



Respiratory syncytial virus prevents the subsequent development of ovalbumin-induced allergic responses by inhibiting ILC2 via the IL-33/ST2 pathway

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Aim: How respiratory syncytial virus (RSV) influences the development of ovalbumin (OVA)-induced asthma remains elusive. As potent T helper (Th)2 cytokine producers, group 2 innate lymphoid cells (ILC2s) are known to serve important functions in the pathogenesis of allergic inflammation. However, how RSV infection affects innate immunity, especially with regard to the function of ILC2s in OVA-induced allergic airway inflammation, is largely unknown. **Materials & methods:** RSV was used to infect adult BALB/c mice intranasally prior to sensitization and subsequent challenge with OVA. ILC2 frequencies and Th2 cytokine production by ILC2s were assessed by flow cytometry. Cytokine levels were detected both by real-time PCR and ELISA. **Results:** Previous infection with RSV attenuated airway inflammation and decreased Th2 cytokine production in mice sensitized and challenged with OVA. Furthermore, previous infection with RSV inhibited the influx of ILC2s into the lung, and constrained their Th2 cytokine production. Adoptive transfer of ILC2s increased asthma-associated airway inflammation in mice previously infected with RSV. These results indicate that previous infection with RSV prevents OVA-induced asthma development via inhibition of ILC2s. Previous infection with RSV attenuated IL-33 production in lung tissue and reduced relative ST2L expression in lung ILC2s, meaning that previous infection with RSV may alter ILC2 function via the IL-33/ST2 signaling pathway. **Conclusion:** These results demonstrate that previous infection with RSV attenuates OVA-induced airway inflammation by inhibiting the recruitment and Th2 cytokine production of ILC2s via the IL-33/ST2 pathway.

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Asthma is a disease of chronic airway inflammation and hyperresponsiveness. Allergic asthma is associated with a number of immunological responses, including eosinophilia, excessive mucus production, goblet cell hyperplasia and smooth-muscle hypertrophy [1]. Asthma is a major public health threat, affecting over 300 million people worldwide [2]. Of patients with asthma, about 80% of children and 50% of adults suffer from allergic asthma [3]. Acute periods of asthma exacerbation are often associated with viral respiratory infections, which are present in up to 85% of children and 80% of adults who are experiencing asthma episodes [4,5]. Respiratory syncytial virus (RSV), which is often associated with asthma attacks, is a negative-sense, single-stranded RNA virus [6]. Whether RSV infection affects immune responses in ovalbumin (OVA)-sensitized mice remains controversial. Most recent studies have found that severe cases of RSV significantly increase the risk of children subsequently developing allergic asthma [7,8]. Recently, several studies have shown that in some contexts, RSV infection may actually be protective against the induction of allergic reactions [9,10]. We have previously demonstrated that RSV can prevent OVA-induced allergic response development via Th2-type $\gamma\delta$ T-cell inhibition [11]. However, whether other innate immune cells also participate in this process as well as the mechanisms by which they function remain unclear.

Group 2 innate lymphoid cells (ILC2s) are an innate lymphoid cell population that produce type 2 cytokines such as IL-5 and IL-13 upon stimulation with epithelium-derived cytokines [12]. ILC2s are thought to mediate asthma development and progression [13]. IL-13-producing ILC2s are upregulated during allergic lung inflammation and promote airway hyperreactivity in OVA-induced asthmatic mice [14]. Moreover, ILC2s are key sources of IL-5 and IL-13 in IL-25/IL-33-induced asthma [15]. How RSV infection affects innate immune responses remains poorly characterized, especially with regard to the function of ILC2s in OVA-induced allergic airway inflammation. Herein, we elucidate the means by which ILC2s inhibit OVA-induced airway inflammatory responses during RSV infection prior to sensitization. Our results demonstrate that previous RSV infection diminishes OVA-induced airway inflammation by inhibiting the recruitment and Th2 cytokine production of ILC2s via the IL-33/ST2 signaling pathway.

Materials & methods

Animals

Female BALB/c mice from the China Medical University (Shenyang, China) laboratory animal center were used at 6–8 weeks of age. Mice were caged in specific pathogen-free conditions, with *ad libitum* food and water access. Mice infected with virus or inoculated with allergens were housed in isolator units containing HEPA filters (AH model; Nihon-Ika, Osaka, Japan) to avoid contamination. All experiments were conducted under the ethical review of the Institutional Animal Care and Use Committee of China Medical University, China.

Virus

Human RSV type A2 (RSV A2) was obtained as a gift from B Wang of the Center for Disease Control and Prevention, Shenyang, China. The virus was replicated using a HEp-2 cell monolayer of American Type Culture Collection (ATCC, VA, USA) in media containing 2% fetal calf serum. When cells exhibited a clear cytopathic effect consistent with maximal viral replication, the remaining cells were collected and sonicated to release viral progeny. Viral titers were measured based on the 50% tissue culture infectious dose, calculated as previously described [16].

Experimental protocol

The protocols for OVA sensitization, airway challenge and RSV infection are described below. Grade V chicken OVA (Sigma-Aldrich, MO, USA) was used to both sensitize and challenge mice as previously described [14]. Briefly, intraperitoneal administration of 20 µg of OVA complexed with 2.25 mg aluminum hydroxide (100 µl, total volume) or phosphate buffered saline (PBS) and alum only (controls), at days 0 and 12 was used to sensitize mice. On days 19–24, mice were challenged with 1% aerosolized OVA (or PBS control) for 20 min per day. In RSV experiments, 7 days prior to OVA sensitization mice were infected intranasally with 10⁶ TCID₅₀ of RSV in a 20 µl volume. Two days after final OVA exposure, mice were sacrificed and tissues were collected for analysis. Mice were categorized into three experimental groups of five mice each: Mock (PBS-sensitized/challenged); OVA (OVA-sensitized/challenged); RSV/OVA (RSV infected prior to OVA sensitization).

Analysis of bronchoalveolar lavage fluid

Bronchoalveolar lavage (BAL) fluid was collected via washing the lungs of sacrificed mice twice with 1 ml phosphate-buffered saline (PBS). Total cellular infiltrate in BAL was assessed using a hemocytometer, Cytospin slides were fixed and stained with Wright–Giemsa stain, and composition was assessed in a blinded manner via counting 200 or more cells using a light microscope.

Flow cytometric analysis

Minced lung tissue was digested at 37°C for 1 h in RPMI-1640 medium (Hyclone, UT, USA) containing 10% fetal calf serum, 40 µg/ml DNase I and 200 µg/ml collagenase D (Roche Molecular Biochemicals, MA, Germany). Lung cells were prepared as single cell suspensions after density-gradient centrifugation using a solution designed for lymphocyte separation.

For ILC2 identification in murine lungs, lung cells were blocked using anti-CD16/32 (Biolegend) and were then stained with isotype control antibodies or specific monoclonal antibodies for 20 min on ice. Antibodies used were as follows: anti-CD45-APC (eBioscience, CA, USA), anti-ST2-PerCP (eBioscience) and FITC-conjugated antilineage 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 TCR δ . Flow cytometry was then used to analyze cells. Pulmonary ILC2s were those cells observed to be CD45⁺Lin⁻ST2⁺.

To assess ILC2 production of cytokines, lung cell digests were stimulated for 4 h *in vitro* using 100 ng/ml of phorbol 12-myristate 13-acetate (Sigma-Aldrich) and 1 ng/ml ionomycin (Sigma-Aldrich) plus 10 mg/ml brefeldin A (Sigma-Aldrich) at 37°C in an incubator (5% CO₂). The Cyto Fix/Perm kit (BD Pharmingen, CA, USA) was then used based on the manufacturer's protocols, and cells were then stained for intracellular IL-4 (PE-IL-4, eBioscience), IL-5 (PE-IL-5, eBioscience) and IL-13 (PE-IL-13, eBioscience).

ILC2 culture & ex vivo stimulation

ILC2s, isolated based on the markers detailed above, were purified with a MoFlo cell sorter (DakoCytomation, CA, USA). Purified ILC2s were more than 90% pure, and these cells were then stimulated for 24 h *in vitro* using 10 ng/ml IL-2 plus 30 ng/ml IL-33 (R&D Systems, MN, USA). *IL-4*, *IL-5*, *IL-13* and *ST2L* mRNA expression in these cells were then analyzed after cell harvesting and RNA preparation.

Gene expression assessment

Total RNA was isolated via extraction from purified ILC2s or total lung homogenates using TRIzol (Life Technologies, CA, USA). The SuperScript III Reverse Transcriptase (Life Technologies) kit was then used to prepare cDNA from this isolated RNA. Power SYBR[®] Green PCR Master Mix (Life Technologies) was next used to perform qPCR with the following target gene primer sets being used: IL-13-F, 5'-AGCATGGTATGGAGTGTGGA-3', IL-13-R, 5'-TTGCAATTGGAGATGTTGGT-3'; IL-4-F, 5'-TGTACCAGGAGCCATATCCA-3', IL-4-R, 5'-TTCTTCGTTGCTGTGAGGAC-3'; IL-5-F, 5'-GGCTTCCTGTCCCTACTCAT-3', IL-5-R, 5'-TCCTCGCCACACTTCTCTTT-3'; IFN- γ -F, 5'-TATCTGGAGGAAGTGGCAAA-3', IFN- γ -R, 5'-GGTGTGATTCAATGACGCTT-3'; IL-33-F, 5'-GATGGGAAGAAGCTGATGGTG-3', IL-33-R, 5'-TTGTGAAGGACGAAGAAGGC-3'; ST2L-F, 5'-CATGGCATGATAAGGCACAC-3', ST2L-R, 5'-GTAGAGCTTGCCATCGTTCC-3'; β -actin-F, 5'-CAACGAGCGTTCCGATG-3', β -actin-R, 5'-GCCACAGGATTCCATACCCA-3'.

A Rotor-Gene Q (QIAGEN) with identical reaction conditions was used for all experiments to assess gene expression. β -actin expression was used as a normalizing control, and fold changes in the resultant mRNA expression were then compared between groups (fold change = $2^{-\Delta\Delta CT}$).

Histological studies

10% neutral-buffered formalin was used to fix small portions of lung tissue, and thin sections (4 μ m) were cut from blocks and hematoxylin and eosin stained to assess peribronchial cell infiltration and periodic acid-Schiff stained to gauge the degree of increased goblet cell growth and mucin expression.

Assay of cytokine production

The levels of cytokine production in BAL fluids were quantified with murine IFN- γ (Catalog No. DY485-05), IL-4 (Catalog No. DY404-05), IL-5 (Catalog No. DY405-05), IL-13 (Catalog No. DY413-05) and IL-33 (Catalog No. DY3626-05) ELISA kits, which were purchased from R&D Systems.

Adoptive transfer of ILC2s

We adoptively transferred ILC2s to RSV/OVA mice to examine the role of ILC2s in OVA-induced allergic airway inflammation in the context of RSV infection. Naive BALB/c mice were intraperitoneally injected with IL-33 (400 ng), daily for 3 days. 24 h after the last injection, lung tissue was harvested and CD45⁺ Lin⁻ ST2⁺ ILC2s were FACS sorted. 2×10^4 purified cells in 200 μ l of PBS were delivered by tail vein injection into each mouse 1 h prior to RSV infection.

Statistical analysis

All data are shown as mean $\pm$ standard error. One-way analysis of variance tests were used to assess differences between multiple groups, using SPSS version 18.0 (SPSS, IL, USA). Significance was established as $p < 0.05$.

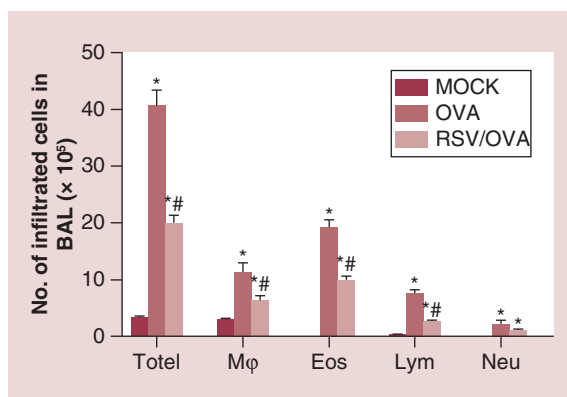


Figure 1. Cellular infiltration in the lungs of respiratory syncytial virus-infected, ovalbumin-sensitized mice.

BALB/c mice were infected intranasally with RSV at an inoculum dose of 2×10^6 TCID₅₀ per mouse before OVA sensitization. BAL fluids were collected 2 days after the last nebulized OVA exposure and analyzed for cellular infiltration. Data are representative of three independent experiments with five mice per group.

*Significant difference ($p < 0.05$ by ANOVA), compared with PBS-inoculated mice.

#Significant difference ($p < 0.05$ by ANOVA), compared with uninfected OVA mice.

BAL: Bronchoalveolar lavage; OVA: Ovalbumin; PBS: Phosphate-buffered saline; RSV: Respiratory syncytial virus.

Results

Previous infection with RSV attenuates OVA-induced airway inflammation

To determine how previous infection with RSV affects OVA-induced airway inflammation, cell numbers in BAL fluid were determined after Wright–Giemsa staining of cells obtained via cytopspin. OVA-sensitized/challenged mice exhibited significant increases in cell numbers, with significantly more lymphocytes, eosinophils, neutrophils and macrophages relative to control mice (Figure 1). Previous infection with RSV was associated with reduced inflammation in OVA-sensitized/challenged mouse lungs. These previously RSV-infected mice had significantly decreased BAL cellularity, with particularly clear decreases in the numbers of macrophages, eosinophils and lymphocytes in their lungs.

Consistent with the increased cellular infiltrate in the BAL fluid, OVA-sensitized and challenged mice presented with markedly higher levels of inflammatory cell infiltration in the perivascular and peribronchiolar connective tissues (Figure 2). In addition, these challenged mice exhibited significantly greater levels of mucus production in the lung relative to normal controls. Previous infection with RSV reduced both this elevated frequency of inflammatory cell infiltration and this excess mucus production, being instead associated with reduced airway epithelial cell hypertrophy. These results suggest that previous infection with RSV protects against airway inflammation associated with OVA challenge.

Previous infection with RSV decreases Th2 cytokine production but increases IFN- γ levels in OVA-sensitized/challenged mice

To determine how previous infection with RSV affects OVA-induced cytokine production, we measured Th1/Th2 cytokine production in the lungs both at the gene expression and protein levels 2 days after final OVA exposure. Concentrations of IL-4 (Figure 3A), IL-5 (Figure 3B) as well as IL-13 (Figure 3C) were all higher in the BAL fluid of the OVA group relative to mock controls, whereas no difference in IFN- γ levels was observed (Figure 3D). Relative to the OVA group, mice previously infected with RSV exhibited reduced concentrations of IL-5 and IL-13 as well as increased IFN- γ levels in BAL fluids. Consistent with these findings, mRNA levels of Th2-type cytokines including *IL-4* (Figure 3E), *IL-5* (Figure 3F) and *IL-13* (Figure 3G), but not the Th1-cytokine, *IFN- γ* (Figure 3H), were elevated in the lungs of OVA-challenged mice. Previous infection with RSV reduced the expression of these Th2-type cytokines, but increased the *IFN- γ* expression level. This suggests that previous infection with RSV thus inhibits Th2 cytokine production in OVA-sensitized and challenged mouse lungs.

Previous infection with RSV inhibits ILC2 influx & Th2 cytokine production in lung tissue

Recent work has highlighted a key role for ILC2s in allergic inflammation [14,17,18]. How previous infection with RSV affects ILC2 responses in the context of an OVA-induced allergic airway inflammation model, however, has not been assessed. Lung ILC2s were identified by FACS using the following cell-surface markers: ILC2s were positive for CD45 and IL-33 receptor (ST2) and negative for lineage markers (CD3, CD4, CD5, CD11b, CD11c, CD49b, Gr1, NK1.1, Fc ϵ RI, Ter119, Siglec F and F4/80). As shown in Figure 4, OVA treatment resulted in a significant increase in total ILC2 numbers and in the frequency of type-2 cytokine-secreting ILC2s, particularly IL-4-, IL-5- and IL-13-producing ILC2s in the lungs of mice that had been OVA-sensitized and challenged. Previous infection with RSV, however, was associated with a decrease in ILC2 influx into the lungs of OVA-challenged mice. Not

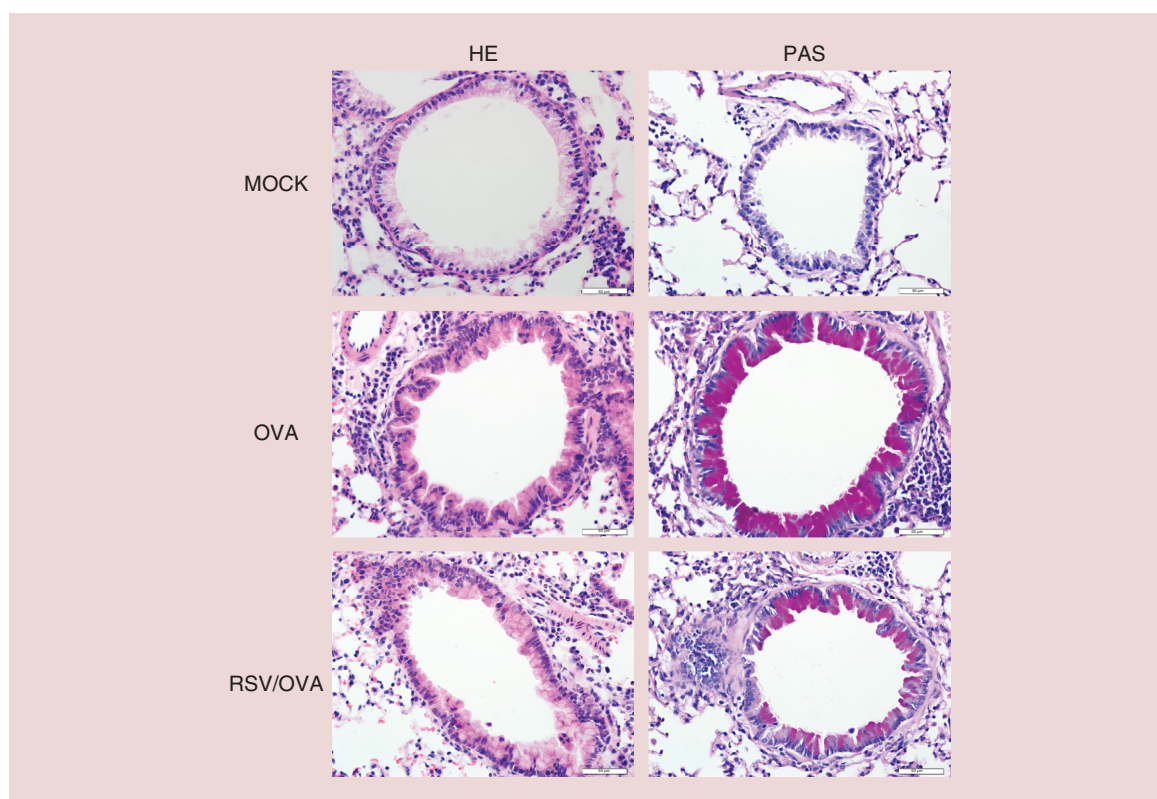


Figure 2. Histological examination of lung tissues from BALB/c mice. Mice were infected intranasally with RSV before OVA sensitization. Lung pathologic lesions were analyzed 2 days after the last nebulized OVA exposure. The section displayed is representative of those from two independent experiments containing five mice per group. Bar: 50 μ m. OVA: Ovalbumin; RSV: Respiratory syncytial virus.

only were ILC2 numbers lower in the lungs of previously RSV infected mice but those ILC2s present in the lung exhibited significantly diminished Th2 cytokine production relative to OVA-sensitized/challenged mice that were not previously infected.

To further assess how previous infection with RSV affects the Th2 cytokine production of ILC2s, ILC2s were sorted from the lungs of mice from the OVA and RSV/OVA groups, and *IL-4*, *IL-5* and *IL-13* expression levels were assessed via real-time PCR. Relative expression of all three of these cytokines was significantly higher in the ILC2s isolated from OVA mice (Figure 4). However, ILC2s from mice previously infected with RSV exhibited decreased *IL-5* and *IL-13* expression, whereas *IL-4* expression was unchanged. These data demonstrate that previous infection with RSV inhibits both ILC2 recruitment and proliferation, and further reduces the ability of these cells to secrete Th2 cytokines in murine lungs during OVA-induced asthma.

Adoptive transfer of ILC2s increases airway inflammation during asthma in mice previously infected with RSV

To further verify the importance of ILC2s for the development and progression of inflammatory allergic airway responses, lung ILC2 cells were collected from naive BALB/c mice and were transferred intravenously into recipient mice 1 h prior to RSV infection. Adoptive transfer of pulmonary ILC2s led to increased airway cellular infiltration and mucus-producing epithelial cell hypertrophy (Figure 5A & B) as well as augmented gene expression and production of IL-4, IL-5 and IL-13 in recipient mouse lungs (Figure 5C & D). These results indicate that lung ILC2 cells serve important proinflammatory drivers of allergen-induced airway inflammation.

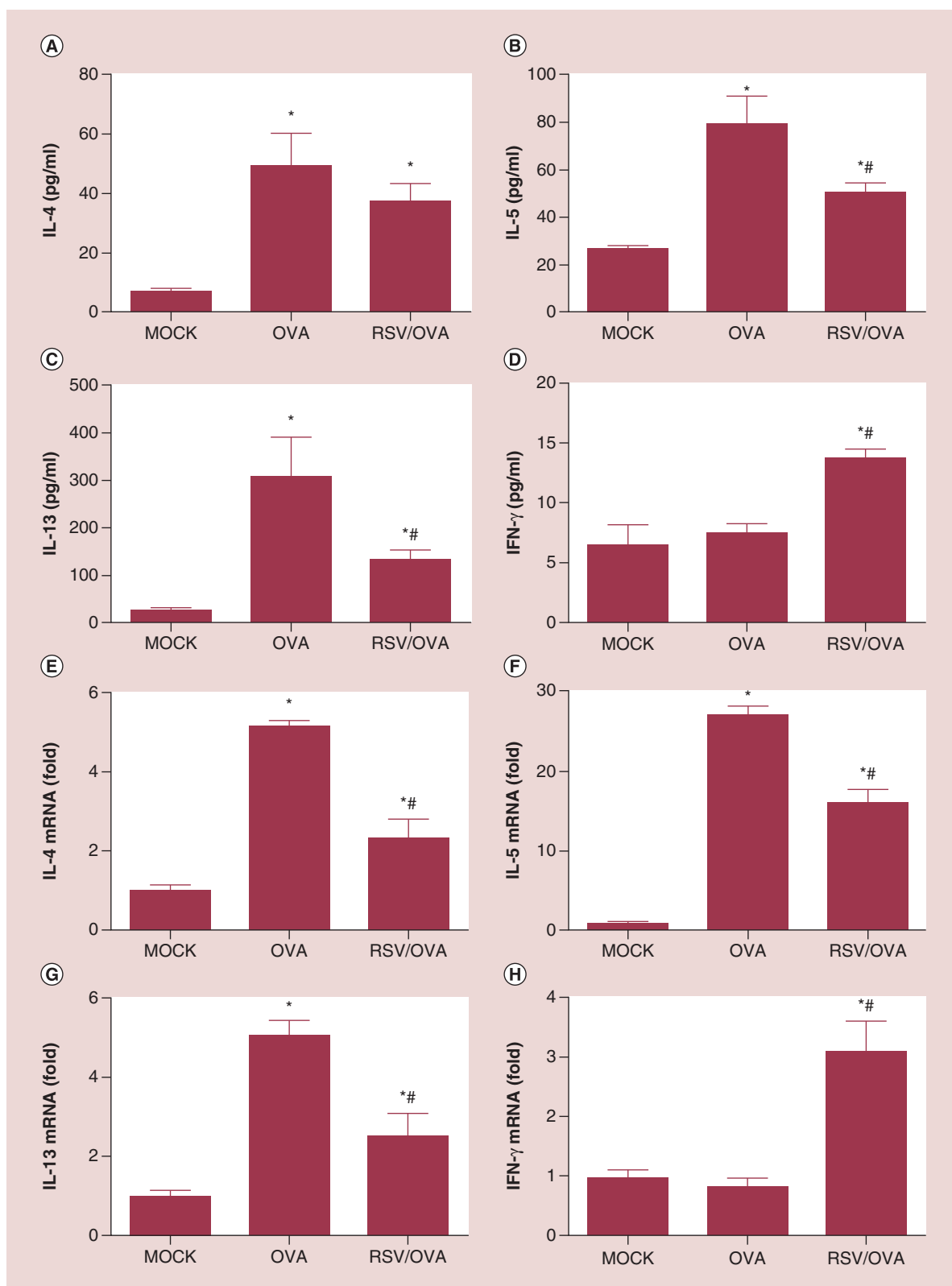


Figure 3. Prior respiratory syncytial virus infection decreases Th2 cytokine production but increases IFN- γ levels in ovalbumin-sensitized/challenged mice. BALB/c mice were infected intranasally with RSV at an inoculum dose of 2×10^6 TCID₅₀ per mouse before OVA sensitization. Protein levels of Th1/Th2 cytokines in BAL fluids were measured using ELISA 2 days after the last nebulized OVA exposure (A–D). mRNA expression of Th1/Th2 cytokines in mouse lung was analyzed using real-time RT-PCR (E–H). Data are representative of three independent experiments with five mice per group.

*Significant difference ($p < 0.05$ by ANOVA), compared with PBS-inoculated mice.

#Significant difference ($p < 0.05$ by ANOVA), compared with uninfected OVA mice.

BAL: Bronchoalveolar lavage; OVA: Ovalbumin; PBS: Phosphate-buffered saline; RSV: Respiratory syncytial virus.

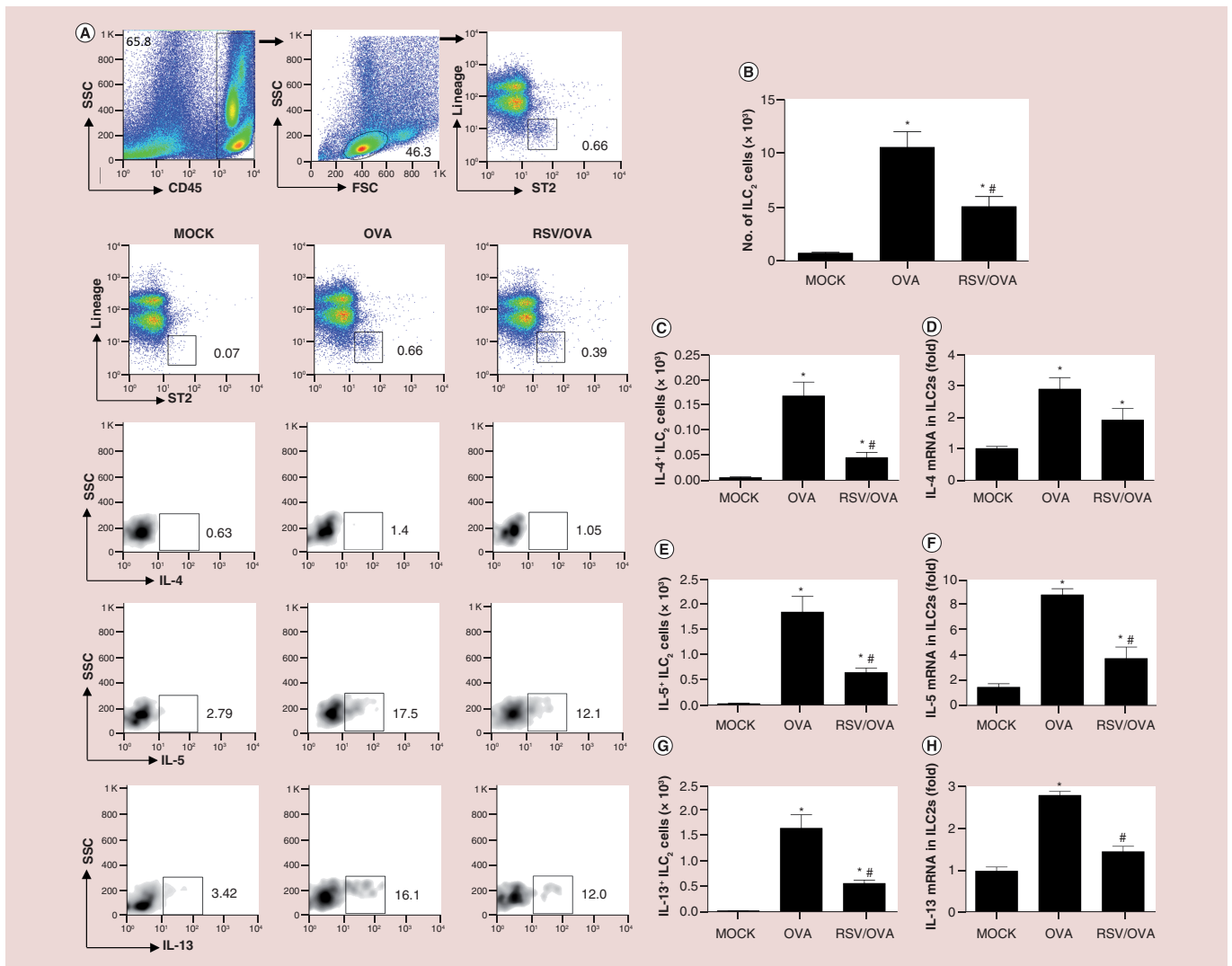


Figure 4. Infection with respiratory syncytial virus prior to ovalbumin sensitization inhibits the influx of ILC₂s into the lungs and reduces Th₂ cytokine production in ILC₂s. BALB/c mice were infected intranasally with RSV at an inoculum dose of 2×10^6 TCID₅₀ per mouse before OVA sensitization. ILC₂s were identified as CD45⁺Lin⁻ ST2⁺ (A). The absolute number of ILC₂s (B) as well as Th₂ cytokine production of ILC₂s (C, E & G) in mouse lung was detected using flow cytometry. For analysis of the expression of IL-4, IL-5, and IL-13 mRNA in pulmonary ILC₂s, CD45⁺Lin⁻ ST2⁺ cells were FACS-sorted from pooled lungs and analyzed immediately using real-time RT-PCR (D, F & H). Data are representative of three independent experiments with five mice per group.

*Significant difference ($p < 0.05$ by ANOVA), compared with PBS-inoculated mice.

#Significant difference ($p < 0.05$ by ANOVA), compared with uninfected OVA mice.

OVA: Ovalbumin; PBS: Phosphate-buffered saline; RSV: Respiratory syncytial virus.

Previous infection with RSV blocks the influx & function of ILC₂s in a murine model of OVA-induced asthma via the IL-33/ST2 pathway

IL-33 is a cytokine related to IL-1 that is thought to support the development of allergic responses and to be especially important for Th₂ responses. ILC₂s have been shown to express ST2L, the portion of the IL-33 receptor responsible for ligand binding and to increase production of Th₂ cytokines upon IL-33 stimulation [19–21]. Our data imply that previous infection with RSV inhibits ILC₂ influx and Th₂ cytokine production in OVA-sensitized/challenged mice lungs. Therefore, we hypothesized that previous infection with RSV may disrupt IL-33/ST2 signaling and suppress ILC₂ activity. BAL fluid and lung tissue were harvested 2 days after final OVA exposure and mRNA and protein levels of IL-33 were measured. IL-33 levels were significantly lower in the BAL fluid and lung homogenates of previously RSV-infected mice (Figure 6A), supporting the hypothesis that IL-33 signaling is a component of the mechanism, whereby RSV infections mitigate OVA-induced airway inflammation. Consistently, *ST2L* mRNA

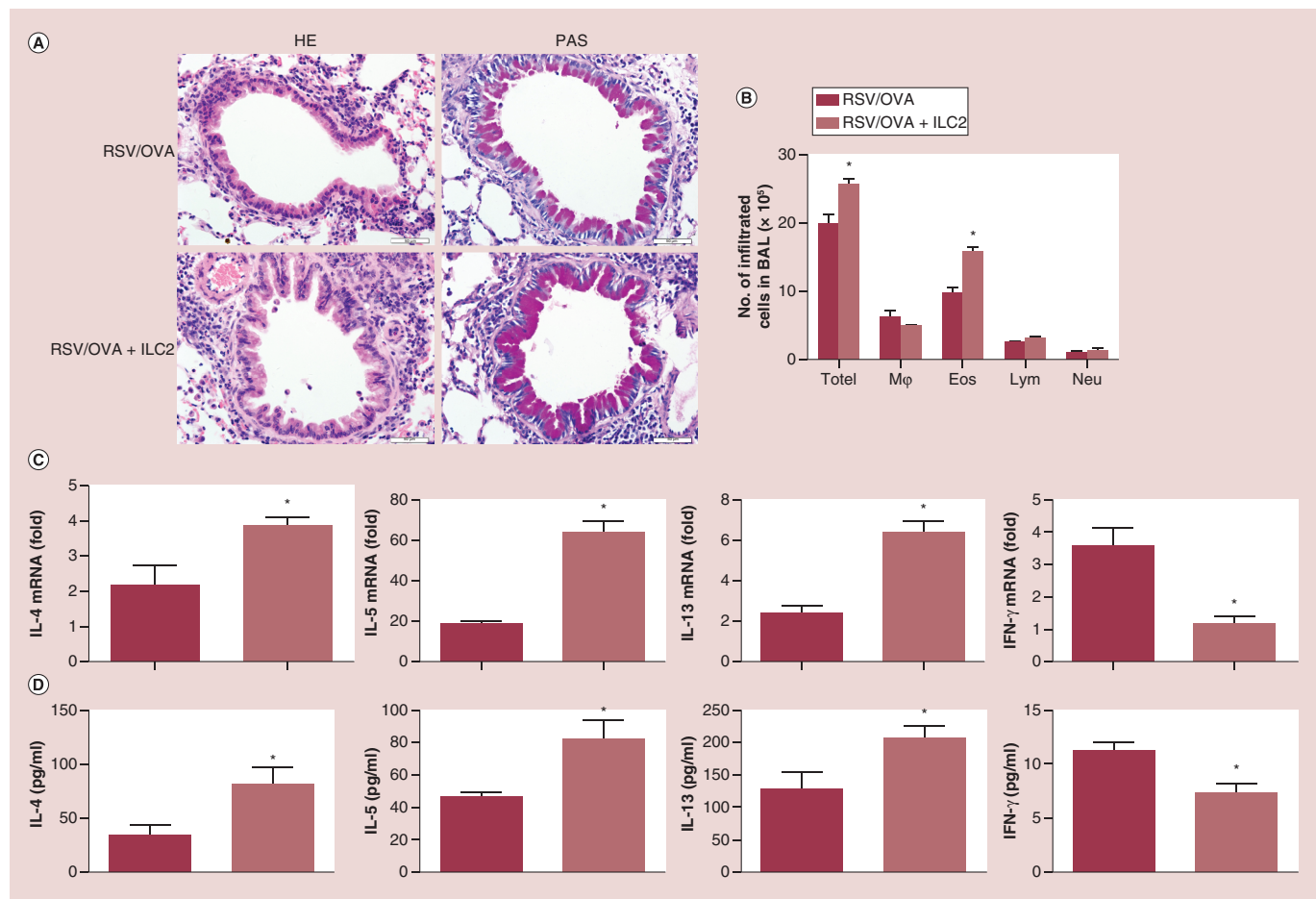


Figure 5. Adoptive transfer of pulmonary ILC2 cells augments airway inflammation in asthmatic mice with prior respiratory syncytial virus infection. Pulmonary CD45⁺Lin⁻ST2⁺ILC2s isolated from naïve BALB/c mice were transferred intravenously into recipient mice 1 hour prior to RSV infection. Histopathology of the lung (A), BAL fluid cells (B), levels of Th2 cytokines in BAL fluids (C), and gene expression of Th2 cytokines in lung (D) were investigated. Data are representative of three independent experiments with five mice per group.

*Significant difference ($p < 0.05$ by ANOVA), compared with non-transferred mice.

BAL: Bronchoalveolar lavage; OVA: Ovalbumin; RSV: Respiratory syncytial virus.

levels in ILC2s were reduced in the lungs of RSV/OVA mice relative to uninfected OVA-induced asthma control mice (Figure 6B). Together these findings demonstrate that previous infection with RSV inhibits the ILC2 lung influx and Th2 cytokine production during asthma via the IL-33/ST2 pathway.

Discussion

The pathogenesis of bronchial asthma is not fully understood. Many studies suggest that a disruption of the Th1/Th2 ratio is the foundation for the development of asthma [22,23]. Other studies have reported that respiratory virus infection can affect this Th1/Th2 balance, thereby potentially leading to the genesis and development of asthma [24]. However, the influence of RSV infection on allergic asthma is unclear. In this study, we report that the absolute number of inflammatory cells, particularly lymphocytes, macrophages and eosinophils in the BAL fluid of RSV/OVA mice was decreased relative to mice not previously infected with RSV (Figure 1). Inflammatory lung tissue infiltration, mucus secretion and goblet cell hyperplasia were all alleviated in mice previously infected with RSV (Figure 2), suggesting that RSV infection prior to OVA sensitization can mitigate the severity of airway inflammation in asthmatic mice. Th2 inflammation, driven by IL-4, IL-5 and IL-13, is thought to be one of the key signaling mechanisms driving the development of asthma [25]. Our study assessed IL-4, IL-5, IL-13 and IFN-γ expression in this mouse model, and our results indicate that both BAL IL-5 and IL-13 protein levels and lung IL-4, IL-5 and IL-13 mRNA levels were decreased in mice previously infected with RSV, whereas levels of IFN-γ

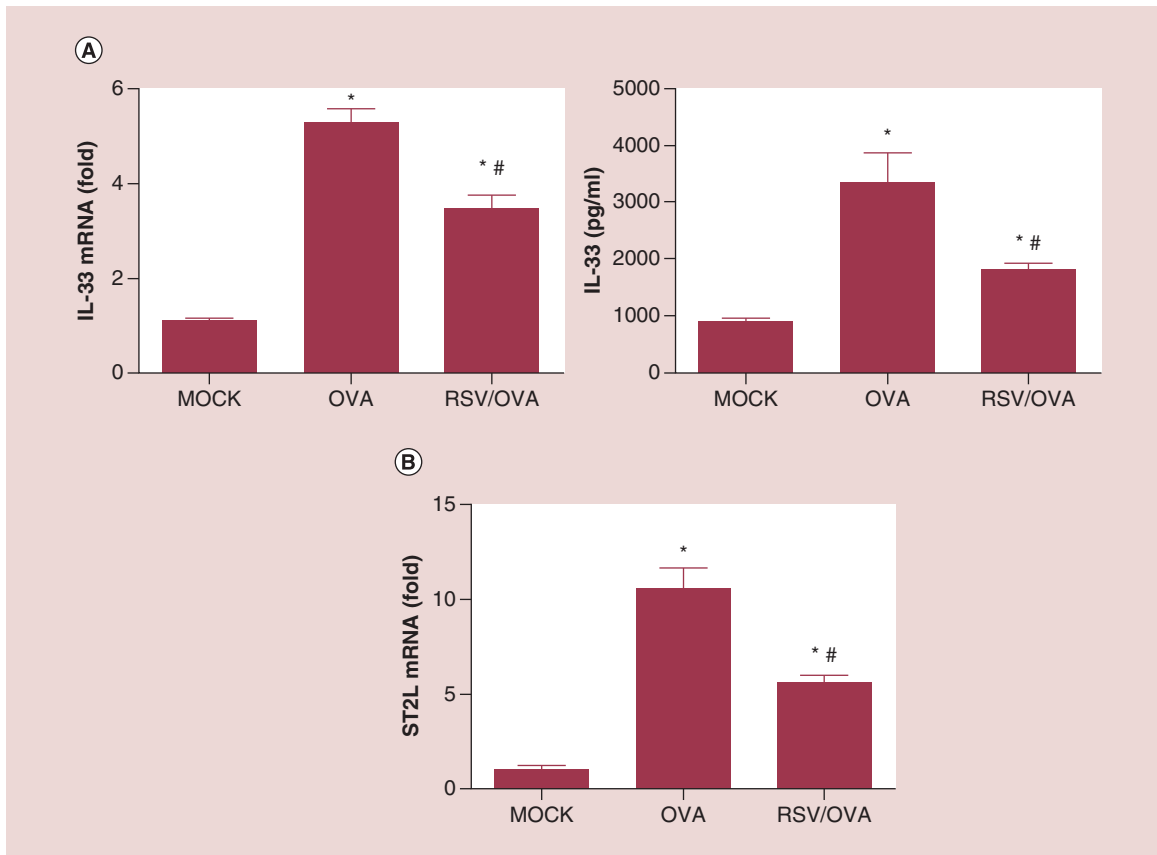


Figure 6. Prior respiratory syncytial virus infection induces a decrease in IL-33 and ST2L mRNA expression in ovalbumin-sensitized mice. BALB/c mice were infected intranasally with RSV at an inoculum dose of 2×10^6 TCID₅₀ per mouse prior to OVA sensitization. Two days after the last nebulized OVA exposure, BAL fluids were collected and assayed for IL-33 protein levels using an ELISA kit; lungs were harvested and analyzed for IL-33 mRNA gene expression (A). CD45⁺Lin⁻ ST2⁺ ILC2s were FACS-sorted from pooled lungs and re-stimulated in vitro with 30 ng/ml of IL-33 and 10 ng/ml of IL-2 for 24 h. The relative expression of mRNA for ST2L (B) in pulmonary ILC2s was analyzed.

*Significant difference ($p < 0.05$ by ANOVA), compared with PBS-inoculated mice.

#Significant difference ($p < 0.05$ by ANOVA), compared with uninfected OVA mice.

BAL: Bronchoalveolar lavage; OVA: Ovalbumin; PBS: Phosphate-buffered saline; RSV: Respiratory syncytial virus.

were significantly elevated in these same mice (Figure 3). These findings support a model wherein RSV infection induces a Th1 response, and thereby inhibits the OVA-induced Th2 reaction in the airway.

While asthma was originally postulated to be linked primarily to Th2 cells, more recent work suggests that type 2 cytokine production by ILC2s may in fact be a key driver of asthma development [14]. ILC2s are a recently identified innate lymphoid population secreting IL-13 and IL-5 as well as other Th2 cytokines as a response to signals from proximal epithelial cells [12]. In this study, we have analyzed the number of ILC2s in the lung tissue of mice subjected to the OVA asthma model system, and we further assessed Th2 cytokine production by these cells. We discovered that, relative to OVA-sensitized/challenged mice, the number of ILC2s in the RSV/OVA group was significantly lower, and fewer of these ILC2s secreted IL-4, IL-15 and IL-13 (Figure 4). These results verify that ILC2s are indeed a component of the means by which previous infection with RSV suppresses inflammation in the lungs of mice with OVA-induced asthma. To further verify the role of ILC2s in the RSV infection-mediated reversal of lung inflammation in asthmatic mice, we transplanted ILC2s into RSV-infected mice with OVA-induced asthma. These experiments revealed that inflammatory cell numbers in the lung were increased after transfer; moreover, alleviation of inflammation in these asthmatic mice was partially impaired (Figure 5). This indicates that ILC2s can promote the genesis and development of inflammatory reactions in the respiratory tract of mice in the context of the OVA asthma model. Furthermore, the ability of previous RSV infection to inhibit inflammation in lungs of mice with OVA-induced asthma is connected to the number and activity of the downregulated ILC2s.

IL-33 is an IL-1-related nuclear cytokine that has been shown to mediate type-2 immune responses [26–28]. IL-33 has been shown to promote the expansion of ILC2s both *in vitro* and *in vivo* via ST2 receptor signaling [29–31]. In this study, RSV infection prior to OVA sensitization attenuated IL-33 production in the lungs (Figure 6A). Relative *ST2L* expression in pulmonary ILC2s was also reduced (Figure 6B), implying that previous infection with RSV may influence the function of ILC2s via the IL-33/ST2 signaling pathway, thus mitigating the severity of airway inflammation in OVA-induced asthmatic mice.

Our findings indicate that RSV infection prior to OVA sensitization can inhibit the influx and activity of ILC2s, reducing their IL-4, IL-5 and IL-13 production via the IL-33/ST2 signaling pathway. Therefore, RSV infection prior to sensitization plays an inhibitory role in the genesis and development of airway inflammation in asthma. Surprisingly, this reveals that, under certain conditions, viral infection is in fact beneficial to asthmatic patients. Even so, RSV infection has been shown in numerous clinical studies to be closely related to asthma. Consequently, the relationship between RSV and asthma remains controversial. In recent years, some studies have shown that the timing of infection relative to the onset of asthma [32], the frequency of RSV infection [10] and age of infected patient [33] may all affect RSV-induced airway inflammation and the development of asthma. In our study, RSV infection was induced prior to OVA-sensitization in adult BALB/c mice. We found that RSV infection downregulated the Th2-type immune response induced by the subsequently introduced allergen, OVA. These conditions conferred some protection from respiratory inflammation in this murine model of asthma.

Conclusion

In conclusion, this study demonstrates that previous infection with RSV alleviates allergic airway inflammation by inhibiting pulmonary ILC2 influx and Th2 cytokine production through inhibition of the IL-33/ST2-signaling pathway, thus offering protection from the respiratory inflammation associated with asthma.

Summary points

- Respiratory syncytial virus (RSV) infection prior to ovalbumin (OVA) sensitization can decrease OVA-induced airway inflammation in adult BALB/c mice.
- Prior RSV infection inhibits ILC2 influx and Th2 cytokine production in the lungs of OVA-sensitized and challenged mice.
- Prior RSV infection diminishes the influx and function of ILC2s by inhibiting the IL-33/ST2-signaling pathway.

Author's contributions

B Liu designed the experiments. D Wang, S Bai, Y Cui, N Zhao, F Qi, J Liu, S Zeng, L Xu and H Hu performed the experiments. D Wang, S Bai, Y Cui, N Zhao, F Qi, J Liu, S Zeng, L Xu, H Hu and B Liu analyzed the data. All the authors contributed to the writing and editing of the manuscript.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

Animal study received China Medical University Animal Care and Use Committee approval.

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