humans (BD-2) efficiently killed L. monocytogenes and fungi (41, 42), and the human homologue of mBD-3 (human BD-2) efficiently killed L. monocytogenes (14). It was recently reported that IL-22 protects hepatocytes from tissue injury via IL-17-mediated regulation of innate and acquired immune response against pulmonare infection. Immunity 16: 279–284. 29. Hiromatsu, K., Y. Yoshikai, G. Matsuzaki, R. Imamura, and T. Suda. 2004. Involvement of IL-17 in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity 24: 179–189.


Roark, C. E., M. Kermal Aydintug, J. Lewis, X. Yin, K. Hahn, W. K. Born. 1994. Immune protection and control of inflammatory tissue necrosis by guest on December 27, 2017 http://www.jimmunol.org/ Downloaded from


