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Letter to the Editor

$\gamma\delta$ T cells enhance TSLP production and ILC2 accumulation in house dust mite-induced allergic airway inflammation

Dear Editor,

Asthma is a chronic allergic inflammation in the airway which is characterized by intense eosinophilic and lymphocytic infiltration, mucus hyper-production, and airway hyper-responsiveness to a variety of stimuli.¹ Allergen-specific Th2 cells have long been thought to orchestrate Th2-type immune responses in the lung and play central roles in the development of the characteristics. However, recent evidence has demonstrated that asthma is a heterogeneous disease mediated by not only acquired but also innate immune cells.¹

One of the innate cell populations shown to be involved in asthma is innate lymphoid cells (ILC), which are characterized by the lack of antigen-specific T cell and B cell receptors and its unique capability of cytokine production upon cytokine stimulation. Among ILCs, group 2 ILCs (ILC2) rapidly produce a large amount of Th2 cytokines, including IL-5, IL-13, and IL-9, in response to epithelial cell-derived cytokines, and contribute to the development of pathological features of asthma.¹ In addition to ILC2s, group 3 ILCs (ILC3s) are implicated in the mechanism of a certain phenotype of asthma.²

Another innate cell population that has been suggested to be involved in asthma is $\gamma\delta$ T cells, which are characterized by the expression of $\gamma\delta$ T cell receptors and the abundance in epithelial or mucosal surfaces including airways.³ In addition, $\gamma\delta$ T cells are shown to be a potent producer of IL-17A, which is associated with severity and neutrophilic inflammation in asthma.³ It is noteworthy that $\gamma\delta$ T cells respond to a series of microbial products, which have a capacity to activate pattern recognition receptors and exacerbate asthma.⁴ These findings suggest that $\gamma\delta$ T cells could be involved in the pathogenesis of asthma. Indeed, it has been reported that eosinophilic inflammation is decreased in mice lacking $\gamma\delta$ T cells in an ovalbumin-induced asthma model^{5,6} and a cockroach allergen-induced asthma model.⁷ However, the roles of $\gamma\delta$ T cells in the pathogenesis of asthmatic responses to the most common allergen, house dust mite (HDM), remain unclear.

Therefore, the purpose of this study is to investigate the role of $\gamma\delta$ T cells in the development of HDM-induced allergic airway inflammation (Supplementary Methods). Wild-type mice (WT mice) were sensitized and challenged with intratracheal administration of HDM, and 48 h after the last HDM challenge,

inflammatory cell infiltration into the lung was evaluated. FACS analysis of single cell suspensions of the lung revealed that frequency of $\gamma\delta$ T cells was increased in the lung upon HDM challenge (Fig. 1A). In contrast, frequency of $\gamma\delta$ T cells was not significantly increased in the lung in mice sensitized and challenged with OVA (Fig. 1B). Although the numbers of CD4⁺ T cells, CD8⁺ T cells, and B cells were also increased in the lung upon HDM challenge, the degree of the increase was most dramatic in $\gamma\delta$ T cells (about 10-fold) (Fig. 1C). Analysis of V γ usage revealed that the majority of $\gamma\delta$ T cells accumulated in the lung upon HDM inhalation expressed V γ 6 (Fig. 1D). Intracellular cytokine staining showed that $\gamma\delta$ T cells preferentially produced IL-17A, whereas CD4⁺ T cells produced IL-5, IL-13, and IFN- γ , in addition to IL-17A (Fig. 1E). FACS analysis of IL-17-producing CD45⁺ cells in HDM-challenged mice revealed that $\gamma\delta$ T cells accounted for about 40% of IL-17A-producing cells, whereas $\alpha\beta$ T cells accounted for 50% of them (Fig. 1F).

To address the roles of $\gamma\delta$ T cells in the development of HDM-induced allergic airway inflammation, $\gamma\delta$ T cell-deficient mice ($TCR\delta^{-/-}$ mice) and littermate WT mice were sensitized and challenged with HDM, and inflammatory cell infiltration into the lung was evaluated. $TCR\delta^{-/-}$ mice showed significantly attenuated eosinophil infiltration into the BALF as compared with WT mice (Fig. 2A). Histological analysis revealed that $TCR\delta^{-/-}$ mice showed less inflammatory cell infiltration to peri-bronchial and perivascular lesion and less PAS-positive goblet cells than WT mice (Fig. 2B and C). Furthermore, $TCR\delta^{-/-}$ mice exhibited significantly attenuated airway hyper-responsiveness (Fig. 2D).

We next evaluated HDM-induced cytokine production of mediastinal lymph node cells. Consistent with attenuated eosinophilic inflammation, lymphocytes from $TCR\delta^{-/-}$ mice produced significantly lower levels of IL-5 and IL-13 than those from WT mice (Fig. 2E). In contrast, IL-17 was undetectable in most of $TCR\delta^{-/-}$ mice and WT mice in this experimental setting (Fig. 2E).

We next evaluated the levels of epithelial cytokines involved in the induction of type 2 inflammation. As shown in Figure 2F, the level of TSLP in the lung was significantly lower in $TCR\delta^{-/-}$ mice than that in WT mice. The level of IL-33 also tended to be lower in $TCR\delta^{-/-}$ mice (Fig. 2F). Consistently, the accumulation of ILC2s was significantly attenuated in the lung in $TCR\delta^{-/-}$ mice as compared with that in WT mice (Fig. 2G). Taken together, these results suggest that $\gamma\delta$ T cells promote epithelial cytokine production and exacerbate allergic airway inflammation.

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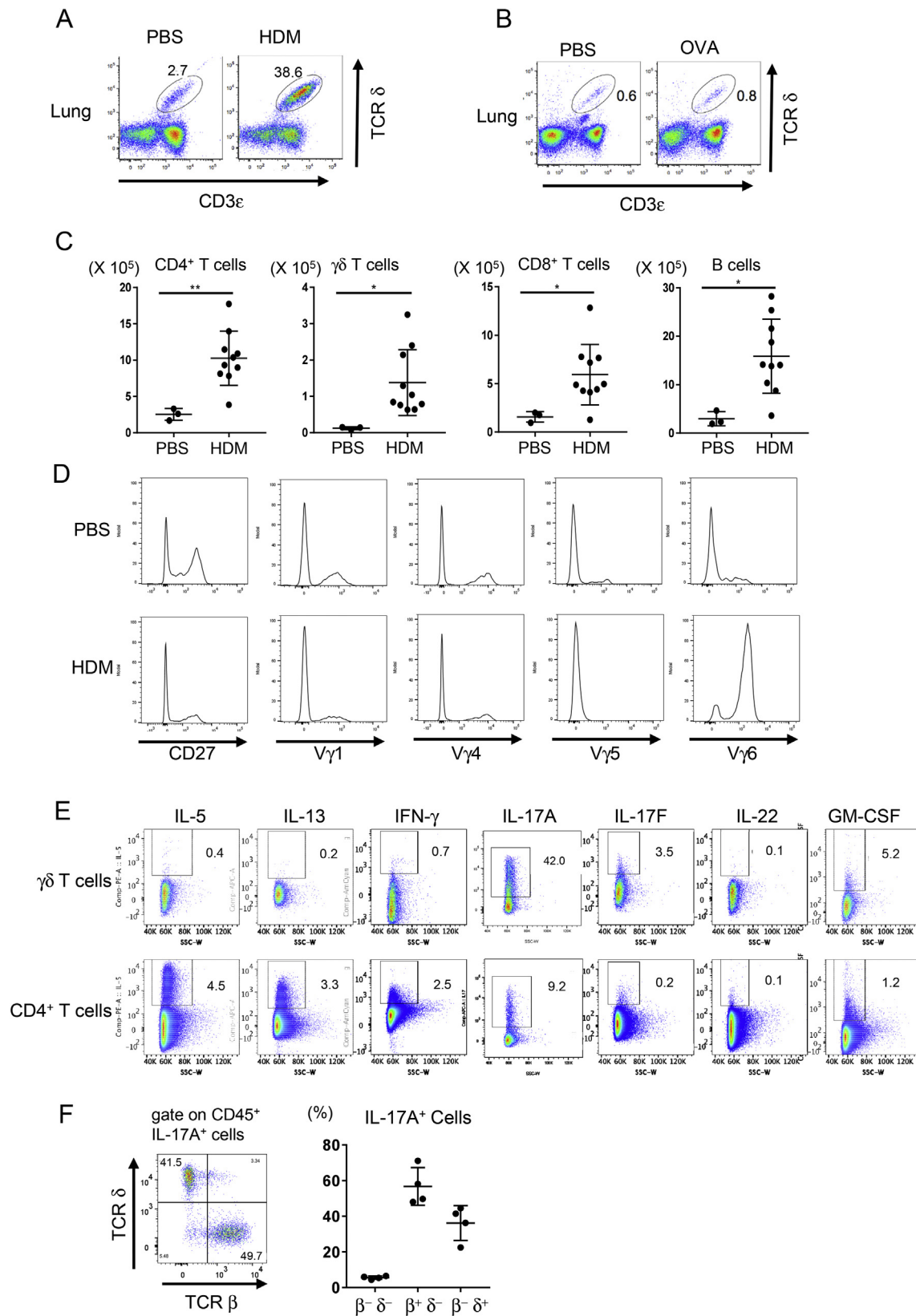


Fig. 1. IL-17A-producing $\gamma\delta$ T cells are accumulated in the lung in HDM-induced allergic inflammation. **(A)** WT mice were sensitized and challenged with HDM or PBS (as a control) (see Online Methods). Shown are representative CD3 ϵ vs. TCR δ staining of CD45⁺ cells in the lung. **(B)** WT mice were sensitized with OVA conjugated with alum and challenged with OVA inhalation (see Online Methods). Shown are representative CD3 ϵ vs. TCR δ staining of CD45⁺ cells in the lung. **(C–F)** WT mice were sensitized and challenged with HDM or PBS. **(C)** Forty-eight hours after the last HDM challenge, the numbers of each cell population in the lung were analyzed. **(D)** The usage of V γ chain of $\gamma\delta$ T cells in the lung was analyzed by FACS. Shown are representative histograms gated on CD3 ϵ ⁺ TCR δ ⁺ cells. **(E)** Shown are representative cytokine profiles of CD3 ϵ ⁺ CD4⁺ cells and CD3 ϵ ⁺ TCR δ ⁺ cells in the lung. **(F)** Shown are representative TCR β vs. TCR δ staining of IL-17A-producing CD45⁺ cells in the lung and the frequencies of TCR β ⁺ TCR δ ⁺ cells, TCR β ⁺ TCR δ ⁺ cells, and TCR β ⁺ TCR δ ⁺ cells among IL-17A-producing CD45⁺ cells. Data are means $\pm$ SEM for 4 mice in each group from 2 independent experiments.

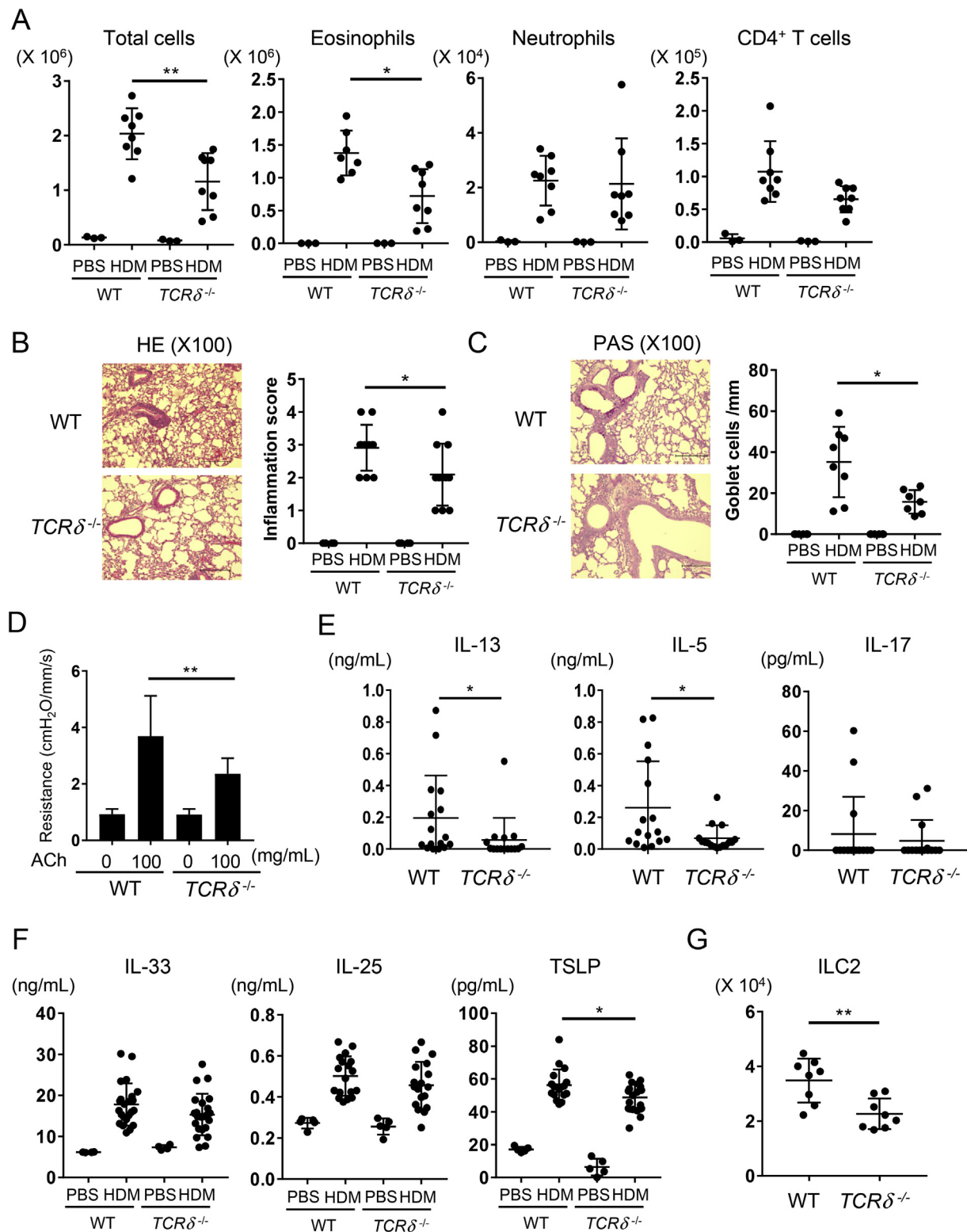


Fig. 2. $\gamma\delta$ T cells exacerbate HDM-induced allergic airway inflammation. (A–F) WT mice and $TCR\delta^{-/-}$ mice were sensitized and challenged with HDM or PBS. (A) The absolute numbers of total cells, eosinophils, neutrophils, and $CD4^{+}$ T cells in BALF were evaluated at 48 h after the last challenge. Data are means $\pm$ SEM for 3–8 mice in each group from 3 independent experiments, * $p < 0.05$, ** $p < 0.01$. (B) Representative photomicrographs of HE-stained lung sections with histological inflammatory scores, Mann–Whitney U test, * $p < 0.05$, bar = 50 μ m. (C) Representative photomicrographs of PAS-stained lung sections with the numbers of goblet cells. Data are means $\pm$ SEM for 3–7 mice in each group from 2 independent experiments, * $p < 0.01$, bar = 50 μ m. (D) Airway resistance to methacholine was measured at 48 h after the last HDM challenge. Data are means $\pm$ SEM for 7 mice in each group, ** $p < 0.01$. (E) Cytokine levels in the supernatants of single cell suspensions of mediastinal lymph node cells stimulated with HDM for 5 days. Data are means $\pm$ SEM for 10 mice in each group from 3 independent experiments, * $p < 0.05$. (F) Levels of IL-25, IL-33, and TSLP in the lung lysate. Data are means $\pm$ SEM for 5–19 mice in each group from 3 independent experiments, * $p < 0.05$. (G) The numbers of ILC2s in the lung. Data are means $\pm$ SEM for 8 mice in each group from 2 independent experiments, * $p < 0.05$.

In this study, we show that $\gamma\delta$ T cells are involved in the induction of allergic airway inflammation in the HDM-induced asthma model. Although the involvement of $\gamma\delta$ T cells in allergic airway inflammation has been shown by previous studies,^{5–7} our results provide a new mechanism in which $\gamma\delta$ T cells enhance epithelial cytokine production and ILC2 accumulation in the lung. We also found that the number of IL-17A-producing $\gamma\delta$ T cells was increased in the lung upon HDM stimulation. Since it has been shown that IL-17A promotes IL-33 and TSLP expression in other experimental systems,^{8,9} IL-17A produced by $\gamma\delta$ T cells could be involved in the enhanced epithelial cytokine production in the HDM-induced asthma model. In addition, we found that V γ 6⁺ cells, which are known to be involved in the inflammatory responses in the skin, joints, and lung,¹⁰ were significantly increased in the lung upon HDM stimulation. This finding is consistent with a previous report showing that V γ 6⁺ cells are relatively abundant in lung and respond to various stimuli including IL-23.¹⁰ Although further studies are needed, our results highlight a key role of $\gamma\delta$ T cells in HDM-induced allergic airway inflammation and propose $\gamma\delta$ T cells as a novel therapeutic target.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2019.05.010>.

Conflict of interest

The authors have no conflict of interest to declare.

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Supplementary Methods

Mice

C57BL/6 mice were purchased from Charles River Laboratories (Kanagawa, Japan). *TCR α ^{-/-}* mice (Jackson Laboratory, Bar Harbor, ME) on a C57BL/6 background were housed in microisolator cages under specific pathogen-free conditions. The Chiba University Animal Care and Use Committee approved animal procedures used in this study.

Murine models of airway inflammation

1) HDM-induced asthma model: Mice were anesthetized with intraperitoneal injection of midazolam, butorphanol, and medetomidine as described previously¹, and then sensitized intratracheally with 50 μ g of HDM (Greer Laboratories, Lenoir, NC) in 25 μ L of PBS twice at a one-week interval. Seven days after the sensitization, mice were challenged with 5 μ g of HDM for 4 consecutive days. Forty-eight hours after the last challenge, the numbers of eosinophils, neutrophils, and lymphocytes recovered from BALF were evaluated². Airway resistance was measured by FlexiVent FX (SCIREQ, Inc, Montreal, Quebec, Canada)³.

2) OVA-induced asthma model: C57BL/6 mice were immunized intraperitoneally twice with 10 μ g of OVA in 4 mg of aluminum hydroxide (alum) at a 2-week interval, and 2 weeks after the second immunization, the sensitized mice were challenged with aerosolized OVA (50 mg/mL) dissolved in 0.9% saline for 20 minutes through a DeVilbiss 646 nebulizer (DeVilbiss Corp, Somerset, Pa) three times at 24-hour intervals.

Histological analysis of lung

Lung sections (3 μ m thick) were stained with hematoxylin-eosin (HE) or periodic

acid-Schiff (PAS) according to standard protocols. Histological score and the number of goblet cells were evaluated as described elsewhere^{4, 5}.

Cytokine production

Single cell suspensions prepared from mediastinal lymph nodes (1×10^6 cells) were stimulated with HDM (30 $\mu\text{g/mL}$) for 5 days in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 50 μM β -mercaptoethanol, and 2 mM L-glutamine (complete RPMI medium) in a round bottom 96-well plate. The levels of cytokines in the culture supernatants were measured by ELISA kits according to the manufacturer's instructions.

ELISA

The amounts of IL-5, IL-13, IL-17A, IFN- γ , TSLP, IL-25, and IL-33 were determined by ELISA kits from R&D systems (Minneapolis, MN).

FACS analysis

Single cell suspensions of the lung were generated as described previously⁶. After Fc receptors were blocked with anti-CD16/32 (Fc γ RII/III) antibody (BD Biosciences), cells were stained with anti-CD45, anti-CD3 ϵ , anti- $\gamma\delta$ TCR, and DAPI. For intracellular cytokine staining, cells were stimulated with PMA (20 ng/mL, Calbiochem, San Diego, CA) and ionomycin (1 $\mu\text{g/mL}$, Calbiochem) for 4 hours in the presence of brefeldin A (10 μM , BD Bioscience, San Diego, CA) and monensin (2 μM , Sigma-Aldrich, St. Louis, MO). After cells were stained with anti-CD45, anti-CD3 ϵ together with anti- $\gamma\delta$ TCR or anti-CD4 for 30 min at 4°C, the cells were fixed and permeabilized with a Fixation/Permeabilization solution kit (BD Bioscience), and stained with indicated anti-cytokine antibody. Data were collected by a FACS Canto II (BD Biosciences) and analyzed by FlowJo software (TreeStar, Ashland, Oregon).

Analysis of the usage of V γ chain

After the blocking with anti-CD16/32 (Fc γ RII/III) antibody, single cell suspensions of the lung were stained with anti-CD45, anti-CD3 ϵ , anti- $\gamma\delta$ TCR, and DAPI. Antibodies used for the analysis of the usage of V γ chain were as follows; TCR-V γ 1 (2.11, Biolegend), TCR-V γ 4 (UC3-10A6, Biolegend), TCR-V γ 5 (536, Biolegend), and TCR V γ 6 (17D1, kindly provided by Dr. Tigelaar, Yale University).

Data analysis

Data are summarized as means $\pm$ SEM. Data were analyzed by unpaired *t* test. Where indicated, data were analyzed by Mann-Whitney U test. *P* values <0.05 were considered significant.

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