Two Types of Interleukin 17A–Producing γδ T Cells in Protection Against Pulmonary Infection With Klebsiella pneumoniae

Tesshin Murakami,1 Shinya Hatano,1 Hisakata Yamada,1 Yoichiro Iwakura,2 and Yasunobu Yoshikai1

1Division of Host Defense, Medical Institute of Bioregulation, Kyushu University, Fukuoka, and 2Center for Animal Disease Models, Research Institute for Biomedical Sciences, Tokyo University of Science, Noda, Japan

Background. Klebsiella pneumoniae frequently causes life-threatening infection in children. Interleukin 17A (IL-17A) is known to be involved in protection against K. pneumoniae infection through activation of neutrophils.

Methods and Results. We found that IL-17A–producing γδ T cells existed more frequently in younger mice on examination of IL-17A–producing lymphocytes in the lung of naive mice at various ages. We hence compared the protective role of IL-17A–producing γδ T cells against pulmonary K. pneumoniae infection in young (3 weeks old) and adult (8–12 weeks old) mice. IL-17A–deficient mice were susceptible to K. pneumonia regardless of age. C57-, Vγ4/6-, or Vδ1-deficient mice were susceptible to K. pneumonia at young age, while interleukin 23p19 (IL-23p19)–deficient mice were susceptible at adult age. IL-17A–producing Vγ1+/Vγ4− γδ T cells expressing canonical Vγ6/Vδ1 genes were dominant over IL-17A–producing Vγ4+ γδ T cells in the lungs of young mice after infection. The IL-17A–producing Vγ1+/Vγ4− γδ T cells expressed an activation marker, CD69, and proliferated in an IL-23–independent manner, while the IL-17A–producing Vγ4+ γδ T cells expressing IL-23 receptor but no CD69 proliferated in IL-23–dependent manner.

Conclusions. These results suggest that 2 types of IL-17A–producing γδ T cells are activated for host defense against K. pneumoniae infection by IL-23–dependent or independent mechanism.

Keywords. IL-17A; CD69; IL-23; Vγ4+ γδ T cells; Vγ1+/Vγ4− γδ T cells; Klebsiella pneumoniae.

Klebsiella pneumoniae is a leading causative agent in community-acquired and nosocomial bacterial pneumonia and frequently causes life-threatening infection in children [1–4]. Host defense against infection with extracellular bacteria such as K. pneumoniae is dependent mainly on neutrophils. The neutrophil-dependent defense mechanism against infection is divided into several phases. Innate immunity consisting of alveolar macrophages resident in the lung are the first to encounter K. pneumoniae after pulmonary infection and produce a variety of factors that are chemotactic for neutrophils, which migrate from the circulation to the infected sites [3]. With regard to adaptive immunity, K. pneumoniae antigen–specific antibody is produced by B cells with help from T-helper type 2 (Th2) cells, and the antibody promotes the accumulation of neutrophils in cooperation with complement to enhance Fc receptor (FcR)–mediated phagocytosis and killing of the bacteria [5]. In addition, IL-17A, a T cell–derived proinflammatory cytokine, was shown to be involved in the mobilization and bactericidal activity of neutrophils in a murine model of K. pneumoniae infection [6]. IL-17R–deficient mice showed markedly decreased neutrophil recruitment to infected tissues and impaired host defense against K. pneumoniae, demonstrating the protective role of IL-17A in K. pneumoniae infection [7]. Although various cell types produce IL-17A, the T-cell receptor (TCR) γδ T-cell subset was reported to be the main source of IL-17A in the lung after K. pneumoniae infection [8, 9]. However, the protective role of IL-17A–producing γδ T cells has not been described for K. pneumoniae infection.

γδ T cells are the first lymphocytes to develop in the thymus. γδ T cells expressing Vγ5, Vγ6, Vγ4 and Vγ1, and Vγ7 TCR (based on the TCR nomenclature by Dr S. Tonegawa [10]) sequentially develop in this order in the fetal thymus around embryonic day 12 and migrate into mucosal epithelia such as skin, intestine, uterus, and lung as tissue-associated cells [10, 11]. Unlike αβ T cells that are functionally differentiated to IL-17A–producing effector cells in the periphery, γδ T cells with effector functions to produce IL-17A develop in the early fetal thymus [12]. It was previously reported that the number of IL-17A–producing γδ T cells in the thymus peaked at the perinatal period and gradually decreased with age [13]. In the periphery, Vγ6+ and Vγ4+ γδ T cells are the main sources of IL-17A [11, 14]. Tissue-resident IL-17A–producing cells are a unique subset that respond to IL-23 immediately after microbial and fungal infection.

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This rapid response suggests that γδ T cells produce IL-17A by a bystander mechanism independent of TCR stimulation during infection.

The current study showed that IL-17A–producing γδ T cells have a critical role in protection against pulmonary infection with *K. pneumoniae* in young mice (3 weeks old) that is IL-23 independent. IL-17A–producing Vγ1^−^ Vγ4^−^ γδ T-cell subsets expressed CD69, CD25, CD137, and Nur77 during infection, suggesting that Vγ1^−^ Vγ4^−^ γδ T cells are activated to produce IL-17A via TCR stimulation, exerting protection against *K. pneumoniae* infection. On the other hand, protection against *K. pneumoniae* in adult mice (8–12 weeks old) was dependent on IL-23. IL-17A–producing Vγ4^+^ γδ T cells expressed IL-23R but no CD69 and expanded in response to IL-23. These results suggest that 2 types of IL-17A–producing γδ T cells are activated during *K. pneumoniae* infection by IL-23–dependent or independent mechanism.

**MATERIAL AND METHODS**

**Mice**

Wild-type (WT) C57BL/6 female mice were purchased from Japan KBT (Tosu, Japan). *Il17a^−/−^, Cβ^−/−^, Cy^−/−^, V81^−/−^, Il23p19^−/−^, and Ifnar^−/−^ mice on a C57BL/6 background were purchased from the Jackson Laboratory (Bar Harbor, ME). Vγ4^+/−^ mice were kindly provided by Dr G. Matsuzaki. IL-17A reporter (*Il17agfp/+*) mice, in which internal ribosome entry site (IRES)–enhanced green fluorescent protein was inserted into the *Il17a* locus without affecting IL-17A production, were described previously [19]. All mice were maintained under specific-pathogen-free conditions and were offered food and water ad libitum. This study was approved by the Committee of Ethics on Animal Experiments in the Faculty of Medicine at Kyushu University (Fukuoka, Japan). Experiments were performed in accordance with the Guidelines for Proper Conduct of Animal Experiments.

**Microorganisms**

*K. pneumoniae* ATCC strain 43816, serotype 2 (ATCC, Rockville, Maryland) was grown in Difco Nutrient Broth (Difco, Detroit, Michigan) for 18 hours at 37°C and 1.12x g. Bacteria were pelleted by centrifugation at 2380×g for 5 minutes and stored at −80°C in 50% glycerol as single-use aliquots.

**Mouse Infection Model**

In survival rate experiments, mice were anesthetized with pentobarbital and intranasally inoculated with *K. pneumoniae* at 1×10^5^, 1×10^6^, or 1×10^7^ colony-forming units (CFU)/mouse, and survival was monitored every 24 hours for up to 20 days. In kinetics experiments, mice were anesthetized with pentobarbital, intranasally inoculated with *K. pneumoniae* at 1×10^8^ CFU/mouse, and euthanized 0, 1, 3, 5, or 7 days after infection. The number of bacteria was counted after overnight culture by plating on Difco Nutrient agar (Difco).

**Lung Histologic Analysis**

Lungs were removed and fixed with 10% neutral buffered formalin and then embedded in paraffin. After the tissues were cut into round slices, the tissue sections were stained with hematoxylin and eosin and examined by microscopy.

**Fluorescence-Activated Cell-Sorting (FACS) Analysis**

Lung cells were prepared as described previously [20]. Cells were stimulated with or without 2, 20, or 200 ng/mL recombinant IL-23 (rIL-23) in a CO2 incubator at 37°C. Total cells were stained with various combinations of monoclonal antibodies: anti-TCRVγ1-αllophycocyanin (APC; clone 2.11), anti-TCRVγ4-phycocerythrin (PE; clone UC3-10A6), anti-TCRVδ-PE/cyanine7 (PE/Cy7; clone GL3), anti-TCRß-PE (clone H57-597), anti-CD11b-violet450 (V450; clone M1/70), anti-CD19-brilliant violet421 (BV421; clone 1D3), and anti-CD25-peridinin chlorophyll protein (PerCP) Cy5.5 (clone PC61), anti-CD45-APC/Cy7 (clone 30-F11), anti-CD69-APC/Cy7 (clone H1.2F3), anti-CD137-PEv (clone 17B5), anti-F4/80-PE/Cy7 (clone BM8), anti-Ly6G-PE (clone 1A8), anti-major histocompatibility complex class II-V500 (clone M5/114,15.2), anti-IL-17A-V450 (clone TC11-18H10), anti-IL-23R-PE (clone 3C9), isotype control mouse immunoglobulin G1-κ PE (clone MOPC-21), and isotype control Armenian hamster IgG-APC/Cy7 (clone HKT888). Dead cells were excluded using propidium iodide (Abcam, Cambridge, United Kingdom) or Zombie Aqua Fixable Viability Kit (BioLegend, San Diego, California). The stained cells were analyzed by a FACS Verse flow cytometer (BD Biosciences). The data were analyzed using CellQuest software (BD Biosciences).

**DNA Purification and Reverse Transcription–Polymerase Chain Reaction (RT-PCR) Analysis**

Green fluorescent protein (GFP)–positive Vγ1^-^ Vγ4^-^ γδ T cells or GFP-positive Vγ4^−^ γδ T cells in the lungs of *Il17agfp/+* mice on day 7 after *K. pneumoniae* infection were sorted by FACS Aria II (BD Biosciences). Total RNA from sorted γδ T cells was purified, and complementary DNA or double-stranded DNA (dsDNA) was synthesized as described previously [21]. For the V repertoire analysis and *Nr4a1* expression, combinations of the primers were shown in **Supplementary Table 1**. PCR analysis was performed with the ABI7500 real-time PCR detection system (Applied Biosystems) and a SYBR Premix DimerEraser qPCR kit (Takara Bio, Kusatsu, Japan). Primer sets yielded a single product of the correct size. Relative expression levels were normalized to β-actin. In some experiments, purified dsDNAs were sequenced using a Taq Dye primer Cycle sequencing kit (Perkin Elmer, Waltham, Connecticut) and an ABI 373A DNA sequencer (Applied Biosystems).

**Statistics**

Statistical analyses of survival curves were performed by the log-rank test, using Prism software (GraphPad Software, La Jolla, California). Statistical analyses of bacterial counts were performed...
RESULTS

Age-Related Change of IL-17A–Producing Lymphocytes in the Lung of Naive Mice

Cells were harvested from the thymus and lungs of WT mice aged 1, 3, 6, or 12 weeks. Absolute and relative numbers of total lymphoid cells, αβ T cells, and γδ T cells are shown in Figure 1. In the lungs, IL-17A–producing γδ T cells were >84% and >55% of the total number of IL-17A–producing lymphocytes in 1-week-old neonatal mice and 3-week-old mice, respectively. However, the number of IL-17A–producing γδ T cells decreased to <18% in 12-week-old adult mice, while the number of IL-17A–producing αβ T cells increased from <2% in 1-week-old mice to 60% in 12-week-old mice. The numbers of IL-17A–producing innate lymphoid cells (ILCs; type III) were almost constant, irrespective of age (Figure 1C and 1D). Thus, consistent with the thymic ontogeny of IL-17A–producing γδ T cells (Figure 1A and 1B), the number of lung IL-17A–producing γδ T cells reached a peak at the perinatal period and rapidly decreased thereafter.

Susceptibility of IL-17A−, Cγ−, Cβ−, Vγ4/6−, or IL-23p19−Deficient Mice to K. pneumoniae Pulmonary Infection

The ontogenetic appearance of IL-17A–producing γδ T cells raises the possibility that the relative contribution of IL-17A–producing γδ T cells to protection against pulmonary infection with K. pneumoniae may differ by age. To explore this, we compared the survival rate at different ages after infection. To determine the median lethal dose (LD50) among WT mice at different ages, 3- and 8-week-old WT mice were intranasally inoculated with K. pneumoniae at various doses. The approximate LD50 was 1.8 × 10³ CFU, or 3.04 × 10³ CFU for 3- and 8-week-old mice, respectively (Supplementary Figure 1). Because the LD50 was not so different for 3- and 8-week-old mice, we compared their survival rates by using WT, Il17a−/−, Cβ−/−, Cγ−/−, Vγ4/6−/−, Vδ1−/−, and Il23p19−/− mice after pulmonary infection with K. pneumoniae at 1 × 10³ CFU/mouse. Consistent with previous reports, both young and adult Il17a−/− mice showed high susceptibility to K. pneumoniae (Figure 2).

Figure 1. Age-related change of interleukin 17A (IL-17A)–producing cells. Cells were harvested from the thymus and lungs of wild-type (WT) mice at 1, 3, 6, 12 weeks of age and cultured with PMA/ionomycin for 5 hours. Brefeldin was added for the last 4 hours of culture. After intracellular staining, IL-17A–producing (IL-17A+) cells were analyzed after gating on CD19<sup>−</sup> cells. CD19<sup>−</sup>T-cell receptor β (TCRβ)<sup>−</sup>TCRδ<sup>−</sup> cells were considered innate lymphoid cells (ILCs). A and C, Absolute numbers of IL-17A–producing αβ T cells (dashed line), IL-17A–producing γδ T cells (solid line), and IL-17A–producing ILCs (dotted line) in thymus and lung. B and D, Percentages of αβ T cells, γδ T cells, and ILCs among IL-17A–producing cells in thymus and lung. Error bars represent mean ± SD (n = 3 mice).
However, young Cγ⁻/⁻, Vγδ/-/γδ, and Vδ1⁻/⁻ mice were more susceptible to *K. pneumoniae*, compared with adult mice (Figure 2). The Vγ6 chain is paired mainly with Vδ1 chains [10]. Thus, these results suggested that IL-17A-producing Vγδ/Vδ1 γδ T cells played an important role in protection against pulmonary infection with *K. pneumoniae* in young mice. However, adult Il23p19⁻/⁻ mice were more susceptible to *K. pneumoniae* (P < .001) than young mice (Figure 2). Furthermore, both young and adult Cγδ/- mice were resistant to *K. pneumoniae* infection (Figure 2). These results suggest that IL-23–dependent IL-17A production by either γδ T cells, αβ T cells, or ILCs may contribute to protection against *K. pneumoniae* infection in adulthood.

Bacterial growth and histopathologic findings were examined in young mice after pulmonary infection with *K. pneumoniae*. The number of bacteria was significantly higher in *Il17a⁻/⁻* (P = .0003) or Cγ⁻/⁻ (P = .016) mice than WT mice on day 3 after infection (Figure 3A). We also examined the morphologic and histologic changes on day 3 after infection. Figure 3B shows representative morphologic analysis after infection. The lungs of *Il17a⁻/⁻* or Cγ⁻/⁻ mice showed severe injury with massive and diffuse cell accumulation in comparison with WT mice after infection. The neutrophil (CD11b⁺CD45⁻F4/80⁻Ly6-G<sup>high</sup>) numbers were significantly lower in the infected lungs of *Il17a⁻/⁻* or Cγ⁻/⁻ mice than WT mice (P = .0079 and P = .0286, respectively; Figure 3C). FACS profiles are shown in Supplementary Figure 2. These results suggest that lung injury induced by *K. pneumoniae* was more severe in the absence of IL-17A–producing γδ T cells.

**Vγ T Repertoire of IL-17A–Producing γδ T Cells in the Lung After *K. pneumoniae* Infection**

To further characterize IL-17A–producing γδ T cells, we infected *Il17gfp⁺/⁺* mice with *K. pneumoniae*. Young (3 weeks old) *Il17gfp⁺/+* mice were intranasally inoculated with *K. pneumoniae* at 1 x 10⁴ CFU/mouse and then euthanized 0, 1, 3, 5, or 7 days after infection. The numbers of bacteria and neutrophils were increased on day 1 and then decreased after infection, and the number of GFP-positive γδ T cells increased gradually on day 7 after infection (Supplementary Figure 3).

The relative number of GFP-positive Vγ4⁺Vγδ⁻ γδ T cells was 30% in naive mice and increased up to 54% at day 7 after infection, while GFP-positive Vγ4⁺ γδ T cells were decreased inversely after infection (Figure 4A). Less than 2% of GFP-positive Vγ1⁺ γδ T cells were present in naive mice, and this level remained low after infection. GFP-positive Vγ1⁺Vγδ⁺ γδ subsets in the lungs of *Il17gfp⁺/+* young mice on day 7 after infection...
were sorted using a FACS Aria II system, and the TCR Vγ and Vδ gene repertoires were analyzed by RT-PCR and nucleotide sequencing. The GFP-positive Vγ1−Vγ4− γδ subset expressed mostly Vγ6- and Vδ1-specific transcripts, and all 36 Vγ6-γδ1 transcripts and 18 of 20 Vδ1-Jδ2 transcripts of the γδ T cells showed no junctional diversity (Figure 4C). This resulted in in-frame invariant canonical sequences, which are preferentially expressed in fetal thymocytes at the late stage (approximately day 17) of gestation and in intraepithelial lymphocytes of reproductive organs [10]. Taken together, these results suggested that K. pneumoniae–induced GFP-positive Vγ1−Vγ4− γδ T cells in the lung expressed a canonical Vγ6/Vδ1 TCR, which was the same as that of fetal γδ T cells [10].

Characteristics of IL-17A–Producing γδ T Cells in the Lung After K. pneumoniae Infection

The surface markers of IL-17A–producing γδ T cells were examined after K. pneumoniae infection. Notably, CD69 expression was detected only on GFP-positive Vγ1−Vγ4− γδ T cells, whereas GFP-positive Vγ4− γδ T cells did not express CD69 (Figure 5A). CD25 and CD137 were related to downstream TCR signaling [22–25] and were also expressed in GFP-positive Vγ1−Vγ4− γδ T cells after infection (Figure 5B). Furthermore, Nr4a1, which encodes nuclear hormone receptor Nur77, upregulated by TCR stimulation but not inflammatory stimuli [26, 27], was abundant in GFP-positive Vγ1−Vγ4− γδ T cells, compared with GFP-positive Vγ4+ γδ T cells, after infection (Supplementary Figure 4). In addition to TCR stimulation, several cytokines, including type I interferon, also induce CD69 and CD25 on T cells in vitro [28–31]. However, CD69 was detected on GFP-positive Vγ1−Vγ4− γδ T cells in Il17gfp/× Ifnra−/− mice after K. pneumoniae infection (Figure 5C).

IL-23R expression was detected on Vγ4+ γδ T cells but not on Vγ1−Vγ4− γδ T cells in the lungs of naive mice, although its expression was downregulated after K. pneumoniae infection (Figure 6A). To confirm the functional expression of IL-23R on γδ T cells, the lung cells of naive young mice were stimulated with mouse rIL-23 in vitro. As shown in Figure 6B, numbers of IL-17A–producing γδ T cells were increased after stimulation. Numbers of Vγ4+ γδ T cells were increased, but Vγ1−Vγ4− γδ T cells showed a decreased frequency among IL-17A–producing γδ T cells after stimulation. Thus, Vγ4+ γδ T cells but not Vγ1−Vγ4− γδ T cells depend on IL-23 for IL-17A production.
The Vγ repertoire of IL-17A–producing γδ T cells was further examined in the absence of IL-23, using Il17gfp/+ × Il23p19−/− mice. As shown in Figure 7, GFP-positive γδ T cells were mainly Vγ1−Vγ4− γδ T cells, and the expression of CD69 was detected in Il17gfp/+ × Il23p19−/− mice after infection, confirming that the production of IL-17A by Vγ1−Vγ4− γδ T cells after K. pneumoniae infection is IL-23 independent.

DISCUSSION

The current study showed that IL-17A–producing γδ T cells have a critical role in protection against K. pneumoniae infection in young mice. It was previously reported that the number of IL-17A–producing γδ T cells in the thymus peaks during the perinatal period and gradually decreases with age [13]. Thus, it is possible that IL-17A–producing γδ T cells are more important in protection at a younger age, which may recapitulate the ontogeny of development in the fetal thymus. After K. pneumoniae infection of young mice, IL-17A–producing cells were mainly Vγ1−Vγ4− γδ T cells expressing canonical Vγ6/Vδ1 genes and had upregulated expression of CD69, CD25, CD137, and Nur77. However, Vγ4+ γδ T cells did not express these activation markers after infection but expressed functional IL-23R before infection. Thus, there are different activation pathways of IL-17A–producing γδ T cells dependent on the TCR Vγ repertoire in the lung after infection.

CD69 and CD25 are the earliest cell surface markers expressed by T cells following TCR/CD3 ligation [22–25], suggesting that Vγ1−Vγ4− γδ T cells may recognize a ligand in the infected lungs and be activated to produce IL-17A. The Vγ6/Vδ1 γδ T cells expressed invariant TCRs, including the nucleotides in the TCR gene junction. The canonical sequence was very simple, with no apparent N-region contribution. Such characteristics led to the hypothesis that this subset of γδ T cells represents a preprogram to recognize a limited set of self-antigens induced by cells infected with K. pneumoniae or common antigens derived from commensal microflora. Therefore, it is of interest to ascertain whether murine Vγ6+ γδ T cells recognize such unique antigens in a manner different from that of γδ T cell subsets with other Vγ repertoires. In addition to TCR stimulation, several cytokines, including type
I IFN, also induce CD69 and CD25 on T cells in vitro [28–31]. Although the present results showed that CD69 expression on Vγ1–Vγ4− γδ T cells occurred after infection in the absence of type IFN receptor signaling, cytokine signaling other than TCR stimulation may be involved in CD69 expression after infection.

Various exogenous signals were reported to induce IL-17A production by γδ T cells. IL-23 and IL-1β, produced by dendritic cells and macrophages after activation through pattern-recognition receptors [32–34], are potent inducers of IL-17A production by γδ T cells. It was shown that >50% of γδ T cells in mucosal tissues constitutively expressed IL-23R in naive adult mice [17, 35]. Another study reported that IL-17A production by γδ T cells in the lung was induced in response to IL-23 at an early stage after infection of adult mice with Candida albicans [16]. Results in the current study revealed that IL-17A–producing Vγ4+ γδ T cells but not Vγ1−Vγ4− γδ T cells expanded in response to IL-23. It has been recently reported that γδ T cells are the major source of gut-protective IL-17 and are independent of IL-23 [36]. Therefore, IL-17A production in Vγ1−Vγ4− γδ T cells may be less dependent than Vγ4+ γδ T cells on IL-23. The reduced dependency on IL-23 for protection against K. pneumoniae infection in young mice as compared to adult mice may be because Vγ1−Vγ4− γδ T cells are the predominant IL-17A–producing cells in young mice after infection. Similar to γδ T cells, Th17 cells express IL-23R, which induces IL-17A production and cell proliferation [17]. Furthermore, resident IL-17A–producing ILCs compose a unique subset that can respond to IL-23 immediately after microbial infection [37]. The current study found that adult TCRγδ-deficient mice and TCRβ-deficient mice were resistant to K. pneumoniae infection. Recently, Xiong et al reported that

![Figure 5. Activation markers of γδ T cells in the lungs of mice intranasally infected with Klebsiella pneumoniae. A, Three-week-old Il17gfp+/+ mice were intranasally inoculated with 20 µL of K. pneumoniae strain 43816 serotype 2 at 1 × 10^3 colony-forming units (CFU)/mouse and euthanized 0, 1, 3, 5, or 7 days after infection. Lung cells were harvested at the indicated days, and the representative histograms show expression of surface markers on Vγ4+ and Vγ1−Vγ4− γδ T-cell repertoires. As a positive control of activation markers, cells were cultured with PMA/ionomycin for 1 hour and brefeldin was added for 1 hour of culture. Solid lines indicate CD69+ cells, and dashed lines indicate the isotype control. B, Eight-to-twelve-week-old Il17gfp+/+ mice were intranasally inoculated with 20 µL of K. pneumoniae strain 43816 serotype 2 at 1 × 10^3 CFU/mouse and euthanized 7 days after infection. The representative histograms show expressions of surface markers on Vγ1−Vγ4− γδ T-cell repertoires. Solid lines indicate cells after gating on CD69+ populations, and dotted lines indicate cells after gating on CD69− cells. C, Eight-week-old Il17gfp+/+ x Ifnra+/+ and Il17gfp+/+ x Ifnra−/− mice were intranasally inoculated with 20 µL of K. pneumoniae strain 43816 serotype 2 at 1 × 10^3 CFU/mouse and euthanized 7 days after infection. The lung cells harvested at the indicated days and the representative histograms show expressions of surface markers on Vγ4+ and Vγ1−Vγ4− γδ T-cell repertoires. Solid lines indicate CD69+ cells, and dashed lines indicate the isotype control. Data represent 3 independent experiments (A; n = 15–32 of each group), 4 independent experiments (B; n = 3 of each group), or 2 independent experiments (C; n = 3–7 of each group).]
ILCs producing IL-17A (type III) contributed to clearance of K. pneumoniae in adult mice [38]. Therefore, resident IL-17A–producing ILCs in adult mice may produce IL-17A in response to IL-23 immediately after infection, exerting their protective roles in K. pneumoniae infection.

Several studies have reported different characteristics between Vγ4+ and Vγ6+ IL-17A–producing γδ T cells. It was recently suggested that the TCR is essential for the development of IL-17A–producing γδ T cells because Zap-70 mutant mice, which express low levels of a kinase essential for TCR signaling, produced very few γδ T cells [27]. They also found that the peripheral γδ T cells in adult mice had a very strong response to IL-23 plus IL-1β without TCR signaling [27]. On the other hand, TCR signaling was not thought to be involved in the development of IL-17A–producing Vγ4+ γδ T cells [39]. More recently, Munoz-Ruiz et al have reported that the relative number of IL-17A–producing Vγ6+ T cells but not IL-17A–producing Vγ4+ T cells decreased in fetal thymus in CD3 double-haploinsufficient mice. They also found that the relative number of IL-17A–producing Vγ6+ T cells in the thymus decreased with age and that, reciprocally, the number of IL-17A–producing Vγ4+ T cells increased with age not only in CD3 double-haploinsufficient mice but also in WT mice [40]. Consistently, we also found the same age-related change in IL-17A–producing Vγ1−Vγ4− γδ T cells in the periphery. These results suggest that TCR signaling was more important in IL-17A–producing Vγ6+ T cells than in IL-17A–producing Vγ4+ T cells for development and maintenance. The present study demonstrated that Vγ1−Vγ4− γδ T cells in young mice did not express IL-23R but expressed CD69 and CD25, suggesting that the TCR is essential for IL-17A responses in the periphery after K. pneumoniae infection. Roark et al reported that a dermis-associated γδ T-cell subset producing IL-17A responded preferentially following immunization with intradermal or subcutaneous complete Freund adjuvant and expressed Vγ4+ TCR or Vγ6+ TCR [41]. Naive dermal γδ T cells were induced to proliferate and secrete IL-17A when cultured with cytokines as the only stimulant [42]. Mice developed severe psoriasis symptoms after imiquimod treatment, which was a better inducer of IL-17A in Vγ4+ cells as compared to Vγ6+ dermal cells [42, 43]. Moreover, IL-23 induced a higher

**Figure 6.** Functional expression of interleukin 23 receptor (IL-23R) on γδ T cells in the lung. A, IL-23R expression on Vγ4+ and Vγ1−Vγ4− γδ T-cell repertoires in wild-type (WT) mice. The lung cells were harvested from 3-week-old naive mice or mice intranasally infected with K. pneumoniae 7 days previously. The representative histograms show expressions of surface markers on Vγ4+ and Vγ1−Vγ4− γδ T-cell repertoires. Data represent 3 independent experiments (n = 3 of each group). B, Effect of exogenous IL-23 on γδ T cells in the lungs of naive WT mice. Cells were harvested from lungs of 3-week-old mice, and single-cell suspensions were prepared. Cells were stimulated with or without 2, 20, or 200 ng/mL recombinant IL-23 in a CO2 incubator at 37°C, and intracellular staining for IL-17A was performed after adding 10 μg/mL brefeldin A for 3 hours incubation. γδ T-cell repertoires are shown after gating on IL-17A–positive cells. The lung cells were harvested and pooled from B–10 mice in each experiment for a stimulation experiment. Data represent 3 independent experiments.
level of IL-17A production by Vγ4+ cells than by Vγ6+ cells [42, 44]. Consistent with these reports, the current study also showed that IL-23 stimulated greater IL-17A production by Vγ4+ cells, compared with Vγ1−Vγ4− cells, in the lungs of naive mice. Thus, bystander stimulation with IL-23 was more important in IL-17A-producing Vγ4+ T cells than in IL-17A-producing Vγ1−Vγ4− T cells for maintenance and activation in the periphery.

In conclusion, the current study found 2 novel findings. First, Vγ6 T cell–mediated protection against pulmonary infection with *K. pneumoniae* is dependent on age, and second, Vγ1−Vγ4− and Vγ4+ Vγ8 T cells are activated to produce IL-17A by different mechanisms during *K. pneumoniae* infection (Supplementary Figure 5).

### Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

### Notes

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Y. Y. had the original idea and designed the study. T. M. and S. H. performed experiments and analyzed data. Y. I. provided *Il17gfp/+* mice. Y. Y. provided experimental help. T. M., S. H., H. Y., and Y. Y. discussed the results. T. M. and Y. Y. prepared the manuscript.

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**Potential conflicts of interest.** All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

### References


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Figure 7. Vγ repertoire of interleukin 17A (IL-17A)–producing γδ T cells in *Il17gfp/+ × Il23p19+/+* and *Il17gfp/+ × Il23p19−/−* mice intranasally infected with *Klebsiella pneumoniae*. A and B, Lung cells were harvested from 3-week-old *Il17gfp/+ × Il23p19+/+* and *Il17gfp/+ × Il23p19−/−* mice intranasally inoculated with 20 μL of *K. pneumoniae* strain 43816 serotype 2 at 1 × 105 colony-forming units/mouse 7 days previously. The representative percentages of each Vγ subset among γδ T cells are shown as circles in the graph and the representative histograms show expressions of Vγ4+ and Vγ1−Vγ4− γδ T-cell repertoires. Solid lines indicate the CD69+ cells, and dashed lines indicate the isotype control. Data represent for 2 independent experiments (n = 3 of each group; A and B). Abbreviation: ND, not detectable.
IL-17A-Producing γδ T Cells and K. pneumoniae