Group 2 innate lymphoid cells mediate the activation of CD4$^+$ T cells and aggravate Th1/Th2 imbalance via MHC II molecules during respiratory syncytial virus infection

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Research Article

Keywords: RSV, CD4+ T cells, Th1/Th2, ILC2s, MHC II

Posted Date: October 25th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-970722/v1

Abstract

Background

Respiratory syncytial virus (RSV), as an important worldwide pediatric respiratory pathogen, can cause pneumonia and bronchiolitis in infants and young children. $CD4^+$ T cells play a crucial role in RSV-induced airway inflammation. However, the underlying mechanism of $CD4^+$ T cell activation during RSV infection is not fully understood.

Methods

The proliferation and activation of $CD4^+$ T cells in the lungs of RSV infected BALB/c mice were measured by flow cytometry. RSV-induced airway inflammation was determined by HE staining and the inflammatory cells in bronchoalveolar lavage fluids (BALF) were counted. The protein levels of IL-4, IL-5, IL-13, and IFN-γ in the supernatant of BALF were detected by ELISA. The mRNA levels of IL-4, IL-5, IL-13, and IFN-γ in the lung homogenates were analyzed by real-time PCR. The expression of major histocompatibility complex II (MHCII), CD80 and CD86 on pulmonary ILC2s were measured by flow cytometry and real-time PCR.

Results

RSV infection may promote the differentiation and activation of $CD4^+$ Th2 cells, resulting in imbalance of Th1/Th2 in the lung tissues. Depletion of $CD4^+$ T cells can obviously reduce airway inflammation caused by RSV infection. Adoptive transfer of pulmonary ILC2s significantly enhanced the number of pulmonary $CD4^+$ T cells and promoted their differentiation to Th2. In fact, RSV infection increased the expression of MHC II and B7 molecules on the surface of pulmonary ILC2s. In vitro co-culture experiments showed that ILC2s may act as a promoter to promote the expansion and differentiation of RSV-infected naïve $CD4^+$ T cells. However, blocking the interaction between ILC2s and $CD4^+$ T cells with anti-MHC II mAbs significantly reduced $CD4^+$ T cell expansion and increased the Th1/Th2 ratio of $CD4^+$ T cells.

Conclusions

Our data suggest that pulmonary ILC2s may function as antigen-presenting cells to induce the activation of $CD4^+$ T cells through MHC II pathway during RSV infection.

Background

Respiratory syncytial virus (RSV), a member of family paramyxoviridae, is the main pathogen causing severe respiratory diseases like pneumonia and bronchiolitis in infants, elderly and immunocompromised

people[1–3]. It has been reported that RSV- induced airway inflammation can affect airway function and result in asthma-like symptoms such as airway hyperresponsiveness, mucus hypersecretion, and eosinophil infiltration, which are closely associated with greater risk of recurrent wheezing and asthma[4].

Increasing evidence suggests that T lymphocytes, especially $CD4^+$ T cells play an indispensable role in RSV-induced airway inflammation[5, 6]. The imbalance of Th1/Th2 cells maybe the key factor for the progression and severity of RSV-related disease. In fact, during RSV infection, $CD4^+$ Th2 cell-derived IL-4 enhances airway hyperresponsiveness[7], IL-5 recruits eosinophil infiltration into the lungs[8, 9], and IL-13 promotes mucus secretion and airway remodeling[10]. Therefore, $CD4^+$ T cell activation is the pivotal event for RSV-induced airway inflammation.

It is no doubt that dendritic cell (DC) is the main cell type which induce $CD4^+$ T cell activation during RSV infection[11]. However, recently, an innate immune cell type, group 2 innate lymphocytes (ILC2s) attract more attention. It has been reported that ILC2s can be activated quickly during RSV infection and mediate airway inflammation by secreting large amounts of type 2 cytokines[12]. Interestingly, ILC2s can regulate the proliferation and activation of $CD4^+$ T cells via OX40/OX40L axis during RSV infection[13]. However, whether ILC2s can affect the biological activity of $CD4^+$ T cells through other pathways during RSV infection is still unclear.

Thus, in this study, by using BALB/c mice that were intranasally inoculated with RSV, the role of ILC2s and its underlying mechanism in regulating $CD4^+$ T cell proliferation and activation was investigated.

Material And Methods

Mice and infection

Female specific-pathogen-free (SPF) BALB/c mice (6–8 weeks of age) were purchased from Liaoning Changsheng Biotechnology Co. Ltd. (Liaoning, China). Mice had fresh water and autoclaved food on a weekly schedule and maintained in individual filter cages at the Laboratory Animal Center, China Medical University. The animal protocol was approved by the Institutional Animal Care and Use Committee of China Medical University. Human RSV type A2 (RSV A2) strain was grown and assayed for infectivity (expressed as a 50% tissue culture infectious dose, TCID 50) in Hep2 cells (ATCC) as previously described[14]. Mice were inoculated intranasally with RSV at an inoculum dose of 2×10^6 TCID 50 in 20 µL of sterile PBS per mouse. Mice were inoculated intranasally with sterile PBS as a control group (expressed as day 0 in figures).

Lung cell preparation

Mice were anesthetized and the pulmonary circulation was flushed with ice-cold PBS to remove intravascular blood pool. Then, lung tissue was minced and incubated in RPMI 1640 (with 200 µg/mL collagenase D and 40 µg/mL DNase I) at 37 °C for 90 min on a rocker. The digested lung tissue was

passed through a 70-μm stainless steel mesh, then red blood cells were lysed with 0.15 M ammonium chloride and 1 mM potassium bicarbonate, the lung cells were resuspended in RPMI 1640 medium.

Isolation of pulmonary ILC2s and splenic $CD4^+$ T cells

Pulmonary ILC2s, defined as $CD45^+$ $linage^-$ $ST2^+$ cells were obtained using a MoFlo high-speed cell sorter (Beckman Coulter). Naïve $CD4^+$ T Cells were purified using naïve $CD4^+$ T Cell Isolation kit (130-104-453, Miltenyi). The purity of these isolated cells was > 85%.

Adoptive transfer experiment

For adoptive transfer experiment, lung cells from mice that were infected with RSV at day 3, were pooled, and $CD45^+$ Lin^- $ST2^+$ cells, which were identified as ILC2s in this study, were isolated. Then 1×10^5 of ILC2s (purity was > 86%) in 100 μL of PBS were transferred by intravenous injection 2 h before RSV infection. Samples were collected at day 6 after RSV infection.

Real-time PCR

ILC2s were sorted from the lungs of RSV-infected mice at various time points. The total RNA was isolated from the lung homogenates or purified ILC2s using TRIzol reagent (Life Technologies) and converted to cDNA with a SuperScript III Reverse Transcriptase using Oligo (dT) primers (Life Technologies). Quantitative real-time PCR was performed using SYBR Green Master Mix (Life Technologies). Primers used for the detection of expression of mRNAs for IL-4, IL-5, IL-13, IFN-γ, MHC II, CD80 and CD86 were below:

IL-4-F: 5′- TGTACCAGGAGCCATATCCA-3′, IL-4-R: 5′- TTCTTCGTTGCTGTGAGGAC-3′; IL-5-F: 5′-GGCTTCCTGTCCCTACTCAT-3′, IL-5-R: 5′- TCCTCGCCACACTTCTCTTT-3′; IL-13-F: 5′-AGCATGGTATGGAGTGTGGA -3′, IL-13-R: 5′- TTGCAATTGGAGATGTTGGT -3′; IFN-γ-F: 5′-TATCTGGAGGAACTGGCAAA -3′, IFN-γ-R: 5′- GGTGTGATTCAATGACGCTT -3′; MHC II-F: 5′-TCTGATTCTGGGGGTCCTCG -3′, MHC II -R: 5′-ATAGGTGCCTACGTGGTCGG -3′; CD80-F: 5′-ACCCCCAACATAAACTGAGTCT -3′, CD80-R: 5′-TTCCAACCAAGAGAAGCGAGG-3′; CD86-F: 5′-TGTTTCCGTGGAGACGCAAG -3′, CD86-R: 5′-TTGAGCCTTTGTAAATGGGCA-3′; β-actin-F: 5′-CAACGAGCGGTTCCGATG-3′, β-actin-R: 5′-GCCACAGGATTCCATACCCA-3′.

Reactions were run using a 7500 Real-Time PCR System (Applied Biosystems) under identical amplification conditions. Results are normalized to β-actin and presented as fold mRNA expression (fold change = $2^{-\triangle\triangle CT}$).

Flow cytometry

Lung cells were first blocked for Fc receptors with Fc Block (CD16/32, BD Bioscience) and then surface-stained with fluorescein-conjugated antibodies in PBS for 30 min at 4 °C under light protection. For intracellular cytokine staining, naïve $CD4^+$ T cells were isolated from the lungs of mice and stimulated

with PMA (25 ng/mL, Sigma) and ionomycin (1 μg/mL, Sigma) for 4.5 h at 37 °C. Brefeldin A (10 μg/mL, Sigma) was added for the last 4 h of culture. After incubation, intracellular staining was performed according to the manufacturer's instructions (BD Pharmingen). Antibodies used were as follows: anti-IFN-γ-PE (Biolegend), anti-MHC II-APC/cy7 (Biolegend), anti-CD80-BV421 (Biolegend), anti-CD86-PE/cy7 (Biolegend), anti-CD3-FITC (Biolegend), anti-CD4-PerCP (Biolegend), anti-CD69-APC/cy7 (Biolegend), anti-IL-4-PE (Biolegend), anti-IL-5-BV421 (eBioscience), anti-IL-13-PE/cy7 (eBioscience), anti-CD45-APC (eBioscience), anti-ST2-PE (eBioscience), and FITC-conjugated anti-lineage antibodies (eBioscience). The lineage marker antibody cocktail was composed of antibodies against: DX5 (or NK1.1), CD3, CD4, CD5, CD8, CD11b, CD19, B220, Gr-1 and TCRδ (BD Bioscience).

Neutralizing Antibody: Anti-mouse MHC Class II (clone: M5/114; InVivoMAb grade; BioXcell, BE0108), anti-mouse CD4 (α-CD4; clone: GK1.5; InVivoMAb grade; BioXcell, BE0003).

ELISA

To measure the protein levels of cytokines in BALF. Supernatant of BALF were measured with ELISA kits (R&D Systems) including IL-4, IL-5, IL-13 and IFN-γ according to the manufacturer's instruction. The absorbances at 450 nm were determined by a microplate reader.

Histopathological examination

Bronchoalveolar lavage fluids (BALF) were collected and centrifuged. Differential counting of cells in the pellet was performed on cytospins. For lung histology, lung tissue sections were processed for hematoxylin and eosin (H&E) staining.

Co-culture experiment

2×10^5 of pulmonary ILC2s from the mice on day 3 after RSV infection and equal numbers of splenic naïve $CD4^+$ T cells from normal mice, were co-cultured in vitro in the presence or absence of 0.5 μg/mL anti-MHC II mAbs. The cells were cultured in a 24-well plate in 500 μL RPMI-1640 medium containing 10% FBS with the presence of IL-2 (20 ng/mL) IL-7 (20 ng/mL) and IL-33 (20 ng/mL) for 72 h, the flow cytometry was used to detect the proliferation of $CD4^+$ T cell in the co-cultures.

Statistical analysis

Differences between control and experimental groups were compared using one-way analysis of variance (ANOVA) to calculate the statistical significance (GraphPad Prism software, version 8.0). *P* values < 0.05 were considered significant.

Results

1. RSV infection induces proliferation and activation of $CD4^+$ T cells in the lungs of BALB/c mice

To explore the effect of RSV infection on the proliferation and activation of $CD4^+$ T cells, we prepared single cell suspensions from the lungs of RSV-infected mice at the indicated time points. The number and percentage of $CD4^+$ T cells were detected by flow cytometry. Results showed that RSV infection significantly increased the percentage and total number of $CD4^+$ T cells in the lung tissues (Fig. 1a, b). The percentage and absolute number of $CD4^+$ Th2 cells, which produce type 2 cytokines like as IL-4, IL-5 and IL-13, were increased significantly (Fig. 1c, d). As a result, the ratio of Th1/Th2 in the lungs was decreased (Fig. 1e). These results suggest that RSV infection may cause the proliferation and activation of $CD4^+$T cells and shift $CD4^+$ T cells to Th2 cells.

2. CD4 $^+$ T cells are the key effector cells mediated RSV-induced airway inflammation

To determine the role of $CD4^+$ T cells in airway inflammation, $CD4^+$ T cells were depleted by intraperitoneal injection of anti-CD4 mAbs 2 hours before RSV infection. Results showed that RSV infection led to massive inflammatory cell infiltration and severe vascular congestion in the lungs of mice. However, when depletion of $CD4^+$ T cells, the infiltration of inflammatory cells in the lung tissue was significantly reduced, and the bronchial and alveolar walls were basically normal (Fig. 2a). By counting the number of inflammatory cells in BALF, it was found that the number of inflammatory cells, such as macrophages, neutrophils, and lymphocytes in $CD4^+$ T cell-depleted group was significantly decreased when compared with that in PBS group (Fig. 2b). ELISA and Real-time PCR analysis showed that RSV infection significantly increased Th1 and Th2 cytokines secretion in BALF of BALB/c mice. In contrast, depletion of $CD4^+$ T cells significantly reduced the levels of Th2 cytokines (IL-4, 5, 13) (Fig. 2c, d). These results suggest that $CD4^+$ T cells are important effector cells which mediate RSV-induced acute pneumonia by producing large amounts of Th2 cytokines.

3. Adoptive transfer of pulmonary ILC2s promotes CD4 $^+$ T cell activation in the lungs of RSV infected mice

As an innate immune cell type, ILC2s have been found to not only mediate airway inflammation by producing abundance of Th2 cytokines[15], but also act as antigen-presenting cells to active $CD4^+$ T cells activation during intestinal helminth infection[16, 17].To investigate whether ILC2s are involved in the RSV-induced proliferation of $CD4^+$ T cells, $CD45^+$ Lin^- $ST2^+$ ILC2s were isolated from the lungs of BALB/c mice on day 3after RSV infection, and transferred intravenously into normal mice two hours before RSV infection.The results showed that compared with corresponding non-transferred group, adoptive transfer of pulmonary ILC2s enhanced the percentage and absolute number of both activated $CD4^+$ T cells and $CD4^+$ Th2 cells (Fig.3 a, b, c, d). The ratio of Th1/Th2 in the lungs wasalso significantly increased by adoptive transfer of pulmonary ILC2s (Fig.3 e).Furthermore, adoptive transfer of pulmonary ILC2s increased the production of Th2-type cytokines in the lungs, resulting in the Th1/Th2 balance to shift to Th2 (Fig.3 f) and aggravated infiltration of inflammatory cells in BALF (Fig.3 g).These results suggest that ILC2s contribute to RSV-induced massive airway inflammation in part by promoting the differentiation of $CD4^+$ T cells into Th2 cells.

Figure 3. Adoptive transfer of pulmonary ILC2s augments $CD4^+$ T cell expansion and Th2 differentiation in the lungs. $CD45^+$ Lin^- $ST2^+$ ILC2s were isolated from the lungs of RSV-infected mice and injected via the tail vein into normal mouse 2 h before RSV infection. Lung cells were isolated on day 6 after RSV infection and assayed by flow cytometry. (a, c) The gating strategies of flow cytometry. (b) The frequencies and absolute numbers of total $CD4^+$ T cells and $CD69^+$ $CD4^+$ T cells. (d) The frequencies and absolute numbers of IFN-γ-, IL-4-, IL-5- and IL-13-producing $CD4^+$ T cells. (e) The ratio of Th1/Th2 in the lungs. (f) The mRNA levels of IL-4, IL-5, IL-13, and IFN-γ in the lung homogenates were detected by real-time PCR. (g) Inflammatory cells in BALF of the tested mice were counted. Data indicate mean ± SD of each group (n = 4/group). Statistically significant differences are shown (* $p < 0.05$, compared with the group of control, # $p < 0.05$, compared with the group of RSV infected, ns =non-significant).

4. RSV infection increases the expression of MHC II and B7 molecules on pulmonary ILC2s.

In order to investigate the underlying mechanism of ILC2s on $CD4^+$ T cell activation, we prepared lung cells from the RSV-infected mice and detected the expression of MHC II and B7 (CD80, CD86), which are closely related to antigen presentation, on the pulmonary ILC2s by flow cytometry. The results showed that the number of ILC2s were peaked on day 3 after RSV infection, in parallel with the elevated number of MHC II-, CD80- and CD86- positive cells in the ILC2s population. (Fig. 4a, b, c, d). The mRNAs for MHC II, CD80, and CD86 in the pulmonary ILC2s peaking on day 3 and maintaining high level even on day 6 after RSV infection (Fig. 4e).

5. ILC2s regulate $CD4^+$ T cell activation in a MHC II dependent manner

To clarify whether ILC2s mediate the proliferation and activation of $CD4^+$ T cells through MHC II, pulmonary ILC2s were sorted on day 3 after RSV infection and co-cultured with naïve $CD4^+$ T cells, which were isolated from the spleen of normal mice for 72 hours. The results showed that the numbers of $CD4^+$ T cells, $CD69^+$ $CD4^+$ T cells as well as $CD4^+$ Th2 cells were increased significantly by co-culture of $CD4^+$ T cells with ILC2s. However, blockade of MHC II molecule by anti-MHCII mAbs remarkably decreased the number of $CD69^+$ $CD4^+$ T cells and $CD4^+$ Th2 cells, but enhanced the percentage and absolute number of IFN-γ^+ $CD4^+$ T cells. (Fig. 5a, b), resulting in the increased ratio of Th1/Th2 (Fig. 5c). These results suggest that MHC II may be a key factor for ILC2s-regulated $CD4^+$ T cell activation.

Discussion

RSV is widely prevalent in the world, and causes inflammatory respiratory diseases, such as bronchiolitis or pneumonia. More than 3 million children are hospitalized and 66,000 to 199,000 children die from RSV-related acute lower respiratory infections every year[18]. As an important cell type of adaptive immune cells, $CD4^+$ T cells play an important role in mediating a strong immune response by producing Th2 cytokines, which mediate inflammation and induce asthma-like symptoms, such as airway hyperresponsiveness, airway mucus secretion and increased eosinophilic infiltration[6, 19]. In addition,

the imbalance of Th1/Th2 response is a key immunological pathogenesis of airway inflammation induced by RSV infection[20, 21]. As some clinical studies have shown, the balance between the Th1 and Th2 immune response has been identified as a critical component in severe RSV disease[22, 23].

In this study, we found that RSV infection significantly increased the total number of $CD4^+$ T cells as well as the percentage and absolute number of $CD4^+$ Th2 cells. As a result, the ratio of Th1/Th2 in $CD4^+$ T cells decreased significantly (Fig. 1). It is suggested that RSV infection can cause the proliferation of $CD4^+$ T cells and Th2 differentiation in the lung tissue, resulting in Th1/Th2 imbalance. In addition, our study confirmed that $CD4^+$ T cells are important effector cells mediating acute pneumonia of RSV, as shown in Fig. 2, inflammatory cells and Th2 cytokine levels in both the lung tissue and BALF were decreased in $CD4^+$ T cell-depleted group. Therefore, in vivo depletion of $CD4^+$ T cells may significantly reduce lung and respiratory tract inflammation. It is concluded that $CD4^+$ T cells play a pro-inflammatory role and may be the main factor for immune-mediated disease during RSV infection.

Another important immune cell type in viral-induced respiratory infection is the recently discovered ILC2s, which is now recognized as a key event in many inflammatory diseases[24–26]. ILC2s and Th2 cells share many similarities, both of which can effectively regulate type 2 immune response by producing similar type 2 cytokines[27]. Therefore, the interaction between the two in type 2 immune diseases deserves attention. In this study, the results from adoptive transfer experiments confirmed that ILC2s may promote the proliferation of $CD4^+$ T cells and Th2 polarization, and further worsen the Th1/Th2 imbalance (Fig. 3). However, the regulatory role of ILC2s in $CD4^+$ T cell activation is not fully understood. We have reported that $CD4^+$ T cells contribute to RSV-induced ILC2 activation partly via producing IL-2[28], but in fact, the regulatory effect of ILC2s on $CD4^+$ T cell activation may precede that. As shown in Fig. 4, on day 3 after RSV infection, the proliferation of ILC2s reached a peak.

Studies have shown that ILC2s may mediate the proliferation of $CD4^+$ T cells through OX40-OX40L and promote the production of type 2 cytokines by $CD4^+$ T cells[13, 29]. In addition, it has been found that ILC2s can drive $CD4^+$ T cells by secreting cytokines and modulating DCs[30]. ILC2s can also act upstream of DCs and are essential for their production of the memory Th2 cell chemoattractant CCL17[31]. ILC2s can up-regulate GATA3 through its expression of PD-L1, which in turn mediates $CD4^+$ T cell activation and increased IL-13 expression[32]. Furthermore, ILC2-derived IL-4 also plays a promotive role in the ILC2 and $CD4^+$ T cell interaction, as co-culture of IL-4-deficient $CD4^+$ T cells with WT ILC2s produced large amounts of Th2 cytokines[29]. Besides the above mechanisms, in this study, ILC2s are likely to exert their regulatory effect on $CD4^+$ T cell through an alternative pathway.

It is well known that DCs are essential for the activation of $CD4^+$ T cells [33, 34],however, some reports suggest that nonprofessional APCs, such as eosinophils and endothelial cells which expressing MHC II and members of B7 family, can also activate T cells by presenting antigens[17]. It is generally known that MHC II molecules are essential for cell-mediated immunity which present antigen to naïve $CD4^+$ T cells to activate them[35]. Because of MHC II molecules are polymorphic, different MHC II alleles link strongly to

defined auto-immune diseases[36]. MHC II molecules on APCs thereby are critical for the initiation of the antigen-specific immune response. Oliphant et al. demonstrated that ILC2s can be act as antigen-presenting cells to induce proliferation and activation of $CD4^+$ T cells in worm-infected mice via MHC II[16]. In this study, we found that pulmonary ILC2s exhibit high levels of MHC II on ILC2s during RSV infection(Fig. 4). Blockade of MHC II by anti-MHC II mAbs significantly reduced the proliferation and activation of $CD4^+$ T cells and inhibited their Th2 differentiation, demonstrating that ILC2s may contribute to $CD4^+$ T cell activation and differentiation via MHC II pathway(Fig. 5).

Conclusions

In conclusion, in this study, we confirmed that pulmonary ILC2s may function as antigen-presenting cells to induce the activation of $CD4^+$ T cells through MHC II pathway during RSV infection, elucidating the interaction and regulatory mechanism of the two in the occurrence and development of airway inflammation will help us understand its pathogenesis and seek new treatment methods.

Abbreviations

MHC II Major histocompatibility complex II

RSV Respiratory syncytial virus

IL Interleukin

IFN-γ Interferon-γ

HE Hematoxylin-eosin

BALF Bronchoalveolar lavage fluids

ELISA Enzyme-linked immunosorbent assay\

PCR Polymerase chain reaction

ILC2s Group 2 innate lymphoid cells

DC Dendritic cell

FBS Fetal bovine serum

PBS Phosphate-buffered saline

$TCID_{50}$ 50% tissue culture infections dose

HEp-2 Human epithelial type 2

RNA Ribonucleic Acid

cDNA Complementary Deoxyribonucleic ACID

Eos Eosinophil

Lym Lymphocyte

Mφ Macrophage

Neu Neutrophil

Lin Lineage

Declarations

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cDNA Complementary Deoxyribonucleic ACID

Eos Eosinophil

Lym Lymphocyte

Mφ Macrophage

Neu Neutrophil

Lin Lineage

References

1. Leader S, Kohlhase K. Recent trends in severe respiratory syncytial virus (RSV) among US infants, 1997 to 2000. J Pediatr. [Journal Article]. 2003 2003-11-01;143(5 Suppl):S127-32.
2. Coultas JA, Smyth R, Openshaw PJ. Respiratory syncytial virus (RSV): a scourge from infancy to old age. THORAX. [Journal Article; Research Support, Non-U.S. Gov't; Review]. 2019 2019-10-01;74(10):986–93.
3. Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. N Engl J Med. [Journal Article; Research Support, U.S. Gov't, P.H.S.]. 2005 2005-04-28;352(17):1749–59.
4. Shi T, Ooi Y, Zaw EM, Utjesanovic N, Campbell H, Cunningham S, et al. Association Between Respiratory Syncytial Virus-Associated Acute Lower Respiratory Infection in Early Life and Recurrent Wheeze and Asthma in Later Childhood. J INFECT DIS. [Journal Article; Research Support, Non-U.S. Gov't]. 2020 2020-10-07;222(Suppl 7):S628-33.
5. Guvenel A, Jozwik A, Ascough S, Ung SK, Paterson S, Kalyan M, et al. Epitope-specific airway-resident CD4+ T cell dynamics during experimental human RSV infection. J CLIN INVEST. [Clinical Trial; Journal Article; Research Support, Non-U.S. Gov't]. 2020 2020-01-02;130(1):523–38.
6. Christiaansen AF, Knudson CJ, Weiss KA, Varga SM. The CD4 T cell response to respiratory syncytial virus infection. IMMUNOL RES [Journal Article; Review]. 2014;2014-08-01(1-3):109–17. 59(.
7. Manson ML, Safholm J, James A, Johnsson AK, Bergman P, Al-Ameri M, et al. IL-13 and IL-4, but not IL-5 nor IL-17A, induce hyperresponsiveness in isolated human small airways. J Allergy Clin Immunol. [Journal Article; Research Support, Non-U.S. Gov't]. 2020 2020-03-01;145(3):808–17.
8. Schwarze J, Cieslewicz G, Hamelmann E, Joetham A, Shultz LD, Lamers MC, et al. IL-5 and eosinophils are essential for the development of airway hyperresponsiveness following acute respiratory syncytial virus infection. J IMMUNOL. [Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.]. 1999 1999-03-01;162(5):2997–3004.
9. Schwarze J, Hamelmann E, Bradley KL, Takeda K, Gelfand EW. Respiratory syncytial virus infection results in airway hyperresponsiveness and enhanced airway sensitization to allergen. J CLIN INVEST. [Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.]. 1997 1997-07-01;100(1):226–33.

10. Bertrand P, Lay MK, Piedimonte G, Brockmann PE, Palavecino CE, Hernandez J, et al. Elevated IL-3 and IL-12p40 levels in the lower airway of infants with RSV-induced bronchiolitis correlate with recurrent wheezing. CYTOKINE. [Journal Article; Research Support, Non-U.S. Gov't]. 2015 2015-12-01;76(2):417–23.
11. Lukens MV, Kruijsen D, Coenjaerts FE, Kimpen JL, van Bleek GM. Respiratory syncytial virus-induced activation and migration of respiratory dendritic cells and subsequent antigen presentation in the lung-draining lymph node. J VIROL. [Journal Article; Research Support, Non-U.S. Gov't]. 2009 2009-07-01;83(14):7235–43.
12. Walker JA, Barlow JL, McKenzie AN. Innate lymphoid cells–how did we miss them? NAT REV IMMUNOL. [Journal Article; Research Support, Non-U.S. Gov't; Review]. 2013 2013-02-01;13(2):75–87.
13. Wu J, Cui Y, Zhu W, Bai S, Zhao N, Liu B. Critical role of OX40/OX40L in ILC2-mediated activation of CD4(+)T cells during respiratory syncytial virus infection in mice. INT IMMUNOPHARMACOL. [Journal Article]. 2019 2019-11-01;76:105784.
14. Liu B, Kimura Y. Local immune response to respiratory syncytial virus infection is diminished in senescence-accelerated mice. J GEN VIROL. [Journal Article; Research Support, Non-U.S. Gov't]. 2007 2007-09-01;88(Pt 9):2552–8.
15. Halim TY, Krauss RH, Sun AC, Takei F. Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. IMMUNITY. [Journal Article; Research Support, Non-U.S. Gov't]. 2012 2012-03-23;36(3):451–63.
16. Oliphant CJ, Hwang YY, Walker JA, Salimi M, Wong SH, Brewer JM, et al. MHCII-mediated dialog between group 2 innate lymphoid cells and CD4(+) T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. IMMUNITY. [Journal Article; Research Support, Non-U.S. Gov't]. 2014 2014-08-21;41(2):283–95.
17. Schuijs MJ, Hammad H, Lambrecht BN. Professional and 'Amateur' Antigen-Presenting Cells In Type 2 Immunity. TRENDS IMMUNOL. [Journal Article; Review]. 2019 2019-01-01;40(1):22-34.
18. Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Singleton RJ, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. LANCET. [Journal Article; Meta-Analysis; Research Support, Non-U.S. Gov't; Review; Systematic Review]. 2010 2010-05-01;375(9725):1545–55.
19. Peebles RJ, Aronica MA. Proinflammatory Pathways in the Pathogenesis of Asthma. CLIN CHEST MED. [Journal Article; Research Support, N.I.H., Extramural; Review]. 2019 2019-03-01;40(1):29–50.
20. Hwang YH, Paik MJ, Yee ST. Diisononyl phthalate induces asthma via modulation of Th1/Th2 equilibrium. TOXICOL LETT. [Journal Article]. 2017 2017-04-15;272:49–59.
21. Asayama K, Kobayashi T, D'Alessandro-Gabazza CN, Toda M, Yasuma T, Fujimoto H, et al. Protein S protects against allergic bronchial asthma by modulating Th1/Th2 balance. ALLERGY. [Journal Article; Research Support, Non-U.S. Gov't]. 2020 2020-09-01;75(9):2267–78.

22. Choi EH, Lee HJ, Yoo T, Chanock SJ. A common haplotype of interleukin-4 gene IL4 is associated with severe respiratory syncytial virus disease in Korean children. J INFECT DIS. [Journal Article]. 2002 2002-11-01;186(9):1207–11.
23. van der Sande MA, Kidd IM, Goetghebuer T, Martynoga RA, Magnusen A, Allen S, et al. Severe respiratory syncytial virus infection in early life is associated with increased type 2 cytokine production in Gambian children. CLIN EXP ALLERGY. [Journal Article; Research Support, Non-U.S. Gov't]. 2002 2002-10-01;32(10):1430–5.
24. Vivier E, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, et al. Innate Lymphoid Cells: 10 Years On. CELL. [Journal Article; Research Support, N.I.H., Extramural; Research Support, Non-U.S. Gov't; Review]. 2018 2018-08-23;174(5):1054–66.
25. Artis D, Spits H. The biology of innate lymphoid cells. NATURE. [Journal Article; Research Support, N.I.H., Extramural; Research Support, Non-U.S. Gov't; Review]. 2015 2015-01-15;517(7534):293–301.
26. Fonseca W, Lukacs NW, Elesela S, Malinczak CA. Role of ILC2 in Viral-Induced Lung Pathogenesis. FRONT IMMUNOL. [Journal Article; Research Support, N.I.H., Extramural; Review]. 2021 2021-01-20;12:675169.
27. Van Dyken SJ, Nussbaum JC, Lee J, Molofsky AB, Liang HE, Pollack JL, et al. A tissue checkpoint regulates type 2 immunity. NAT IMMUNOL. [Journal Article]. 2016 2016-12-01;17(12):1381–7.
28. Han X, Bai S, Cui Y, Zhu W, Zhao N, Liu B. Essential role of CD4(+) T cells for the activation of group 2 innate lymphoid cells during respiratory syncytial virus infection in mice. IMMUNOTHERAPY-UK. [Journal Article; Research Support, Non-U.S. Gov't]. 2019 2019-10-01;11(15):1303–13.
29. Drake LY, Iijima K, Kita H. Group 2 innate lymphoid cells and CD4+ T cells cooperate to mediate type 2 immune response in mice. ALLERGY. [Journal Article; Research Support, N.I.H., Extramural; Research Support, Non-U.S. Gov't]. 2014 2014-10-01;69(10):1300–7.
30. Halim TY, Steer CA, Matha L, Gold MJ, Martinez-Gonzalez I, McNagny KM, et al. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. IMMUNITY. [Journal Article; Research Support, Non-U.S. Gov't]. 2014 2014-03-20;40(3):425–35.
31. Halim TY, Hwang YY, Scanlon ST, Zaghouani H, Garbi N, Fallon PG, et al. Group 2 innate lymphoid cells license dendritic cells to potentiate memory TH2 cell responses. NAT IMMUNOL. [Journal Article; Research Support, Non-U.S. Gov't]. 2016 2016-01-01;17(1):57–64.
32. Schwartz C, Khan AR, Floudas A, Saunders SP, Hams E, Rodewald HR, et al. ILC2s regulate adaptive Th2 cell functions via PD-L1 checkpoint control. J EXP MED. [Journal Article]. 2017 2017-09-04;214(9):2507–21.
33. Lambrecht BN, Salomon B, Klatzmann D, Pauwels RA. Dendritic cells are required for the development of chronic eosinophilic airway inflammation in response to inhaled antigen in sensitized mice. J IMMUNOL. [Journal Article; Research Support, Non-U.S. Gov't]. 1998 1998-04-15;160(8):4090–7.

34. Lambrecht BN, De Veerman M, Coyle AJ, Gutierrez-Ramos JC, Thielemans K, Pauwels RA. Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. J CLIN INVEST. [Journal Article; Research Support, Non-U.S. Gov't]. 2000 2000-08-01;106(4):551–9.
35. Unanue ER, Turk V, Neefjes J. Variations in MHC Class II Antigen Processing and Presentation in Health and Disease. ANNU REV IMMUNOL. [Journal Article; Review; Research Support, N.I.H., Extramural; Research Support, Non-U.S. Gov't]. 2016 2016-05-20;34:265–97.
36. Fernando MM, Stevens CR, Walsh EC, De Jager PL, Goyette P, Plenge RM, et al. Defining the role of the MHC in autoimmunity: a review and pooled analysis. PLOS GENET. [Journal Article; Research Support, N.I.H., Extramural; Review]. 2008 2008-04-25;4(4):e1000024.

Figures

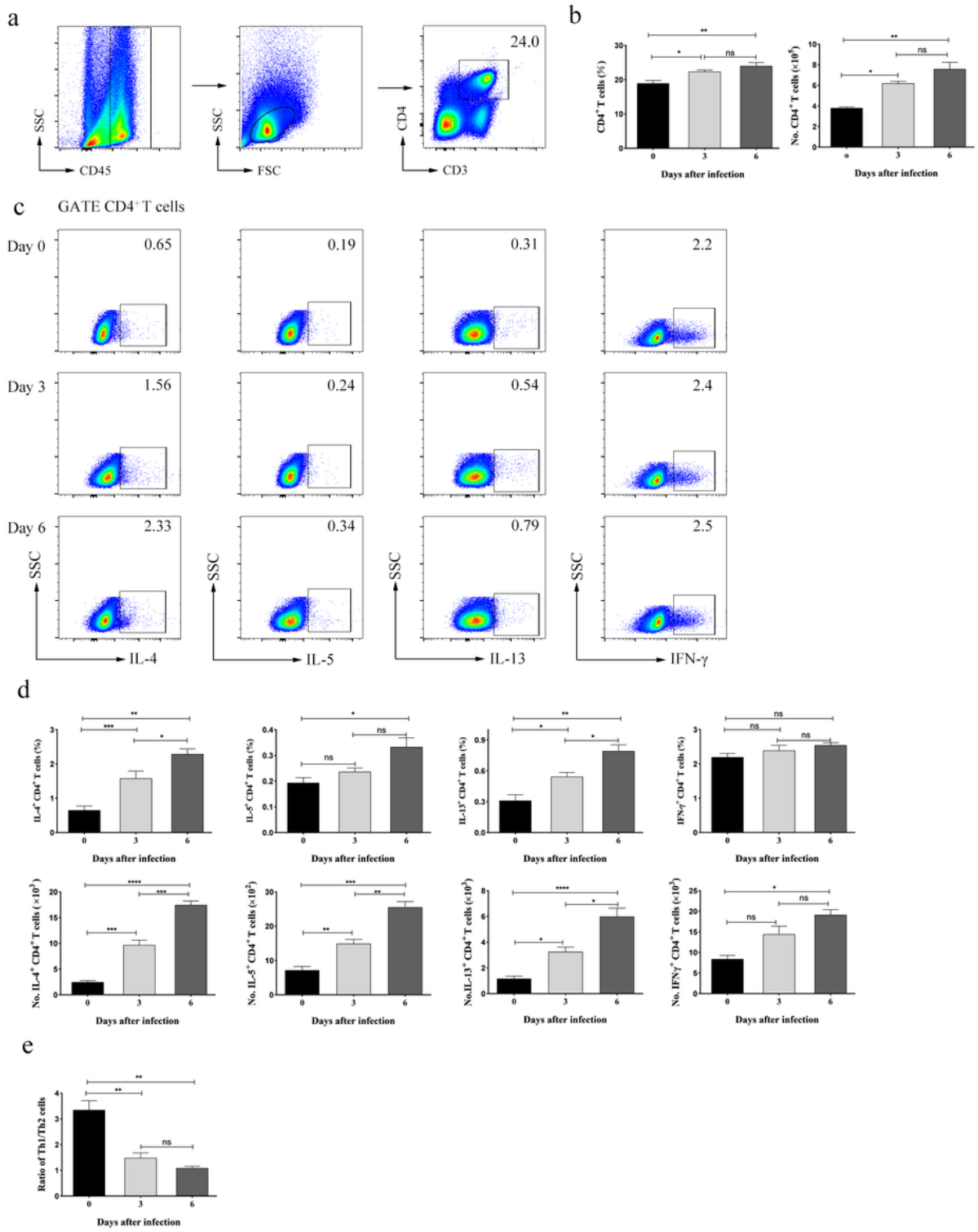

Figure 1

RSV infection induces CD4+ T cell activation. (a–c) The gating strategies of flow cytometry. (b) The frequency and absolute numbers of total CD4+ T cells (d) and IFN-γ-, IL-4-, IL-5- and IL-13-producing CD4+ T cells in the lungs of RSV-infected mice. (e) The ratio of IFN-γ-producing CD4+ T cells/IL-4-producing CD4+ T cells in the lungs on day 6 after RSV infection. Data indicate mean ± SD of each group (n =

5/group). Statistically significant differences are shown (∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001, ****p < 0.0001, ns =non-significant).

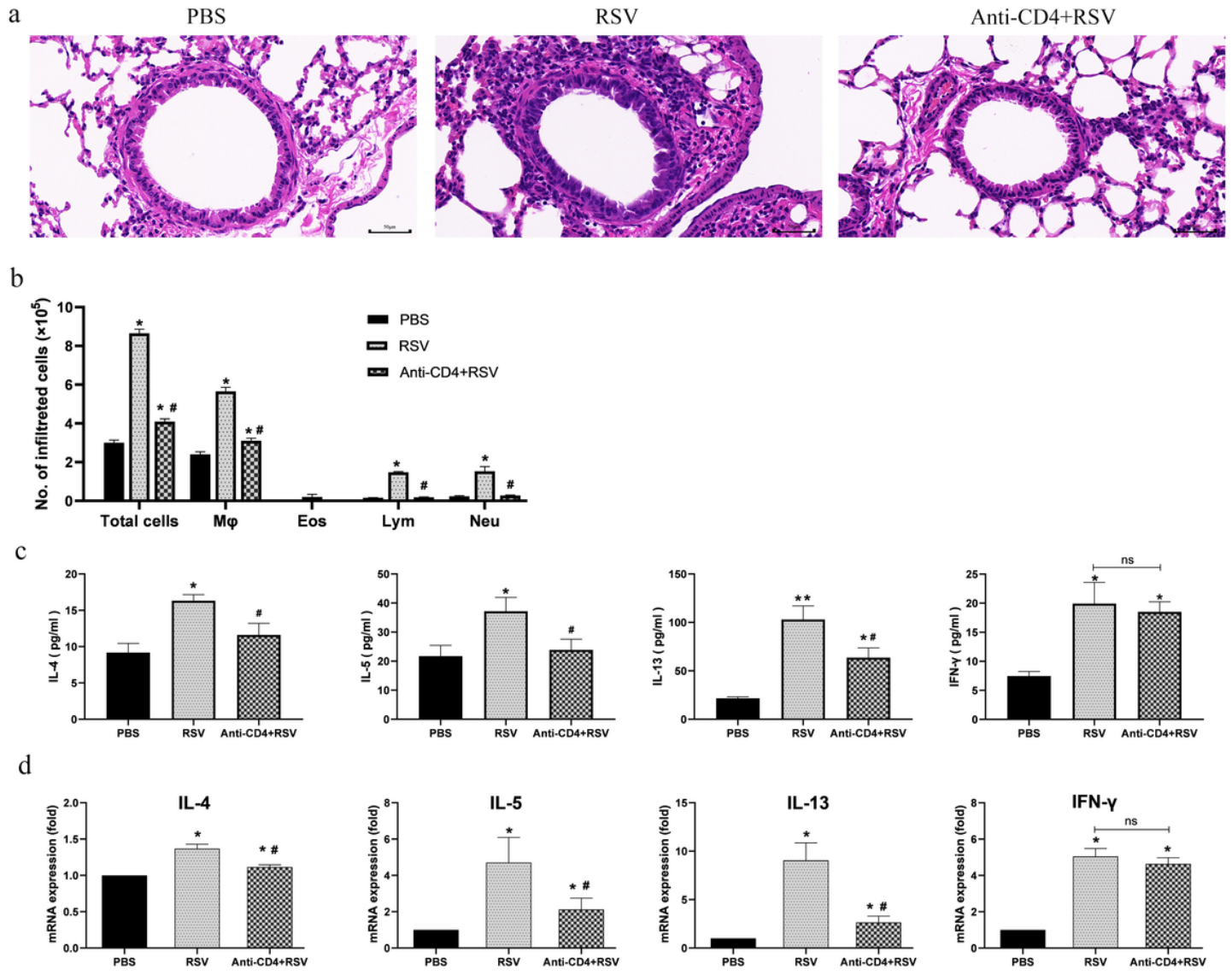

Figure 2

In vivo depletion of CD4+ T cells alleviates RSV-induced airway inflammation. BALB/c mice were intraperitoneally injected with anti-CD4 mAbs at a dose of 250 μg per mouse 24h before RSV infection. On day 6 after RSV infection, the mice were sacrificed, and bronchoalveolar lavage fluid (BALF) and lung tissue were harvested. (a) RSV-induced airway inflammation was determined by HE staining. (b) Inflammatory cells in BALF of the tested mice were counted. (c) The protein levels of IL-4, IL-5, IL-13, and IFN-γ in the supernatant of BALF were determined by ELISA. (d) The mRNA levels of IL-4, IL-5, IL-13, and IFN-γ in the lung homogenates were detected by real-time PCR. Data indicate mean ± SD of each group (n = 4/group). Statistically significant differences are shown (*p < 0.05 , compared with the group of control, #p < 0.05, compared with the group of RSV infected, ns =non-significant).

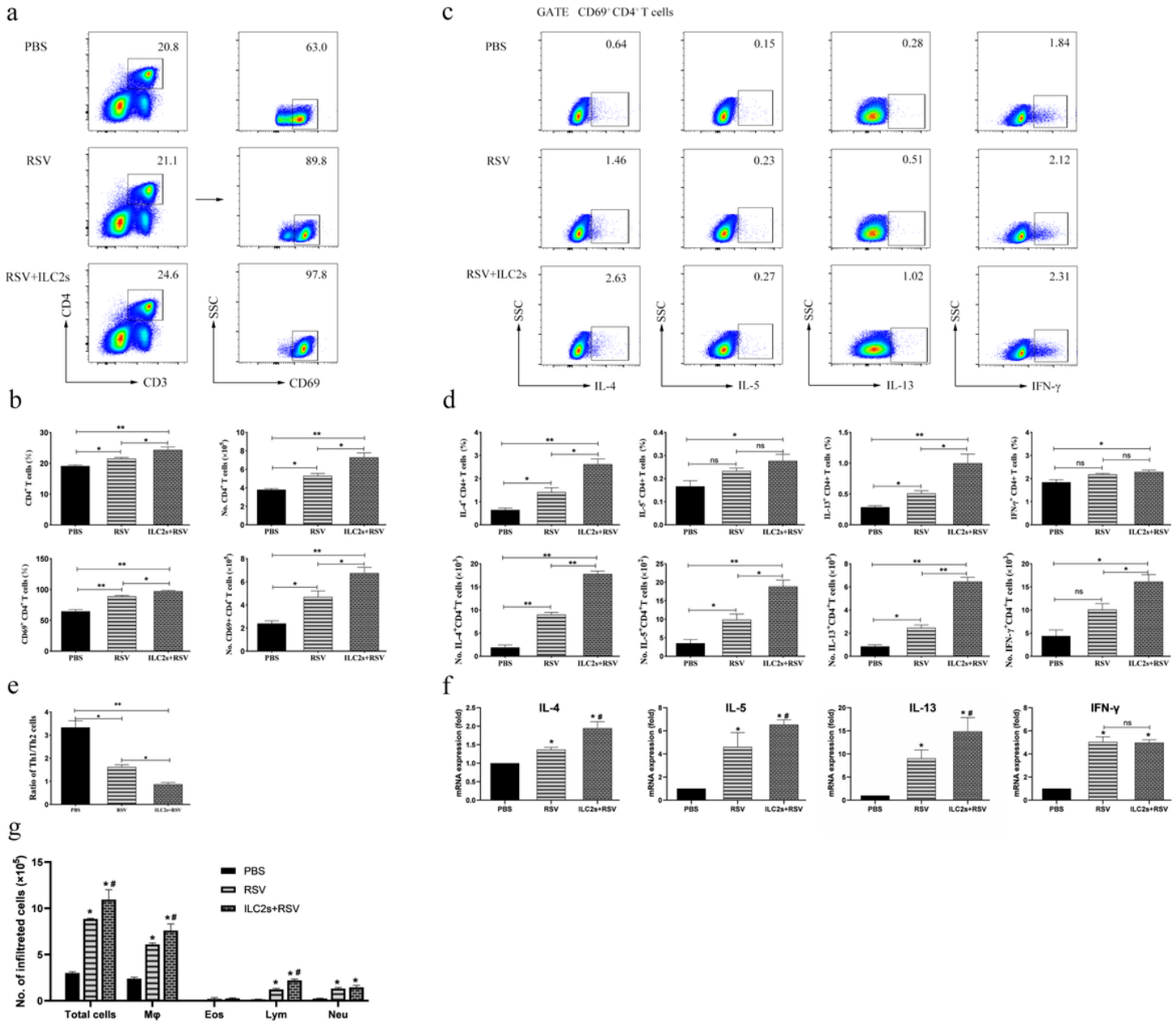

Figure 3

Adoptive transfer of pulmonary ILC2s augments CD4+ T cell expansion and Th2 differentiation in the lungs. CD45+ Lin- ST2+ ILC2s were isolated from the lungs of RSV-infected mice and injected via the tail vein into normal mouse 2 h before RSV infection. Lung cells were isolated on day 6 after RSV infection and assayed by flow cytometry. (a, c) The gating strategies of flow cytometry. (b) The frequencies and absolute numbers of total CD4+ T cells and CD69+ CD4+ T cells. (d) The frequencies and absolute numbers of IFN-γ-, IL-4-, IL-5- and IL-13-producing CD4+ T cells. (e) The ratio of Th1/Th2 in the lungs. (f) The mRNA levels of IL-4, IL-5, IL-13, and IFN-γ in the lung homogenates were detected by real-time PCR. (g) Inflammatory cells in BALF of the tested mice were counted. Data indicate mean ± SD of each group (n = 4/group). Statistically significant differences are shown (* $p < 0.05$, compared with the group of control, # $p < 0.05$, compared with the group of RSV infected, ns =non-significant).

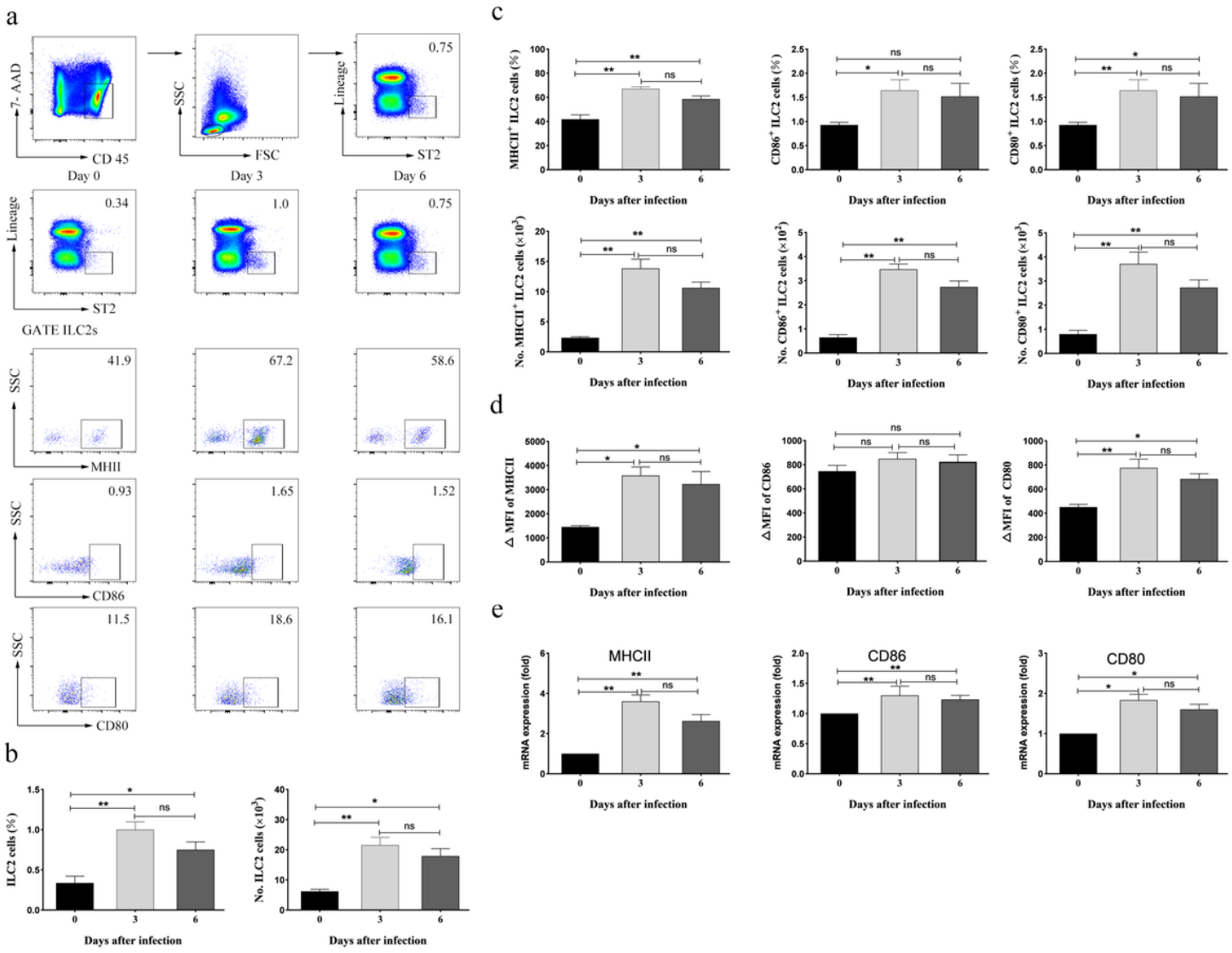

Figure 4

Adoptive transfer of pulmonary ILC2s augments CD4+ T cell expansion and Th2 differentiation in the lungs. CD45+ Lin- ST2+ ILC2s were isolated from the lungs of RSV-infected mice and injected via the tail vein into normal mouse 2 h before RSV infection. Lung cells were isolated on day 6 after RSV infection and assayed by flow cytometry. (a, c) The gating strategies of flow cytometry. (b) The frequencies and absolute numbers of total CD4+ T cells and CD69+ CD4+ T cells. (d) The frequencies and absolute numbers of IFN-γ-, IL-4-, IL-5- and IL-13-producing CD4+ T cells. (e) The ratio of Th1/Th2 in the lungs. (f) The mRNA levels of IL-4, IL-5, IL-13, and IFN-γ in the lung homogenates were detected by real-time PCR. (g) Inflammatory cells in BALF of the tested mice were counted. Data indicate mean ± SD of each group (n = 4/group). Statistically significant differences are shown (* $p < 0.05$, compared with the group of control, # $p < 0.05$, compared with the group of RSV infected, ns =non-significant).

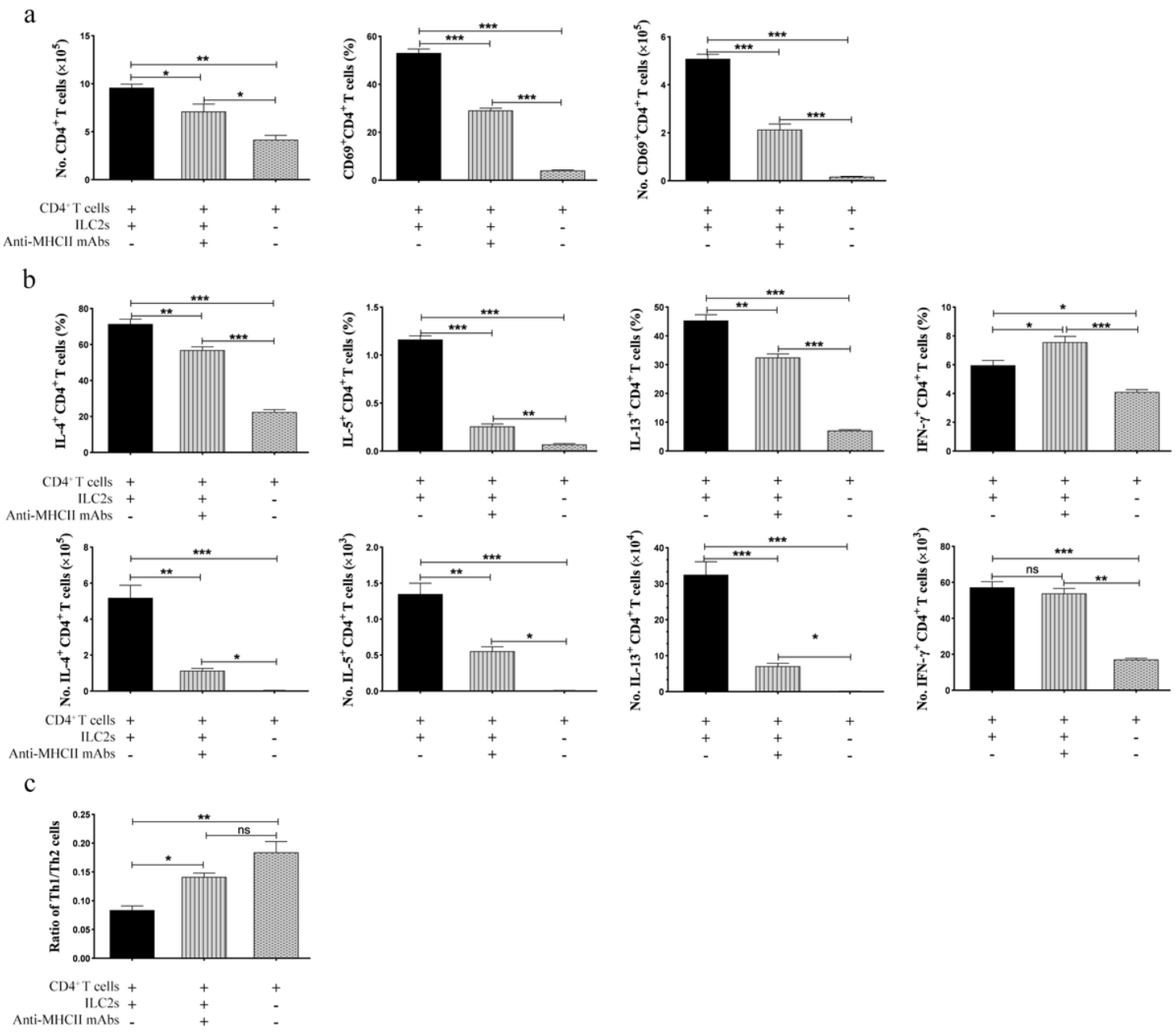

Figure 5

MHC II contributes to ILC2-regulated CD4+ T cell activation. ILC2s were sorted from the lungs of mice on day 3 after RSV infection and co-cultured with naïve splenic CD4+ T cells in vitro in the presence or absence of anti-MHC II mAbs (0.5μg/mL) for 72 hours. (a) The frequencies and absolute numbers of CD4+ T cells and CD69+ CD4+ T cells. (b) The frequencies and absolute numbers of IFN-γ-,IL-4-, IL-5- and IL-13-producing CD4+ T cells. (c) The ratio of Th1/Th2 in the cultured CD4+ T cells. Data indicate mean ± SD of each group (n = 5/group). Statistically significant differences are shown (∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001, ns =non-significant).