



# Adenovirus-mediated interleukin-35 gene transfer suppresses allergic airway inflammation in a murine model of asthma

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## Abstract

**Objective and design** Asthma is thought to result from the generation of T helper type 2 (Th2) responses, leading to bronchial inflammation. Interleukin (IL)-35 is a recently described member of IL-12 cytokine family that plays a critical role in influencing Th cell differentiation and inflammatory processes. The aim of this study was to examine the effect of adenovirus expressing IL-35 (AdIL-35) on allergic airway hyperresponsiveness (AHR) and inflammation in a mouse model of asthma.

**Methods** BALB/c mice were subjected to an established model of allergic airway disease. AdIL-35 was administered intranasally and the effect of IL-35 on Th2 responses, pulmonary inflammation, goblet cell metaplasia, and AHR were assessed.

**Results** Transfer of AdIL-35 significantly reduced the severity of AHR and numbers of inflammatory cells and levels of IL-4, IL-5, IL-13, and IL-17 in bronchoalveolar lavage fluid, compared with administration of a control virus. Moreover, AdIL-35 elevated the numbers of CD4+CD25+Foxp3+ regulatory T cells in the lungs.

Histological analysis showed that AdIL-35 inhibited allergic lung tissue inflammation and mucus hypersecretion.

**Conclusion** These results demonstrate that adenovirus-mediated delivery of interleukin-35 gene can mitigate allergic airway inflammation in experimental asthma and suggest that IL-35 may offer a novel therapeutic approach to treat allergic asthma.

**Keywords** IL-35 · Allergic asthma · Eosinophils · Th17 cells · Regulatory T cells · Hyperresponsiveness · Airway inflammation

## Introduction

Allergic disease is a major health problem, and the prevalence of allergic asthma is increasing worldwide. Asthma is a chronic airway inflammation that is characterized by intense eosinophil infiltrates, mucus hypersecretion, airway remodeling, and airway hyperresponsiveness (AHR) [1, 2]. These pathognomonic features are mediated mainly by antigen-specific CD4+ T helper type 2 (Th2) cells and their cytokines such as interleukin (IL)-4, IL-5, and IL-13 [1, 3]. These Th2 cytokines induce, prolong, and amplify the inflammatory response by enhancing the production of allergy-specific immunoglobulin E (IgE); enhancing recruitment, growth, and differentiation of eosinophils; and directly causing AHR [4, 5]. Other CD4+ Th effectors subsets, such as Th1, Th17, and CD4+CD25+Foxp3+ regulatory T (Treg) cells also participate in disease pathogenesis [6]. Depending on the specific disease model and stage of inflammation, Th1 cells can either exacerbate allergic symptoms and asthma, and the expression of the Th1 cytokine interferon (IFN)- $\gamma$

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correlates with antigen-induced airway hyperresponsiveness and eosinophilic infiltration [7], or dampen allergic inflammation [8]. Th17 cells are correlated with neutrophilic airway inflammation and resistance to corticosteroid treatment in moderate-to-severe asthmatics [9]. Treg cells inhibit the development of allergy via several mechanisms, including suppression of other effector Th1, Th2, and Th17 cells; suppression of eosinophils, mast cells, and basophils; Ab isotype change from IgE to IgG4; suppression of inflammatory dendritic cell (DC); and suppression of inflammatory cell migration to tissues [10]. Apart from T lymphocytes, the process of airway inflammation involves various types of cells, such as eosinophils, neutrophils, mast cells, and DCs [11].

IL-35 is the most recently identified member of the IL-12 cytokine family, a heterodimeric cytokine composed of an Epstein–Barr virus-induced gene 3 (EBI3) chain and an IL-12p35 chain [12, 13]. IL-35 is highly expressed in Treg cells, but not in effector or naive T cells and suppresses inflammatory responses of immune cells. It has been reported that IL-35 induces proliferation of Treg cell [14], but inhibits the proliferation of CD4+CD25<sup>−</sup> T cells and reduces the activity of the Th17 cell populations in vitro [13]. In animal studies, administration of recombinant IL-35 can inhibit experimental colitis [15, 16] and clinical manifestation of collagen-induced arthritis or cease further disease exacerbation [13, 17]. It has been identified that IL-35 induces regulatory B cells that suppress autoimmune and infectious diseases [18, 19]. Intramuscular injection of IL-35 plasmid DNA can attenuate dust mite allergen-specific murine CD4+Th2 cell-mediated airway inflammation [20]. Wong et al. [21] reported recently that plasma IL-35 concentration was significantly elevated in asthmatic patients, but other studies showed that IL-35 protein and mRNA levels were decreased in allergic asthmatics [22, 23]. Dong et al. have demonstrated that IL-35 can act as a crucial regulatory cytokine to inhibit the development of allergic airway inflammation via suppressing the formation of inflammatory DC [24]. Moreover, IL-35 production by inducible costimulator-positive Treg cells can suppress IL-17 production and reverse IL-17-dependent AHR in mice [25].

In the present study, we evaluated the effect of adenovirus expressing mouse IL-35 (AdIL-35) on allergic airway inflammation and AHR in a mouse model of asthma. Our results demonstrate that intranasal (i.n.) delivery of AdIL-35 may inhibit allergic airway inflammation and decrease the severity of AHR in experimental asthma by down-regulation of antigen-driven Th2-mediated responses, inducing augmentation of CD4+CD25+Foxp3+ Treg cells and suppression of Th17 cells. It provides direct evidence supporting the role of IL-35 in the regulation of allergy-induced pulmonary allergic inflammation.

## Materials and methods

### Animals

Female BALB/c mice, 8–10 weeks of age and free of murine-specific pathogens, were obtained from the Shanghai Experimental Animal Center of Chinese Academy of Sciences (Shanghai, China). The mice were housed in a controlled environment with a 12 h light/dark cycle, had free access to food and water, and were maintained on ovalbumin (OVA)-free diets. All experimental animals used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the Ningbo University or Shantou University.

### Sensitization and antigen challenge

Mice were sensitized and challenged as described previously with a slight modification [26]. Briefly, mice were sensitized on days 1 and 14 by intraperitoneal (i.p.) injection of 20 µg of OVA (Grade V; Sigma-Aldrich, St. Louis, MO) emulsified in 2 mg of aluminum hydroxide gel (alum, Sigma) in a total volume of 200 µl. On days 28, 29, and 30 after the initial sensitization, the mice were challenged for 30 min daily with an aerosol of 1 % (wt/vol) OVA in sterile phosphate-buffered saline (PBS) (or with PBS as a control) using an ultrasonic nebulizer (Yuyue, Jiangsu, China). Twenty-four hours after the last exposure to OVA or PBS, mice were anesthetized and connected to a computer-controlled small animal ventilator (flexiVent, SCIREQ, Montreal, Canada), and AHR to intravenous methacholine (Mch) (Sigma) was measured with the pulmonary resistance (RL) as described previously [27]. Bronchoalveolar lavage fluid (BALF) and tissues were prepared for further analyses.

### Adenoviral vectors and administration of AdIL-35

IL-35 was constructed by linking EBI3 and IL-12p35 with a linker peptide (3 × GGGGS) in a pcDNA3.1 vector (Invitrogen). Briefly, EBI3 and IL-12p35 cDNA PCR fragments were amplified from mouse splenocyte cDNA, using primer sequences as follows—EBI3 sense: 5'-CTC GGA TCC GAC ATG TCC AAG CTG CTC TTC-3', and EBI3 anti-sense: 5'-GCC ACC GCC GCT TCC ACC GCC ACC GGG CTT ATG GGG TGC-3'; IL-12p35 sense: 5'-GGC GGA AGC GGC GGT GGC GGC AGC AGG GTC ATT CCA GTC-3', and IL-12p35 anti-sense: 5'-CCG CTC GAG GGC GGC GGC CGC ATA GCC CAT CAC CCT GTT GA-3'. A BamH I site (shown in italics) was introduced into the EBI3 sense primer. An Xho I site (shown in italics) was introduced into the IL-12p35 anti-sense primer.

The additional ATG for initiation of translation is shown in bold style. Linkers were introduced into the EBI3 anti-sense and the IL-12p35 sense primer, respectively. The two PCR-amplified cDNA fragments of mouse IL-35 were linked by overlapping PCR using EBI3 sense primer and IL-12p35 anti-sense primer. The PCR fragment, which encodes the EBI3, 3 × GGGGS linker, and IL-12p35 cDNA, was then inserted into the BamH I and Xho I sites of the pcDNA3.1 vector to generate the pcDNA3.1-mIL-35 vector.

Recombinant adenoviral vector was constructed using the Ad-Easy system that has been described previously [28]. Briefly, the IL-35 cDNA was inserted into a recombinant adenoviral shuttle vector (pAdTrack-CMV) and cotransformed into *Escherichia coli* BJ5183 cells with an adenoviral backbone plasmid (pAdEasy-1). Ad0 was identical with AdIL-35 structurally, but contained no transgene in the expression cassette. The linearized recombinant plasmid was transfected into the adenovirus packaging 293 cell line. Viruses were purified from infected cells 48 h after infection by three freeze–thaw cycles followed by successive banding on cesium chloride density gradient centrifugation. The purified virus was dialyzed and stored at  $-80^{\circ}\text{C}$  until needed. Viral titers were measured by standard end-point dilution assay using the 293 cells. Furthermore, IL-35 expression was confirmed by measuring the culture supernatants of the infected 293 cells (data not shown).

Mice were anesthetized and held supine at an angle of  $45^{\circ}$  with pressure applied to the lower mandible to immobilize the tongue and prevent swallowing. Twenty-four hours before the first challenge with OVA (day 27), AdIL-35 ( $1 \times 10^9$  pfu) was introduced to the nasal planum using a micropipette. Control mice received the same dose of control virus (Ad0). The dose of adenovirus ( $1 \times 10^9$  pfu) was chosen as previously described [28]. The dose ( $1 \times 10^9$  pfu/mice) can avoid serious inflammation by vectors and induce adequate target transgene expression [28].

### Bronchoalveolar lavage

Mice were anesthetized with a sodium pentobarbital, and the lungs were lavaged three times with 1.5 ml saline. Total cells and cell differentials were counted using a hemocytometer and Diff-Quik stain (Fisher Scientific, Pittsburgh, PA), respectively. The supernatant was stored at  $-80^{\circ}\text{C}$  for cytokine analysis.

### Histopathology

Mouse lungs were fixed in Carnoy's solution and embedded in paraffin using standard methods. Four-micrometer-

thick sections were stained with hematoxylin and eosin (H&E) or periodic acid–Schiff/alcan blue (PAS/AB).

### Enzyme-linked immunosorbent assay (ELISA)

Levels of IL-4, IL-5, IL-13, IFN- $\gamma$ , and IL-17 in BALF supernatants were measured using ELISA kits (Biosource, Camarillo, CA) according to the manufacturer's instructions. Quantifications of IL-35 in BALF supernatants were evaluated using commercially available ELISA (R&D Systems, Minneapolis, MN).

### Collection of lung cells and flow cytometry

Collection of lung cells was performed as described previously [29]. Briefly, lungs were removed, cut, and minced. Complete RPMI-1640 medium was added to the minced lungs and incubated for 60 min. The samples were filtered through 52- $\mu\text{m}$  nylon mesh. The single-cell suspensions were washed with complete RPMI 1640 medium, and erythrocytes were lysed with  $\text{NH}_4\text{Cl}$ –Tris buffer. Single-cell suspensions from lungs were resuspended at  $1 \times 10^6$  cells/ml and stained with FITC-labeled anti-CD4 and PE-labeled anti-CD25 mAbs (BioLegend, San Diego, CA). For Foxp3 expression, cells were stained for surface markers and then fixed, permeabilized, and stained with APC-labeled anti-Foxp3 (eBioscience, San Diego, CA) as recommended by the manufacturer. Flow cytometry was performed with an FACSCalibur (Becton-Dickinson, Mountain View, CA, USA) using CELLQuest software. Absolute numbers of cells were calculated by multiplying the percentage of positive staining cells in the total acquired events by the total number of cells isolated from the tissue analyzed.

### Statistical analysis

Data are presented as mean  $\pm$  standard error of the mean (SEM). Significant differences among groups were identified by ANOVA. Individual comparisons between groups were confirmed by the Student's *t* test. Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA). A *P* value of less than 0.05 was considered to be statistically significant.

## Results

### IL-35 expression in BALF after i.n. delivery of AdIL-35

To elucidate the effect of IL-35 administration on airway inflammation in OVA-sensitized mice, we applied AdIL-35

for gene transfer to induce a high level of IL-35 transgenic expression in the mouse lung. We examined the kinetics of the IL-35 expression after a single dose i.n. delivery of AdIL-35. BALF samples were collected at the indicated times after the i.n. instillation of AdIL-35 or control virus (Ad0). We found that the level of IL-35 started to increase at day 1, peaked at day 2 after the i.n. delivery of AdIL-35, and gradually decreased thereafter. However, it still remained high even 10 days after administration of AdIL-35 (Fig. 1). In contrast, there was no detectable IL-35 protein in the Ad0-treated mice. Of note, at the dose of  $1 \times 10^9$  pfu, there was no evidence of vascular congestion and hypersecretion of mucus in bronchioles as shown by lung H&E staining (data not shown).

### Effect of AdIL-35 on allergic airway inflammation

Total cell numbers in BALF were increased 24 h after the final antigen challenge in OVA-sensitized mice than in OVA-sensitized PBS-challenged mice. The increase of total cell numbers was associated with macrophage, eosinophils, lymphocytes, and neutrophils (Fig. 2a). As compared with Ad0, administration of OVA-sensitized and challenged mice with i.n. delivery of AdIL-35 reduced significantly the increase in total cell numbers in BALF ( $P < 0.01$ ). Specific decreases were observed in the number of eosinophils, lymphocytes, and neutrophils in BALF, compared with mice treated with Ad0 ( $P < 0.05$ ). These data demonstrate that IL-35 can modulate the inflammatory response by reducing the number of inflammatory cells in the airways of allergic mice.

Next, we assessed the impact of i.n. delivery of AdIL-35 on allergic AHR in a murine experimental model of

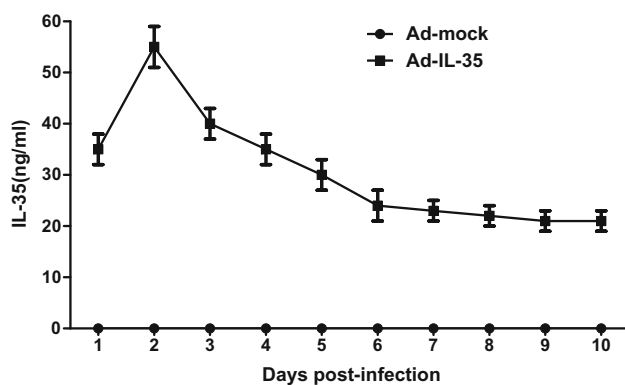


**Fig. 1** IL-35 protein expression in BALF after i.n. administration of AdIL-35. Groups of mice received a single i.n. delivery of 25  $\mu$ L of either AdIL-35 or Ad0 ( $1 \times 10^9$  pfu) on day 0. At different time points, BALF was collected from the mice and individually analyzed for the presence of IL-35 protein by a standard ELISA. The graph represents mean  $\pm$  SEM ( $n = 5$  per group) from at least three different samples

asthma. Antigen challenge of OVA-sensitized mice led to heightened AHR, as determined by increased airway resistance in response to doses of intravenous methacholine (Fig. 2b). Similarly, the airway resistance was increased in OVA-sensitized and OVA-challenged mice pretreated with Ad0. However, BALB/c mice that were sensitized and challenged with PBS revealed no increase in airway responsiveness. In contrast, pretreatment with AdIL-35 decreased significantly AHR as compared to Ad0- and OVA-treated groups (Fig. 2b).

Histological analysis revealed typical pathologic features of asthma-like inflammation in the OVA-sensitized and challenged mice stained with hematoxylin and eosin (H&E). OVA-exposed mice increased the numbers of inflammatory cells and interstitial proliferation in the lung tissue compared with the PBS control. AdIL-35 reduced the numbers of inflammatory cells in the peribronchial and alveolar regions (Fig. 2c). Furthermore, OVA-exposed mice showed goblet cell hyperplasia and mucus overproduction in the airways, which was significantly reduced by AdIL-35, as shown with periodic acid–Schiff/alcan blue (PAS/AB) staining (Fig. 2c). In contrast, Ad0 had no effect on the appearance of inflammatory cells in lung tissue or mucus hypersecretion. Taken together, these data suggest that IL-35 can inhibit inflammatory cell infiltration and goblet cell hyperplasia.

### Effect of AdIL-35 on Th1/Th2 and Th17 cytokines production in BALF

To determine the effect of AdIL-35 on airway inflammation, IFN- $\gamma$ , IL-4, IL-5, IL-13, and IL-17 concentrations in BALF were measured by ELISA 24 h after the last OVA challenge. AdIL-35 markedly decreased IL-4, IL-5, IL-13, and IL-17 production following OVA challenge ( $P < 0.05$ ) (Fig. 3). The production of IFN- $\gamma$  in BALF was not significantly changed by AdIL-35 (data not shown). Administration of OVA-exposed mice with Ad0 had no apparent effect on the concentrations of IL-4, IL-5, IL-13, IL-17, and IFN- $\gamma$ .

### Effect of AdIL-35 on the number of regulatory T cells in mice lung

To investigate whether AdIL-35 induce Treg cells, we analyzed the expression of CD4, CD25, and Foxp3 on lung cells of AdIL-35-treated mice. Flow cytometric analysis revealed that the proportion of CD4+25+Foxp3+ Treg cells in CD4+ gated lung cells populations was significantly elevated in AdIL-35-treated mice compared with the Ad0-treated groups (Fig. 4). Such increases in the percentage of CD4+CD25+Foxp3+ Treg cells were not

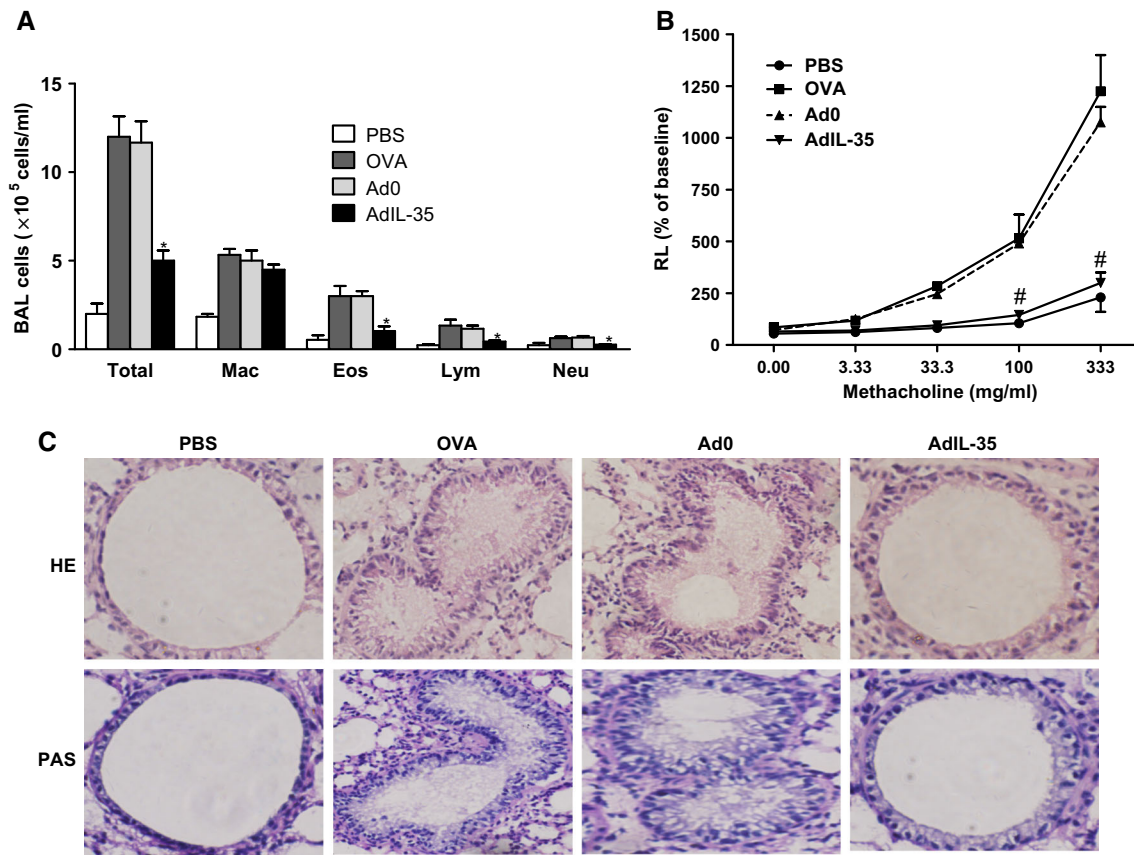


**Fig. 2** AdIL-35 reduces allergic airway inflammation in a mouse model of asthma. **a** AdIL-35 reduces inflammatory cell infiltration in BALF of OVA-sensitized/challenged mice. The number of total and differential cells of BALF from PBS-sensitized and -challenged mice (PBS), OVA-sensitized/challenged mice (OVA), OVA-sensitized/challenged mice with the administration of Ad0 and with the administration of AdIL-35 were determined 24 h after the last challenge. Data are representative of three separate experiments with similar results and expressed as the mean  $\pm$  SEM ( $n = 6-10$  per group). \*Significant differences ( $P < 0.05$ ) between sensitized/challenged control groups and sensitized/challenged AdIL-35 groups. **b** Effect of AdIL-35 on airway responsiveness to intravenous methacholine. RL was measured by means of forced oscillation.

Data are representative of three separate experiments with similar results and expressed as the mean  $\pm$  SEM ( $n = 6-10$  per group). #Significant differences ( $P < 0.05$ ) between AdIL-35 groups and Ad0 groups. **c** AdIL-35 reduces lung tissue eosinophilia and mucus secretion in OVA-sensitized/challenged mice. Lung tissue was fixed, sectioned at 4  $\mu$ m thickness and detected with H&E staining for tissue eosinophilia or PAS/AB staining for goblet cells and mucus production by light microscopy (original magnification:  $\times 400$ ). Lung tissues from PBS challenge, OVA challenge, Ad0 control and AdIL-35. Mice were processed 24 h after the last OVA challenge. A total of 6–10 mice in each group were analyzed and representative findings are shown

observed in Ad0-treated or OVA-sensitized and challenged groups compared with the PBS control groups (Fig. 4).

## Discussion

In this study, we investigated whether adenovirus-mediated delivery of IL-35 gene can affect AHR and allergic airway inflammation in a mouse model of asthma. We demonstrated that transfer of IL-35 gene significantly reduced the severity of AHR, numbers of inflammatory cells, and levels of IL-4, IL-5, IL-13, and IL-17 in BALF. Moreover, administration of AdIL-35 elevated the numbers of CD4+CD25+Foxp3+ Treg cells in the lungs. Histological

analysis showed that IL-35 inhibited allergic lung tissue inflammation and mucus hypersecretion.

The recruitment of differential leukocytes to the airways and lungs may worsen the inflammation status; however, decrease of cells infiltration into BALF may alleviate allergic asthmatic inflammation [30]. In this study, total infiltrating leukocytes, eosinophils, lymphocytes, and neutrophils in BALF were reduced by adenovirus-mediated IL-35 gene delivery. Our results suggest that IL-35 inhibits infiltrating leukocytes into the local tissues of sensitized and challenged mice. On the other hand, increased mucus production by goblet cells in the airway epithelium is associated with airway inflammation and asthma. The data presented here demonstrated that administration with

**Fig. 3** Effect of AdIL-35 on cytokine production in BALF. IL-4, IL-5, IL-13, and IL-17 concentrations in BALF from PBS-sensitized/challenged mice (PBS), OVA-sensitized/challenged mice (OVA), OVA-sensitized/challenged mice with the administration of Ad0 and with the administration of AdIL-35 were assessed 24 h after the last challenge. Mean  $\pm$  SEM of 6 mice per group representing data from three experiments are shown. \*Significant differences ( $P < 0.05$ ) between sensitized/challenged control groups and sensitized/challenged AdIL-35 groups

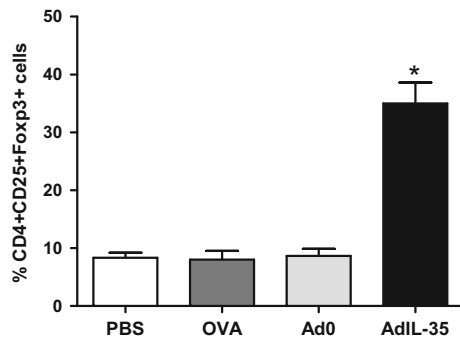
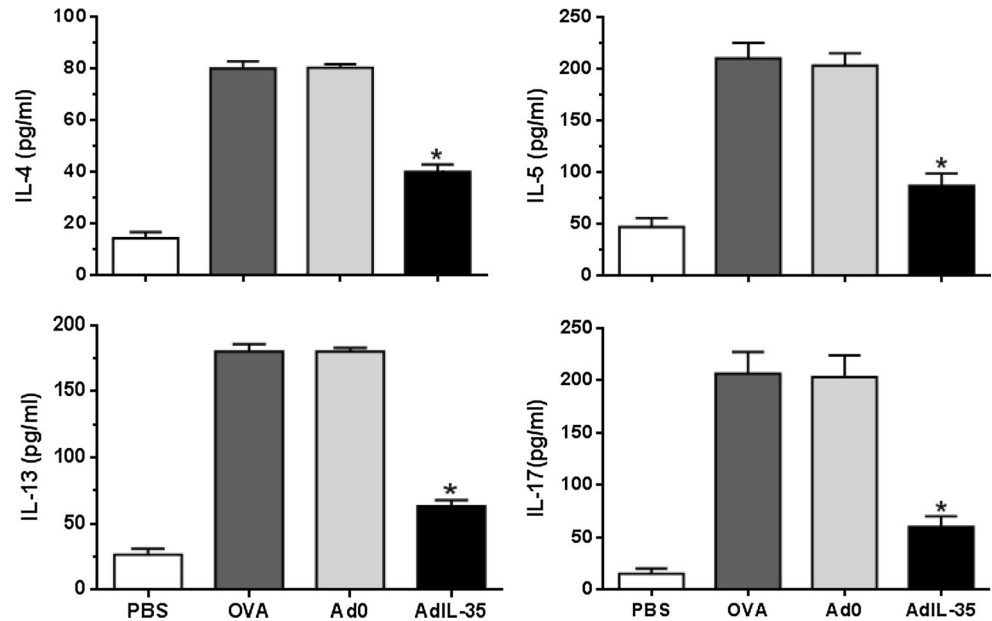


**Fig. 4** AdIL-35 increases lung Treg cells in BALB/c mice. Lung cells from PBS-sensitized/challenged mice (PBS), OVA-sensitized/challenged mice (OVA), with the administration of Ad0 and with the administration of AdIL-35 mice, were stained with specific antibodies to CD4, CD25, and Foxp3 for quantification of CD4+CD25+Foxp3+ Treg cells, and then analyzed using flow cytometry. \*A significant increase ( $P < 0.05$ ) in CD4+CD25+Foxp3+ Treg cell numbers was observed following administration with AdIL-35 compared with the PBS, OVA, or Ad0 groups

AdIL-35 reduced goblet cell hyperplasia in the airways after antigen challenge. Previous studies have implicated IL-4 and IL-13 in increasing airway mucus secretion by goblet cells [31, 32]. Also, IL-5 contributes to the increased mucus production in the airways of allergic mice [33]. Thus, the decrease in the goblet cells observed in mice treated with IL-35 may be due to the inhibition of Th2 cytokines.

Th2 cytokines include IL-4, IL-5, and IL-13, whereas Th1 cytokines include IFN- $\gamma$ . IL-4, IL-5, and IL-13 produced by Th2 cells have been postulated to have central roles in the initiation and maintenance of allergic

inflammation [34]. The development of airway inflammation and allergen-induced AHR is also associated with increased levels of IL-4, IL-5, and IL-13 in BALF, consistent with the development of a Th2-mediated allergic response [35–37]. Our present data showed that AdIL-35 significantly reduced the levels of IL-4, IL-5, and IL-13 in BALF from sensitized and challenged mice, thus indicating its effect in attenuating the inflammatory response.

Th17 cells secrete primarily IL-17A, IL-17F, and IL-22 [38]. In a mouse model of asthma, IL-17A and IL-17F have been shown to induce neutrophilic airway inflammation [39, 40]. In addition, elevated IL-17A has also been correlated to increased AHR in response to methacholine in asthmatics [41]. Furthermore, it has been demonstrated that Th17 cells significantly enhance Th2 cell-mediated eosinophilic airway inflammation in mice [42]. Importantly, studies using murine asthma models have shown that IL-17 receptor-deficient (IL-17R $^{-/-}$ ) mice exhibit reduced recruitment not only of neutrophils, but also of eosinophils into the airways [43], resulting from the impaired priming of Th2 responses in IL-17R $^{-/-}$  mice [43]. It has also been demonstrated that IL-17A $^{-/-}$  mice exhibit reduced Th2 responses to antigen sensitization [44]. On the other hand, it has been demonstrated that the neutralization of IL-17A at the effector phase of airway inflammation enhances eosinophilic inflammation [40, 43]. Our data showed that transfer of IL-35 gene significantly reduced the levels of IL-17 in BALF of sensitized and challenged mice. This is consistent with the previous findings that IL-35 reversed established IL-17-dependent allergic airways disease in mice [25].

Treg cells are key immune suppressive cells which mediate immune tolerance in autoimmune, inflammatory diseases and cancer. The frequencies of CD4+CD25+Foxp3+ Tregs in allergic asthma patients were lower than in healthy controls [23]. In experimental models, CD4+CD25+Foxp3+ Treg cells can reduce established lung eosinophilia, Th2 infiltration, and expression of IL-5 and IL-13, but have no effect on established airway hyper-reactivity [45]. Furthermore, it has been shown that IL-35 promoted the proliferation of CD4+CD25+ T cells and inhibited the proliferation of CD4+CD25- T cells in vitro [13, 23]. A recent study demonstrated that treatment of allergic mice with house dust mite peptides increased the proportion of Foxp3+ Treg cells among those expressing IL-10, but it was not associated with increased IL-35 [46]. In the current study, we demonstrated that AdIL-35 significantly increased the proportion of CD4+CD25+Foxp3+ Treg cells in the lung of OVA-sensitized and challenged mice. These results indicate that our model was not only eosinophilic, but also neutrophilic.

In conclusion, transfer of IL-35 gene can mitigate allergic airway inflammation through significantly reduced severity of AHR and numbers of inflammatory cells and levels of IL-4, IL-5, IL-13, and IL-17 in BALF. Moreover, IL-35 elevated the numbers of CD4+CD25+Foxp3+ Tregs cells in the lungs. The effects of IL-35 presented in this study may be applicable to understanding the pulmonary immune response to allergens and for the development of new potential therapeutics.

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