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Vγ4 γδ T Cell-Derived IL-17A Negatively Regulates NKT Cell Function in Con A-Induced Fulminant Hepatitis

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Con A-induced fulminant hepatitis is a well-known animal model for acute liver failure. However, the role of γδ T cells in this model is undefined. In this report, using TCR δ−/− mice, we demonstrated a protective role of γδ T cells in Con A-induced hepatitis model. TCR δ−/− mice showed significantly decreased levels of IL-17A and IL-17F in the Con A-treated liver tissue, and reconstitution of TCR δ−/− mice with wild-type (Wt) but not IL-17A−/−, γδ T cells significantly reduced hepatitis, strongly suggesting a critical role of IL-17A in mediating the protective effect of γδ T cells. Interestingly, only Vγ4, but not Vγ1, γδ T cells exerted such a protective effect. Furthermore, depletion of NKT cells in TCR δ−/− mice completely abolished hepatitis, and NKT cells from Con A-challenged liver tissues of TCR δ−/− mice expressed significantly higher amounts of proinflammatory cytokine IFN-γ than those from Wt mice, indicating that γδ T cells protected hepatitis through targeting NKT cells. Finally, abnormal capacity of IFN-γ production by NKT cells of TCR δ−/− mice could only be downregulated by transferring Wt but not IL-17A−/−, Vγ4 γδ T cells, confirming an essential role of Vγ4-derived IL-17A in regulating the function of NKT cells. In summary, our report thus demonstrated a novel function of Vγ4 γδ T cells in mediating a protective effect against Con A-induced fulminant hepatitis through negatively regulating function of NKT cells in an IL-17A–dependent manner, and transferring Vγ4 γδ T cells may provide a novel therapeutic approach for this devastating liver disease.

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Materials and Methods

**Mice**

All experiments were performed with age- (6–8 wk) and sex-matched mice (male or female mice were used). C57BL/6J (B6 wild-type [Wt]) mice were purchased from Academy of Military Medical Science (Beijing, China). B6.129P2-Tcrdtm1Mom (B6 TCR δ−/−) mice were purchased from The Jackson Laboratory (Bar Harbor, ME), and C57BL/6 IL-17A−/− mice (29) were kindly provided by Dr. Richard Flavell.

**Reagents**

Con A (type IV) was purchased from Sigma Chemical (St. Louis, MO). Recombinant mouse (rm) IL-2 was purchased from R&D Systems (Minneapolis, MN), and rmIL-17 was from eBioscience (San Diego, CA).
ROLE OF γδ T CELLS IN CON A-INDUCED HEPATITIS

FTTC-conjugated anti-mouse TCR β, aliphycocyanin-conjugated anti-mouse CD4 (clone GK1.5), anti-mouse NK1.1 (clone PK136), purified anti-mouse IL-17A (clone 17E), hamster anti-mouse TCR Vγ1 mAb 2.11, and hamster anti-mouse TCR Vγ4 mAb UC3 were from Sungeone (Tianjin, China); Alexa Fluor 647-conjugated anti-mouse γδ TCR (clone U7C7) and aliphycocyanin-conjugated anti-mouse IFN-γ (clone XMGl.1.2) were purchased from BioLegend (San Diego, CA). PE-conjugated anti-mouse IL-17A (clone TC11-18H10) was purchased from BD Biosciences (San Jose, CA).

T cell-mediated hepatitis model and survival study

To induce hepatitis, we injected Wt, TCR δ−/−, and IL-17A Δ/Δ mice i.v. with Con A (10 mg/kg body weight). For survival study, the dose of Con A was increased to 20 mg/kg body weight. Mice were closely monitored and euthanized before the end point was reached.

Assay for serum transaminase activity

Mice serum samples were obtained at different time points after Con A injection. Serum alanine aminotransferase (ALT) activities were measured using commercial available test kit (Rong Sheng Biotech, Shanghai, China) based on methods recommended by the International Federation of Clinical Chemistry.

Histology

Liver tissues were harvested after treatment of Con A, fixed in 10% formalin, and embedded in paraffin. Five-micrometer sections were affixed to slides, stained with H&E, and images were acquired on a Leica DM5000 microscope using 10× objective. The liver damage extent was quantified by the necrosis area as a percentage of total area using Image-Pro Plus (IPP) 6.0 software (Media Cybernetics, Silver Spring, MD). Three different fields were randomly chosen in every slide, and average necrosis percentage was calculated.

Liver mononuclear cell preparation

Mouse livers were removed and pressed through a 200-gauge stainless-steel mesh. The liver cell suspension was collected, suspended in PBS, and centrifuged at 50 × g for 5 min. Supernatants containing mononuclear cells (MNCs) were collected and resuspended in 40% Percoll (GE Healthcare). The cell suspension was gently overlaid onto 70% Percoll and centrifuged for 30 min at 1260 × g. MNCs were collected from the interphase and washed twice in PBS.

Adoptive transfer experiments

For expansion of γδ T cells, liver lymphocytes were isolated 2 h after Con A treatment and stimulated with PMA (50 ng/ml; Sigma, St. Louis, MO) and ionomycin (1 μg/ml; Sigma) in the presence of GolgiPlug (BD Biosciences, San Jose, CA) for 4.5 h. Cells were stained first with Abs against surface molecules and then fixed and permeabilized as described previously (15). For IFN-γ staining, liver lymphocytes from different groups of mice were isolated 12 h after Con A treatment, and stimulated with plate-coated anti-CD3 and soluble anti-CD28 and IL-2 for 6 h in the presence of GolgiPlug. Cells were then stained with FITC–anti-mouse TCR β and PE–anti-mouse NK1.1 followed by fixation, permeabilization, and intracellular staining as described previously (15).

Cell depletion

Mice were administered with an i.v. injection of 50 μl anti-asialo GM1 (ASGM1; Wako Pure Chemical Industries, Osaka, Japan) diluted in 200 μl pyrogen-free PBS to deplete NK cells. To deplete both NK and NKT cells, we injected mice i.v. with 200 μg anti-NK1.1 mAb (PK136; American Type Culture Collection) diluted in 200 μl pyrogen-free PBS. Depletion was confirmed by flow cytometry. For neutralizing effect of IL-17A in vivo, anti-mouse IL-17A Ab (100 μg/mouse) was injected i.v. 1 h before Con A treatment. Mice with cell depletion were then treated with Con A as described earlier.

Real-time PCR for gene transcription

Total RNA was extracted from liver MNCs by using TRizol Reagent (Invitrogen, Carlsbad, CA) and reverse-transcribed by Quantscript RT Kit (Tiangen, Beijing, China). mRNA expression was quantified by SYBR Premix HotMaster Taq (Tiangen, Beijing, China), and ribosomal protein large P0 (RPLP0) (30) was used as an internal normalizing gene. The primer sequences used were as follows: IL-17A forward: 5′-TGA AGG CAG CAG CGA TCA-3′; reverse: 5′-GGA AGT CCT TGG CCT CAG TGT-3′; IL-17F forward: 5′-CCG CAT TCA GCA AGA AAT CC-3′; reverse: 5′-CTC CAA CCT GAA GAA ATT AGA ACA G-3′; IFN-γ forward: 5′-ATG AAC GCT ACA CAC TGC ATC-3′; reverse: 5′-GCC TTC TTT TGG CAG TTC CTC CTC-3′; IL-4 forward: 5′-GAA AAC TCC ATG CTT GAA GAA-3′; reverse: 5′-TCT TCC TAT GTG GTG TGT TGG T-3′; TNF-α forward: 5′-CTA CTG AAC TGG GTG AT-3′; reverse: 5′-CAG GCT TGT CAC TGG AAT T-3′; T-bet forward: 5′-TCT TAT TGG GGA AGC TAA AG-3′; reverse: 5′-GGC TGG TAC TTG TGG AGA GA-3′; GATA-3 forward: 5′-AGA GGT CGA CCT ATT TTA C-3′; reverse: 5′-AGA GAT CGT ACA GCA GC-3′; FASL forward: 5′-CAG CTT CAG ATG CAA GTG AG-3′; reverse: 5′-CAA GGA CAG AAC TCT GAC GCT GAC-3′; RPLP0 forward: 5′-GAA ACT GCT GCC TCA CAG CCG-3′; reverse: 5′-CTG GCA CAG TGA CCT CAC ACG-3′.

Statistics

Data are presented as mean values ± SD. Statistical significance between two groups was evaluated by two-tailed unpaired Student t test using InStat version 3.06 software for Windows (GraphPad, San Diego, CA). For comparing values obtained in three or more groups, we used one-factor ANOVA, followed by S-N-K’s post hoc test. The difference of survival was compared and analyzed using the log-rank test, performed by GraphPad Prism 4 for Windows (GraphPad). Throughout the text, figures, and figure legends, the following terminology is used to denote statistical significance: *p < 0.05, **p < 0.01.

Results

γδ T cells play a protective role in Con A-induced fulminant hepatitis

To define the role of γδ T cells in Con A-induced hepatitis, sex- and age-matched B6 Wt and TCR δ−/− mice were treated with high dose of Con A (20 mg/kg body weight), and the survival rate of mice was observed and recorded. In comparison with B6 Wt mice, TCR δ−/− mice died much earlier and no mice survived after 20 h (Fig. 1A), indicating an essential role of γδ T cells in protection against Con A-induced liver damage. To further dissect the protective role of γδ T cells in this model, we administered different doses of Con A to B6 Wt or TCR δ−/− mice, and a lower dose of Con A (10 mg/kg body weight) was found to be the best to demonstrate significant differences between Wt and TCR δ−/− mice (data not shown). Thereafter, all the following experiments were performed using this lower dose. On treatment with Con A, hepatitis appeared significantly earlier and lasted much longer in TCR δ−/− mice in comparison with Wt mice, as indicated by the serum ALT level (Fig. 1B). This was further evidenced by liver histopathology at 18 h after treatment of Con A (Fig. 1C, 1D). Significantly bigger areas of necrosis in liver tissues were observed in TCR δ−/− mice.

To further define the role of γδ T cells in Con A-induced hepatitis, we adopted another approach. B6 TCR δ−/− mice were reconstituted with in vitro-activated Wt γδ T cells as described in Materials and Methods. Adoptive transfer of γδ T cells into TCR δ−/− mice significantly reduced hepatitis (Fig. 1E), further confirming the protective role of γδ T cells. These results collectively demonstrated a protective role of γδ T cells in Con A-induced hepatitis model.

IL-17A is a critical cytokine for protection against Con A-induced hepatitis

IL-17 cytokine family has multiple members and it has been shown to be involved in many inflammatory diseases. To define the role of IL-17A in Con A-induced hepatitis, we treated sex- and age-matched B6 Wt and IL-17A Δ/Δ mice with Con A and measured serum level of ALT at different time points. In the absence of IL-
γδ T cells play a protective role in Con A-induced hepatitis. A, Sex- and age-matched B6 Wt and TCR δ−/− mice (n = 15 for each group) were i.v. injected with Con A (20 mg/kg body weight), and the rates of death were observed every 30 min and recorded. One representative experiment of three repeated experiments is shown (*p < 0.05). B, Sex- and age-matched B6 Wt and TCR δ−/− mice (n = 12 for each group) were i.v. injected with Con A (10 mg/kg body weight), and at different time points after Con A treatment, serum samples were obtained for measuring the level of ALT. One representative experiment is shown (**p < 0.01). C, Liver tissues at 18 h after Con A treatment were fixed for H&E staining, and one representative tissue staining is shown. N, necrosis area. Scale bars, 200 μm. D, Percentage of necrosis is calculated and shown. n = 3 mice/group. **p < 0.01. E, B6 TCR δ−/− mice (n = 6) were reconstituted with in vitro-activated total γδ T cells (1 × 10^6 cells/mouse) as described in Materials and Methods, followed with Con A treatment. Serum samples were obtained at different time points after Con A treatment, and the levels of ALT were measured. One of three repeated experiments is shown (*p < 0.05, **p < 0.01).

γδ T cells are the primary source of IL-17A in this hepatitis model

Based on our findings as described earlier that both γδ T cells and IL-17A played important roles in protection against Con A-induced hepatitis, we hypothesized that γδ T cells might be the primary source of IL-17A. To test our hypothesis, we treated sex- and age-matched B6 Wt or TCR δ−/− mice with Con A (10 mg/kg), and at 2 h after treatment, liver lymphocytes were collected for RNA extraction and an array of cytokines were analyzed by real-time PCR. In comparison with Wt mice, liver lymphocytes of TCR δ−/− mice expressed significantly lower level of IL-17A and IL-17F (Fig. 3A). To further confirm the primary source of IL-17A, we isolated liver lymphocytes from Wt mice at 2 h after Con A treatment and activated them, followed by intracellular staining as described in Materials and Methods. Interestingly, >50% of liver γδ T cells expressed IL-17A, and few CD4^+ T and TCR αβ^+ T cells showed IL-17A^+ (Fig. 3B), suggesting the notion that γδ T cells, but not Th17 or other αβ^+ T cells (such as NKT cells), were the predominant source of IL-17A in Con A-induced hepatitis.

In contrast, no significant changes were observed between these two groups of mice for IFN-γ, IL-4, TNF-α, T-bet, GATA-3, and FASL (Fig. 3C), suggesting a critical role of γδ T cells in providing the primary source of IL-17A in Con A-induced hepatitis model.

γδ T cell-derived IL-17A is critical for protective immune response against Con A-induced hepatitis

To test the hypothesis that the protective effect of γδ T cells is through providing IL-17A, we administered B6 TCR δ−/− mice with rmIL-17 as described earlier and monitored serum ALT level and liver histopathology. Indeed, injection of IL-17A significantly reversed Con A-induced hepatitis in TCR δ−/− mice (Fig. 4A), supporting a critical role of IL-17A in γδ T cell-mediated protective responses on Con A challenge.

To further test the critical role of IL-17A derived from γδ T cells, we adopted a similar approach as described in Fig. 1E. Splenic γδ T cells were isolated either from Wt or IL-17A−/− mice, activated in vitro, and then transferred into TCR δ−/− mice (1 × 10^6 cells/mouse). At 24 h after transfer, these recipients were then treated with low dose of Con A as described earlier. Serum samples were collected at different time points for monitoring the ALT level, and liver tissues were collected at 18 h of Con A treatment for histopathology staining. Interestingly, TCR δ−/− mice reconstituted with Wt γδ T cells showed significant protection. In sharp contrast, reconstitution of TCR δ−/− mice with IL-17A−/− γδ T cells did not offer any protection (Fig. 4B); the severity of liver damage in these reconstituted mice was almost the same as untreated TCR δ−/− mice, strongly supporting a critical role of γδ T cell-derived IL-17A in mediating the protective effect of γδ T cells in Con A-induced hepatitis model.

Only Vγ4, but not Vγ1, γδ T cells offer protection against Con A-induced hepatitis

Peripheral γδ T cells are mainly composed of two subsets: Vγ1 and Vγ4. These two subsets of γδ T cells have divergent functions in many different disease models (9). Based on the results
Obtained earlier that IL-17A was a critical mediator for the protective effect of γδ T cells, and studies by others (27, 28, 31) and our own unpublished work that Vγ4 γδ T cells are the main producer of IL-17, we hypothesized that Vγ4 γδ T cells might be the critical one to play the protective effect. Vγ1 and Vγ4 γδ T cells were sorted from B6 Wt mice, cultured as described earlier, and used for IL-17 family cytokine analysis by real-time PCR to test this hypothesis. Only Vγ4, but not Vγ1, γδ T cells expressed high levels of IL-17A and IL-17F (Fig. 5A). Consistently, we found the main source of IL-17A in Con A-treated liver was the Vγ4+ subset (Fig. 5B). To directly compare the protective effect of Vγ1 and Vγ4 γδ T cells in vivo, we reconstituted TCR δ−/− mice with either Vγ1 or Vγ4 γδ T cells isolated from B6 Wt mice followed by Con A administration as described earlier. Only Vγ4 γδ T cells offered significant protection against Con A-induced hepatitis. In contrast, reconstitution with Vγ1 γδ T cells did not show any protective effect (Fig. 5C). To test whether the protective effect of Vγ4 γδ T cells was dose dependent, we transferred different amounts of Vγ4 γδ T cells to TCR δ−/− mice followed by Con A treatment. We showed that at least 1 million Vγ4 γδ T cells were required for the protective effect (Fig. 5D). The differential effects of these two subsets of γδ T cells were further confirmed by liver histopathology sections (Fig. 5E, 5F). To further prove the critical role of IL-17A in mediating the protective effect of Vγ4 γδ T cells, we reconstituted TCR δ−/− mice with either Wt or IL-17A−/− Vγ4 γδ T cells as described earlier, and monitored serum ALT level and liver histopathology. Consistently, only Wt, but not IL-17A−/−, Vγ4 γδ T cells showed significant protection. Reconstitution of Vγ4 γδ T cells significantly reduced serum ALT level (Fig. 5G). Therefore, our results determined a critical role of Vγ4 γδ T cells in protection against Con A-induced hepatitis through IL-17A production.

Vγ4 γδ T cell-derived IL-17A regulates IFN-γ secretion by NKT cells

NKT cells play an essential role in the pathogenesis of Con A-induced hepatitis through producing inflammatory cytokines, especially IFN-γ (2). We hypothesized that the protective effect of γδ T cells might be through targeting NKT cells. To test our hypothesis, we treated TCR δ−/− mice with an anti-NK1.1 (clone PK136) or ASGM1 Ab to deplete NK1.1+ (include NK and NKT cells) or NK cells, respectively (32). These mice were then treated with Con A and the serum ALT level was measured at 18 h

FIGURE 2. IL-17A is required for protection against Con A-induced liver damage. A, Sex- and age-matched B6 Wt and IL-17A−/− mice (n = 15 for each group) were i.v. injected with Con A (10 mg/kg body weight), and at different time points after Con A treatment, serum samples were obtained for measuring the level of ALT. One of three repeated experiments is shown (**p < 0.01). B, Liver tissues of B6 Wt or IL-17A−/− mice at 18 h after Con A treatment were fixed and stained with H&E. One representative staining for each strain of mice is shown. Scale bars, 200 μm. C, Percentage of necrosis is shown. n = 3 mice/group. **p < 0.01. D, B6 IL-17A−/− mice (n = 5) were treated with i.p. injection of rmIL-17 (1 μg/mouse) or PBS vehicle 30 min before and 1 h after Con A treatment, and serum ALT levels were measured from serum samples 18 h after Con A treatment. One representative experiment is shown (**p < 0.01). E, Liver tissues of B6 IL-17A−/− mice that received PBS or rmIL-17 at 18 h after Con A administration were fixed and stained with H&E. One representative staining is shown. Scale bars, 200 μm. F, Percentage of necrosis is shown. n = 3 mice/group. **p < 0.01. G, For IL-17A blocking, B6 Wt mice (n = 5) were treated with i.v. injection of either anti-mouse IL-17A Ab, 100 μg/mouse, or control Ab 1 h before Con A treatment. Serum samples were collected at different time points after Con A administration for analysis of the ALT level. One representative experiment is shown (*p < 0.05, **p < 0.01). N, necrosis area.
after treatment. Depletion of NK1.1\(^+\), but not NK, cells in TCR \(\delta^{-/-}\) mice significantly decreased Con A-induced liver damage, indicating an essential role of NKT cells in the Con A-induced hepatitis model, even in the absence of \(\gamma\delta\) T cells (Fig. 6A). Based on the fact that NKT cell-derived IFN-\(\gamma\) is critical for the pathogenesis of Con A-induced liver damage, we hypothesized that the protective effect of \(\gamma\delta\) T cell-derived IL-17A might negatively regulate IFN-\(\gamma\) production by NKT cells. To test our hypothesis, we treated sex- and age-matched B6 Wt and TCR \(\delta^{-/-}\) mice with Con A as described earlier. NKT and NK cells were then analyzed by real-time PCR. In contrast, NKT cells from mice reconstituted with IL-17A \(\delta^{-/-}\) mice showed a similar level of IFN-\(\gamma\) as those in TCR \(\delta^{-/-}\) mice (Fig. 6C). To further confirm the regulatory effect of V\(\gamma4\) \(\gamma\delta\) T cells on the ability of NKT cells to produce IFN-\(\gamma\), we sorted NKT cells at 12 h after Con A treatment and activated them for intracellular cytokine staining. In our preliminary studies, at this time point, the biggest differences were observed between Wt and TCR \(\delta^{-/-}\) mice in their IFN-\(\gamma\) production (data not shown). In the absence of \(\gamma\delta\) T cells, NKT cells produce a significantly higher level of IFN-\(\gamma\), which was inhibited by transferring Wt, but not IL-17A \(\delta^{-/-}\), V\(\gamma4\) \(\gamma\delta\) T cells (Fig. 6D), suggesting a critical role of IL-17A in mediating the protective effect of V\(\gamma4\) \(\gamma\delta\) T cells through targeting IFN-\(\gamma\) production by NKT cells.

**Discussion**

Con A-induced fulminant hepatitis is a well-known animal model for studying the pathophysiological mechanisms of acute liver failure, a devastating liver disease with significant mortality worldwide and without effective therapeutic approaches. Activated T cells, especially NKT cells, have been defined to play a critical role in promoting liver damage through producing cytokines, such as IFN-\(\gamma\). However, the controlling mechanisms for NKT cells, especially for their cytokine production, have been elusive. In this report, we demonstrated for the first time, to our knowledge, that \(\gamma\delta\) T cells, especially V\(\gamma4\) \(\gamma\delta\) T cells, played a critical role.
Each group) followed with the treatment of Con A as described previously. Reconstituted with different numbers of cultured Vγ1, Vγ4, and lymphocytes were isolated from pooled liver tissues of Con A-treated B6 TCRδ-/- mice, followed by Con A treatment as described earlier. The levels of ALT were measured. One representative experiment is shown (**p < 0.01). E, Liver tissues of Con A-treated B6 TCRδ-/- mice at 18 h after Vγ1 or Vγ4 γδ T cell (1 × 10⁶ cells/mouse) transfer as described previously were fixed and stained with H&E. One representative staining for each group is shown. N, necrosis area. Scale bars, 200 µm. F, Percentage of necrosis is also shown (n = 3 mice/group, **p < 0.01). G, B6 TCRδ-/- mice were reconstituted with cultured Vγ4 γδ T cells from either Wt or IL-17A-/- mice, or with PBS, and then followed by Con A administration. The levels of ALT from serum samples at 18 h after treatment were measured. One representative experiment is shown (**p < 0.01).

At 18 h after Con A treatment, serum samples were collected for determination of ALT level. One representative experiment is shown (***p < 0.01). E, Liver tissues of Con A-treated B6 TCRδ-/- mice at 18 h after Vγ1 or Vγ4 γδ T cell (1 × 10⁶ cells/mouse) transfer as described previously were fixed and stained with H&E. One representative staining for each group of mice is shown. N, necrosis area. Scale bars, 200 µm. F, Percentage of necrosis is also shown (n = 3 mice/group, **p < 0.01). G, B6 TCRδ-/- mice were reconstituted with cultured Vγ4 γδ T cells from either Wt or IL-17A-/- mice, or with PBS, and then followed by Con A administration. The levels of ALT from serum samples at 18 h after treatment were measured. One representative experiment is shown (**p < 0.01).
our knowledge, the interaction between these two cell types from this study, especially in the liver, which is a special organ with enriched immune cells, may have significant impact on understanding the immune pathology of other liver diseases. Further studies are granted to study the role of γδ T cells in other cell type-mediated liver damage and viral hepatitis.

What could be the critical mediator between γδ T and NKT cells? We hypothesized that it would be a cytokine. Indeed, our study firmly established that it was IL-17A deriving from Vγ4 γδ T cells that played a critical role in mediating the protective immune responses against Con A-induced liver damage. Our conclusion was supported by results obtained from multiple approaches. First, rIL-17 reconstituted the resistance against liver damage on Con A treatment in TCR δ−/− mice (Fig. 4A). Second, in comparison with those of Wt mice, liver tissues of TCR δ−/− mice showed a significantly decreased level of IL-17A (Fig. 3A), and Vγ4 γδ T cells expressed a high level of IL-17A (Fig. 5A, 5B).

Third, only transferring Wt, but not IL-17A−/−, Vγ4 γδ T cells into TCR δ−/− mice offered protection against Con A-induced liver damage (Fig. 5G). Finally, consistent with findings described earlier, the regulation of the function of NKT cells by γδ T cells was IL-17A dependent (Fig. 6). Therefore, our results firmly established that Vγ4 γδ T cells provided the primary source of IL-17A and played a protective role in Con A-induced liver damage through targeting NKT cells. These results were also consistent with previous findings that Vγ4 γδ T cells were the main source of IL-17A on pathogen infection (27, 28). Interestingly, there were few, if any, CD4+ IL-17A+ cells, indicating a minor role of Th17 cells in this model. Given the critical role of NKT cells in Con A-induced hepatitis, and based on the fact that IL-17A–producing NKT cells express TCR αβ, but not αK1.1 (39), we also analyzed IL-17A production by αβ T cells. Similarly, a very low percentage of TCR αβ+ IL-17A+ T cells was detected in the Con A-treated liver (Fig. 3B), indicating that the critical role of NKT cells in this model was not through providing IL-17A. The protective role of IL-17A demonstrated in this article was also supported by a previous report that IL-17A protected against α-galactosylceramide-induced hepatitis in mice (40). However, in several other previous reports, there was no significant role for IL-17A in the Con A-induced hepatitis model using IL-17A−/− mice (41). The reason for such a discrepancy is unclear at the present time. We speculated that the main reason for such a discrepancy was due to the environment of the animal facility, which, in turn, resulted in the different intestine microbes, and consequently led to the differential phenotypes. It has been reported that intestine microbes can influence IL-17A production by CD4+ T cells and experimental autoimmune encephalomyelitis (EAE) phenotype (42, 43). Further studies are needed to determine whether treatment of these mice with antibiotic will alter the phenotype.

Differing from those IFN-γ–producing Vγ4 γδ T cells as described in our previous studies (15–17), we speculated that these IL-17A–producing Vγ4 γδ T cells were “Ag-naive” γδ T cells. Further studies are needed to further define the functional differences between these two subtypes of Vγ4 γδ T cells and whether the interactions between these subtypes do exist.

Which function of NKT cells was regulated by γδ T cells? Because it has been well established that IFN-γ is a critical cytokine in the pathogenesis of Con A-induced hepatitis (3, 5), and it was reported that IL-17A can downregulate T-bet and IFN-γ in CD4+ T cells (44), we hypothesized that Vγ4-derived IL-17A might downregulate IFN-γ production by NKT cells. Indeed, in the absence of γδ T cells, NKT cells expressed a significantly higher level of IFN-γ (Fig. 6B, 6D), and transferring Wt, but not IL-17A−/−, Vγ4 γδ T cells into TCR δ−/− mice completely decreased IFN-γ expression from NKT cells (Fig. 6C, 6D). We speculated that Vγ4-derived IL-17A might act on NKT cells to alter IFN-γ–producing programs. Further studies are needed to give us a better clue about the detail of molecular mechanisms.
In summary, our study has demonstrated a critical role of γδ T cells, especially Vγ4 γδ T cells, in protection against Con A-induced hepatitis. This protective effect was mediated by Vγ4 γδ T cell-derived IL-17A, which, in turn, targeted NK cells and negatively regulated IFN-γ production by NK T cells. Thus, for the first time, to our knowledge, our results define a critical role of γδ T cells in Con A-induced hepatitis and show that transferring γδ T cells may provide a novel therapeutic approach for this devastating liver disease.

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Disclosures

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