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CD4⁺CD25⁺ Regulatory T Cells Decreased CD8⁺IL-4⁺ Cells in a Mouse Model of Allergic Asthma

Ping Li¹, Ze Li¹, Guqin Zhang², Jiong Yang², and Junwen Chen¹

¹ *Department of Respiratory and Critical Care Medicine, Affiliated Xiangyang Hospital of Hubei University of Medicine, Xiangyang, China*

² *Department of Respiratory and Critical Care Medicine, Zhongnan Hospital of Wuhan University, Wuhan, China*

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ABSTRACT

Interleukin (IL)-4-producing-CD8 (cytotoxic T cells, Tc) contribute to lung eosinophilia and airway hyper-responsiveness (AHR) to an antigen. CD4⁺CD25⁺ regulatory T cells (Tregs) attenuate airway inflammation and AHR. This study investigated whether Tregs decrease Tc2 frequencies in ovalbumin (OVA)-induced asthma model of mice.

Female C57BL/6 mice were sensitized with OVA intraperitoneally and challenged with OVA intranasally to induce allergic asthma model. Tregs were sorted by fluorescence activated cell sorting (FACS) and magnetic activated cell sorting (MACS) microbeads. OVA-sensitized mice were injected with Tregs or phosphate buffer saline (PBS) by tail vein ahead of the first challenge. Airway inflammation and airway hyper-responsiveness (AHR) were evaluated by histological analysis and invasive method, respectively. OVA-specific IgE and cytokine levels were detected by ELISA. Flow cytometry was used to detect the percentages of Tc1 and Tc2. Gata3 and T-bet mRNA was determined by quantitative PCR (qPCR).

OVA-sensitized and challenged mice displayed typical asthma features, which included eosinophilic airway inflammation, higher levels of Th2 cytokines and AHR. Gata3 mRNA, Tc2 frequencies and OVA-specific IgE levels were significantly increased in OVA-sensitized and challenged mice. Compared to PBS treatment, Tregs decreased Tc2 frequencies, airway inflammation, Th2 cytokine levels and AHR in OVA-sensitized and challenged mice. IL-13 levels were negatively correlated with Tc1 frequencies and with IFN γ levels in experimental mice.

Our results demonstrated that Tregs could prevent airway inflammation and AHR by decreasing Tc2 frequencies and cytokine levels in OVA-induced asthma model of mice, supporting Treg might be as a potent therapeutic target for alleviating airway inflammation and AHR.

Keywords: Asthma; Interferon-gamma; Interleukin-13; IL-4⁺CD8⁺ cells; Regulatory T cells

Corresponding Author: Junwen Chen, MD;
Department of Respiratory and Critical Care Medicine, Affiliated
Xiangyang Hospital of Hubei University of Medicine, Xiangyang,

China. Tel: (86 710) 3420 035, Fax: (86 710) 3420 008, E-mail:
ping_li19@163.com

INTRODUCTION

Allergic asthma is a chronic inflammatory disease and is characterized by airway inflammation, AHR (airway hyper-responsiveness) to inhaled harmless allergen, and airway remodeling. The crucial role of T helper 2 (Th2) cells in asthma has been well established. Th2 cells involve in allergic inflammation by producing interleukin (IL)-4, IL-5, and IL-13. By contrast, antigen-primed CD8⁺ T cells (cytotoxic T cells, Tc), also secreting cytokine profiles similar to Th2 cells, could exacerbate Th2-driven airway inflammation and AHR.¹

Tc cells could differentiate into different cell types, including IFN(interferon)- γ -producing Tc1, IL-4- and IL-13-producing Tc2, IL-9-producing Tc9, IL-17-producing Tc17, and CD8⁺ regulatory cells.² Tc2 cells might contribute to lung eosinophilia and AHR to antigen.³ Tc9 cells alone could not induce the crucial features of asthma but promote Th2-mediated airway inflammation by increasing eosinophils infiltration into the airways and lungs.⁴ IL-13 up regulation and IFN- γ downregulation from Tc cells have been linked with the development of airway inflammation and AHR in antigen-induced asthma model of mice.^{5,6} Tc cells have been increased in bronchoalveolar lavage fluid(BALF) from asthmatic patients and were more resistant than Th cells to corticosteroids.^{7,8} Tc1 cells exerted anti-vital response by producing IFN- γ which could attenuate acute exacerbation in asthmatic patients. Collectively, these data from human and animals demonstrated Tc cells played important roles in the development of airway inflammation and AHR.^{7,9,10}

Regulatory T cells (Tregs) exert immune regulatory functions through generating inhibitory cytokines (IL-10, transforming growth factor (TGF)- β and IL-35), contact-dependent mechanisms,¹¹ ATP (adenosine triphosphate) hydrolysis or/and adenosine production.^{12,13} Our previous results showed that CD4⁺CD25⁺ Tregs from asthmatic patients were insufficient in numbers and defective in function, which led to the failure of restraining excessive Th2 cell immune response to environment allergen.¹⁴ Adoptive transfer of CD4⁺GFP⁺Foxp3⁺ Tregs into the OVA-sensitized and challenged mice significantly attenuated airway inflammation and AHR by lowering Th2 and Th17 cytokine levels and decreasing (GATA3/GATA binding protein 3) and retinoic acid-

related orphan receptor γ t (ROR γ t) mRNA.¹⁵ These results confirmed Tregs prevented airway inflammation and AHR by dampening the abnormal immune response.

Under an atopic environment rich in IL-4, IFN- γ ⁺CD8⁺ T (Tc1) cells were prone to convert into IL-13⁺CD8⁺ T (Tc2) cells.¹⁶ In this study, we detected Tc2 frequencies and investigated whether Tregs decreased Tc2 frequencies in OVA-induced asthma model of mice. Furthermore, we analyzed the correlations between IL-13 levels and Tc1 frequencies or IL-13 and IFN- γ levels.

MATERIALS AND METHODS

Mice

Female C57BL/6 mice (Age, 6-8 weeks; Weight, 18-22g) were obtained from the Experimental Animal Center of Wuhan University (Wuhan, China). All mice were housed under specific pathogen-free (SPF) conditions. These mice were provided with autoclaved food and acidified water under a 12 h light/dark cycle. All animal experimental protocols were approved by the Institutional Animal Ethics Committee from Wuhan University (N. WHZN-2011-13).

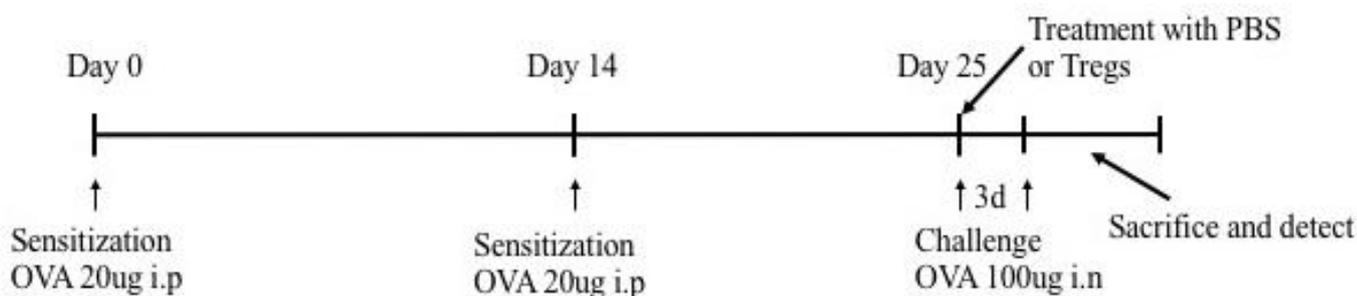
Animal Experimental Protocol

Murine model of allergic asthma was performed as previously described.¹⁷ Briefly, experimental mice were intraperitoneally sensitized with 20 μ g ovalbumin (OVA, Sigma-Aldrich, St Louis, USA) and 2 mg alum (Pierce, Rockford, USA) dissolved in 200 μ L PBS(phosphate buffer saline) on day 0 and 14. OVA-sensitized mice were challenged with 100 μ g OVA dissolved in 50 μ L PBS on day 25-27 by intranasal administration. Control mice were sensitized with PBS and challenged with OVA.¹⁸ The schematic diagram of OVA-induced asthma model of mice was shown in Figure 1. In some experiments, OVA-sensitized mice received an intravenous injection of 1×10^6 CD4⁺CD25⁺ Tregs in 200 μ L PBS or an equal volume of PBS without cells 1 hour ahead of the first challenge on day 25. There were three groups in our experiments (n=5~6 mice in each group, three times).

Cell preparation for CD4⁺CD25⁺ Tregs

CD4⁺CD25⁺Tregs were sorted from the spleen of male C57BL/6 mice (Age, 6-10 weeks) using an EasyStepTM mouse CD4⁺ T cell isolation kit (Stem Cell Technologies Inc, Canada) combination with

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Animal experimental protocols

Figure 1. The schematic diagram of ovalbumin (OVA)-induced asthma model of mice

Sensitization: OVA via intraperitoneal (i.p.) injection (day 0 and 14). **Challenge:** OVA via intranasal (i.n.) administration (day 25, 26, and 27). **Tregs (regulatory T cells) or phosphate buffer saline (PBS) treatment:** via tail vein injection 1 hour ahead of the first challenge. Airway hyper-responsiveness (AHR) was measured and bronchoalveolar lavage (BAL) was performed after the last OVA challenge (day 29). Mice were sacrificed to evaluate airway inflammation, cytokine levels, cell frequencies, cell numbers, and mRNA expression.

fluorescence activating cell sorter (FACS, BD FACS Calibur, USA), as reported previously.¹⁵ The purity of cells was typically greater than 90% by FACS (fluorescence activating cell sorter).

Measurement of Airway Hyper-responsiveness (AHR)

Twenty-four hours after the last OVA challenge, AHR to inhaled methacholine (Sigma-Aldrich, St Louis, USA) was performed using the FinePointe RC system (Wilmington, NC) by the invasive method. The mice were anesthetized via intraperitoneal (i.p.) injection of pentobarbital (50 mg/kg, Shanghai, China). The mice were inserted with a tracheostomy tube and then connected to the FinePointe RC system. Airway resistance to aerosolized PBS and methacholine was recorded. The concentrations of methacholine ranged from 3.12mg/mL to 50 mg/mL.^{19,20}

Collection of BALF and Lung Histopathology

The mice were anesthetized and the total lungs were lavaged with 1.5 mL PBS (0.5 mL each, three times) plus 1 mM sodium Ethylene Diamine Tetra-acetic Acid (EDTA, Beijing, China). The cells in BALF were collected by centrifugation at 300×g for 8 min at 4°C. The total number of pooled cells was counted by a hemacytometer. And these cells were stained with Wright-Giemsa (Nanjing, China) for cellular profiles. Different cells including macrophages, eosinophils, neutrophils, and lymphocytes were counted by light microscopy (Olympus, Japan).

The mice were sacrificed and the lungs were resected. Right lung tissues were fixed in 4% paraformaldehyde buffer and embedded in paraffin blocks. The paraffin blocks were cut into 5 μm sections. Sections were stained with hematoxylin and eosin (H&E) for evaluating airway inflammation through light microscopy.

Cell Preparation and Flow Cytometric Analysis

The lung mononuclear cells were obtained as previously described.²¹ Briefly, lung tissues were cut into small pieces, digested in 1 mg/mL collagenase I (Invitrogen, Carlsbad, CA) at 37°C for 1 hour. The cell suspension was filtered through a 100-μm nylon net filter. The mononuclear cells were obtained by centrifugation at 800×g for 20 min in a lymphoprep gradient (TBD, Tianjin, China).

Lung mononuclear cells were seeded at a density of 1×10^6 cells/well in a six-well plate and cultured in an RPMI-1640 medium plus 10% fetal bovine serum (FBS, Hyclone, USA). These cells were stimulated with 50 ng/mL phorbol myristate acetate (PMA) and 500 ng/mL ionomycin (both from Sigma-Aldrich, St Louis, USA) for 2 hours and then blocked for an additional 4 hours in the presence of 3 μg/mL brefeldin A (eBioscience, San Diego, CA, USA). These pooled cells were stained with FITC-conjugated anti-mouse CD3 Ab and PE-cy5-conjugated anti-mouse CD8 Ab for 30 min at 4°C in the dark. Surface stained cells were fixed, permeabilized and then stained with PE-conjugated anti-mouse IL-4 and IFN-γ Abs. All these

antibodies were purchased from eBioscience. All samples were analyzed by FACS Aria II (BD, USA).

Detection of Cytokines and OVA-Specific IgE Levels

The levels of IL-4, IL-5, IL-13, and IFN- γ in BALF were measured by ELISA kits (eBioscience, San Diego, CA, USA) according to the manufacturer's protocol. The lower limit of detection was 15 pg/mL for IFN- γ and 4 pg/mL for IL-4, IL-5, and IL-13. OVA-specific IgE levels in sera were detected by ELISA, as previously described.²¹ The absorbance intensity was read at 450 nm using a microplate reader (Tecan, Clontarf, Australia). OVA-specific IgE levels in sera were expressed as optical density (OD). Sample concentrations for these cytokines were calculated using software ReaderFit.

RNA Preparation and Real-time Quantitative PCR Analysis

Total RNA was extracted from left lungs using TRIzol reagent (Invitrogen, CA, USA) according to the manufacturer's protocols. The first-strand cDNA was synthesized using a ReverTra Ace qPCR RT Master Mix kit (Toyobo, Tokyo, Japan). Real-time quantitative PCR (qPCR) was performed using an SYBR Premix Ex TaqTM kit (Takara, Tokyo, Japan) following the manufacturer's protocols. The value of the cycle threshold (CT) reflected the number of PCR cycles. The differences in CT values of the target and housekeeping genes were used to delta CT (Δ Ct). Relative fold changes of target genes were calculated using $2^{\Delta\Delta Ct}$ algorithm. glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene. The primer sequences were described as follows: GAPDH sense: 5'-tgtgt ccgtc gtgga tctga-3', anti-sense: 5'-ttgtc gttga agtcg cagga g-3'; Gata3 (GATA binding protein 3) sense: 5'-aggga catcc tgcgc gaact gt-3', anti-sense: 5'-catct tccgg ttccg ggtct gg-3'; T-bet (T-box 21) sense: 5'-caaca acccc ttgc caaag-3', anti-sense: 5'-tcccc caagc agttg acagt-3'.

Statistical Analysis

Data statistical analysis was conducted using SPSS 17.0 software. All continuous variables were expressed as means + standard deviation (SD) or only means. The normality of data was determined by the One Sample Kolmogorov-Smirnov test. Differences among the three groups were analyzed using One-Way

ANOVA (Analysis of Variance), followed by Post Hoc analysis comparing the means between two groups. LSD (Least Significant Difference) test was used under the condition of variance homogeneity and Dunnett's T3 test was used under the condition of variance heterogeneity. Correlation analysis was analyzed by Pearson's test. $p < 0.05$ was considered as statistical difference.

RESULTS

CD4⁺CD25⁺ Cells Attenuated Airway Inflammation in OVA-Induced Asthma Model of Mice

In PBS-sensitized mice, no eosinophils infiltration into the airways were found in H&E-stained sections (Figure 2a). In OVA-sensitized mice, eosinophils around bronchi and vessels were observed in H&E-stained sections (Figure 2b) and the number of eosinophils in BALF was increased (Figure 2d). Compared to PBS treatment, CD4⁺CD25⁺ cells significantly attenuated peri-bronchial and peri-vascular inflammation (Figure 2c). In addition, CD4⁺CD25⁺ cells significantly decreased the number of total and different inflammatory cells in BALF (Figure 2d).

CD4⁺CD25⁺ Cells Decreased AHR in an OVA-induced Asthma Model of Mice

Airway resistance was determined 24 hours after the last challenge. Airway resistance in all mice was increased in a dose-dependent manner when exposed to methacholine. OVA-sensitized mice showed higher airway resistance than PBS-sensitized mice. Compared to that in PBS-treated mice, AHR were decreased in the mice treated with CD4⁺CD25⁺ cells (Figure 3).

CD4⁺CD25⁺ Cells Reduced OVA-specific IgE Levels in Sera

IgE, whose synthesis mainly depends on Th2 cells, is the hallmark of allergic asthma. Therefore, we examined whether CD4⁺CD25⁺ cells decreased OVA-specific IgE levels in sera. As shown in Figure 4, OVA-specific IgE levels were significantly increased in experimental mice compared to control mice, which indicated OVA-induced airway inflammation was successful. Adoptive transfer of CD4⁺CD25⁺ cells significantly reduced OVA-specific IgE levels compared to PBS treatment in experimental mice.

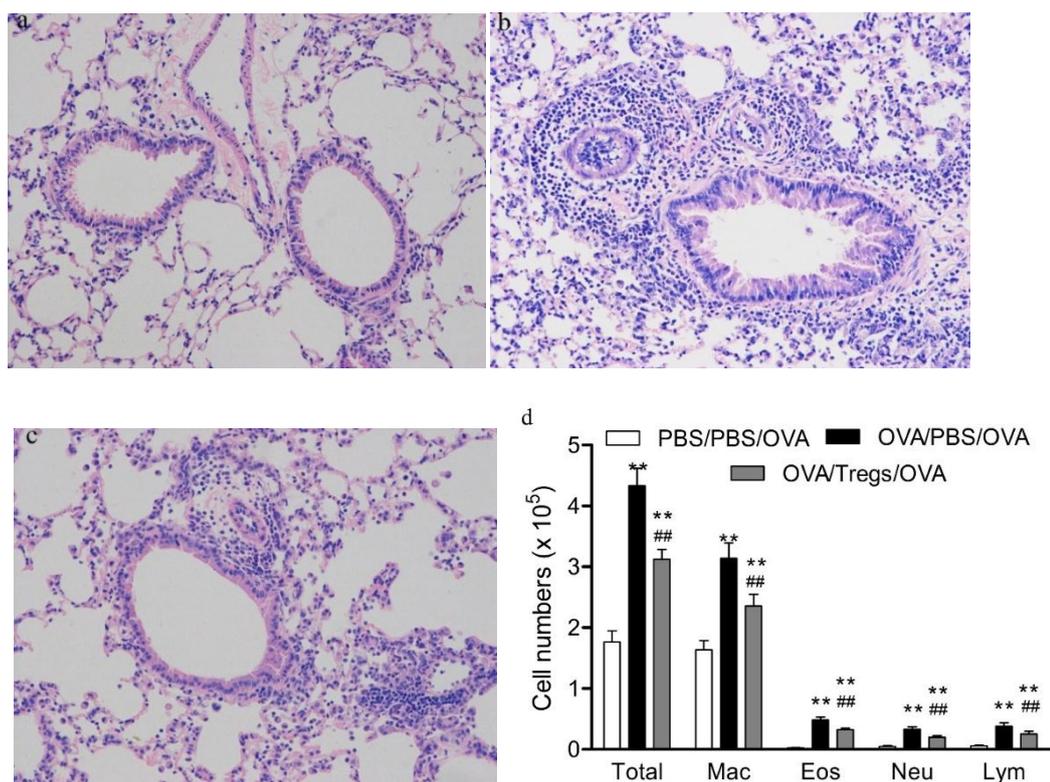


Figure 2. CD4⁺CD25⁺ cells attenuated airway inflammation

a-c. Representative micrographs of airway inflammation were displayed by H&E (×200). (sensitization/treatment/challenge) a: PBS/PBS/OVA b: OVA/PBS/OVA c: OVA/Tregs/OVA d. The cells in BALF were pooled 24h after the last challenge. Numbers for macrophages (Mac), eosinophils (Eos), lymphocytes (Lym) and neutrophils (Neu) in BALF were counted. All data were described as means + SD (n=5-6 mice per group). ***p*<0.01 comparison to PBS/PBS/OVA group; ## *p*<0.01 comparison to OVA/PBS/OVA group. (One-Way ANOVA was used to compare the means among three groups, followed by Post Hoc analysis comparing the means between two groups. The data conform to normal distribution.)

OVA: ovalbumin; Tregs: CD4⁺CD25⁺ regulatory T cells; PBS: phosphate buffer saline; BALF: bronchoalveolar lavage fluid

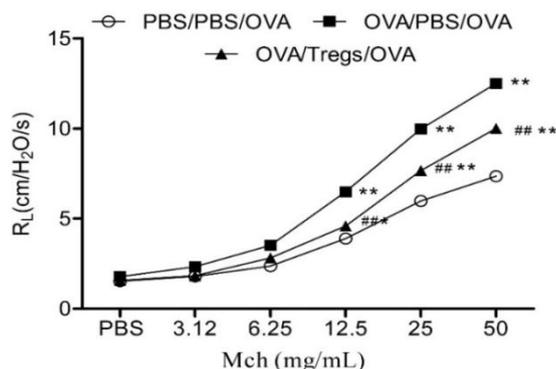


Figure 3. CD4⁺CD25⁺ cells decreased AHR (airway hyper-responsiveness) to aerosolized methacholine in ovalbumin(OVA)-induced asthma of mice. PBS: phosphate buffer saline

Airway resistance (R_L) to aerosolized methacholine was determined by an invasive method. All data were described as means (n=5-6 mice per group). (sensitization/treatment/challenge) **p*<0.05, ***p*<0.01 comparison to PBS/PBS/OVA group; ## *p*<0.01 comparison to OVA/PBS/OVA group. (One-Way ANOVA was used to compare the means among three groups, followed by Post Hoc analysis comparing the means between two groups.)

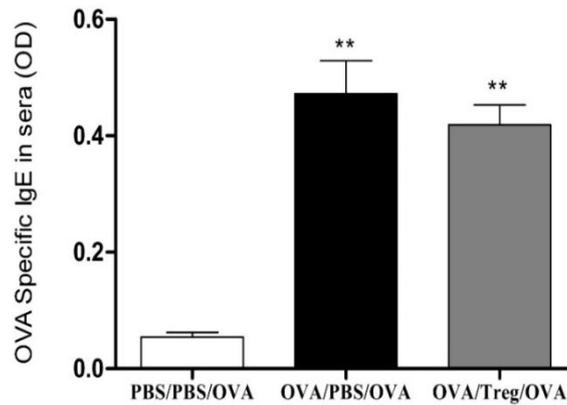


Figure4. CD4⁺CD25⁺ cells reduced the levels of ovalbumin(OVA)-specific IgE in sera of experimental mice
 OVA-specific IgE levels in sera were determined by ELISA and expressed as OD value. All data were described as means + SD (n=5-6 mice per group). (group: sensitization/ treatment/challenge) ***p*<0.01 comparison to PBS/PBS/OVA group. (One-Way ANOVA was used to compare the means among three groups, followed by Post Hoc analysis comparing the means between two groups.)

CD4⁺CD25⁺ Cells Decreased IL-4, IL-5 and IL-13 Levels and Increased IFN-γ Levels in BALF

As shown in Figure 5, experimental mice displayed higher levels of IL-4, IL-5, and IL-13 in BALF than control mice. The levels of IL-4, IL-5 and IL-13 were significantly decreased in OVA-sensitized mice treated

with CD4⁺CD25⁺ cells compared to mice treated with PBS. OVA-sensitized mice displayed lower levels of IFN-γ in BALF than PBS-sensitized mice. Whereas, the lower levels of IFN-γ in BALF were elevated when OVA-sensitized mice were treated with CD4⁺CD25⁺ cells (Figure5b).

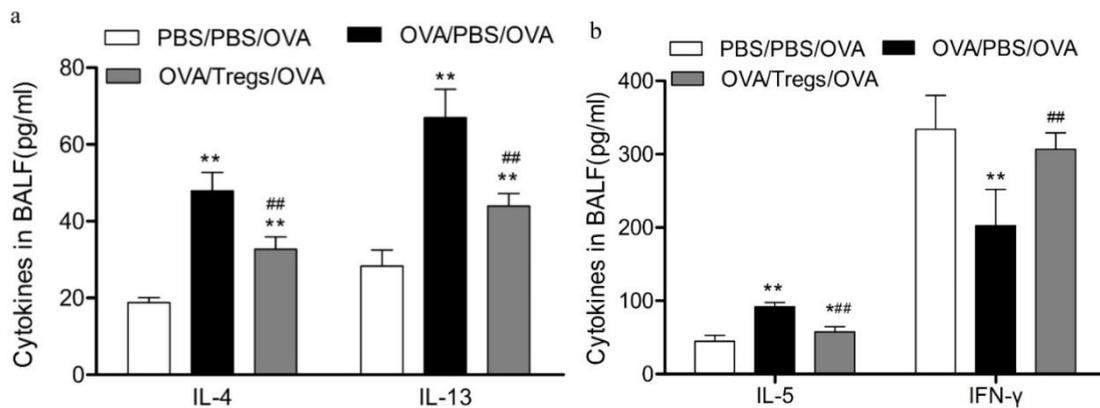


Figure 5. CD4⁺CD25⁺ cells decreased IL-4, IL-5 and IL-13 levels and increased IFN-γ levels in bronchoalveolar lavage fluid (BALF)

The supernatant in BALF was collected by centrifugation. The levels of cytokines in BALF were measured by ELISA. All data were described as means+SD (n=5-6 mice per group). (sensitization/treatment/challenge) **p*<0.05, ***p*<0.01 comparison to PBS/PBS/OVA group; ## *p*<0.01 comparison to OVA/PBS/OVA group. (One-Way ANOVA was used to compare the means among three groups, followed by Post Hoc analysis comparing the means between two groups.)

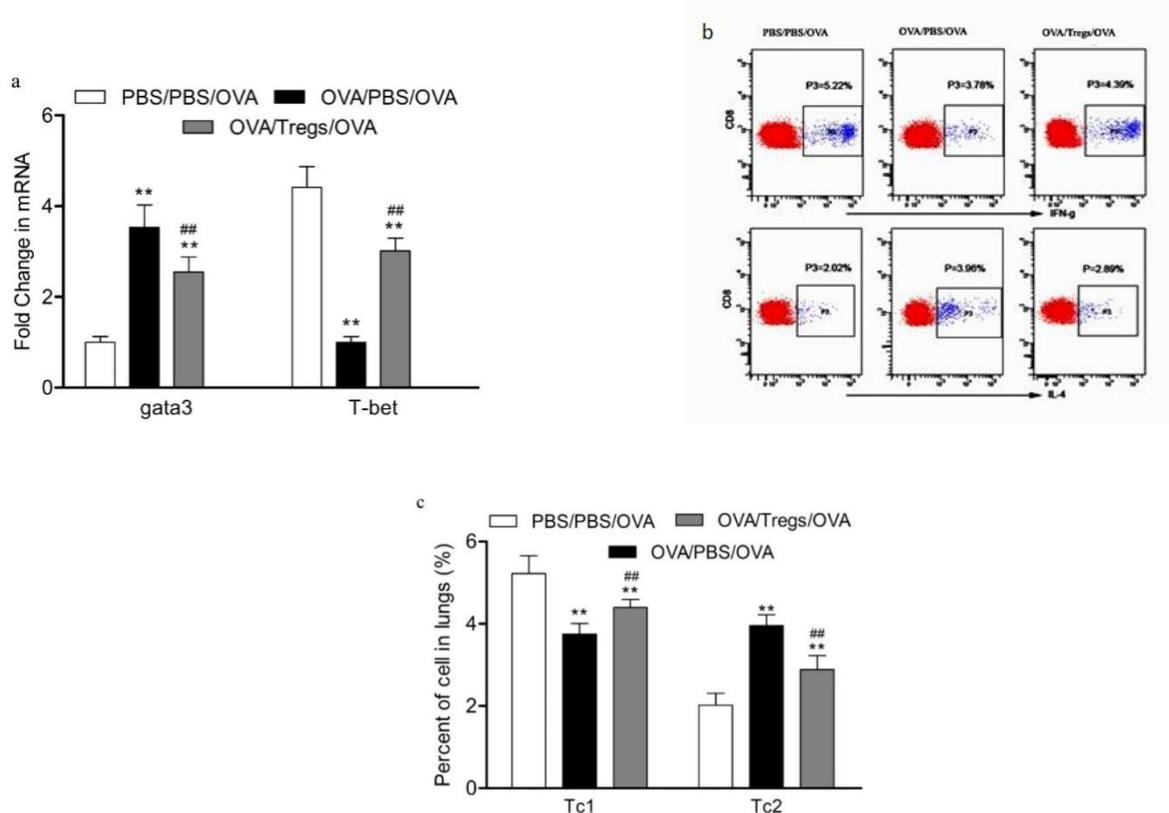


Figure 6. CD4⁺CD25⁺ cells reduced GATA3 mRNA expression and the frequencies of CD8⁺IL-4⁺ cells in the lungs from experimental mice

a: The expression for T-bet and GATA3 was detected by quantitative real time PCR analysis. **b.** Representative plots of CD3⁺CD8⁺IL-4⁺ (Tc2) and CD3⁺CD8⁺IFN- γ ⁺ cells (Tc1) in the lung mononuclear cells from different groups were shown. **c.** The frequencies of Tc1 and Tc2 cells in the lungs were calculated by flow cytometry. All data were described as means+SD (n=5-6 mice per group). (sensitization/ treatment/ challenge) ***p*<0.01 comparison to PBS/PBS/OVA group; ## *p*<0.01 comparison to OVA/PBS/OVA group. (One-Way ANOVA was used to compare the means among three groups, followed by Post Hoc analysis comparing the means between two groups.)

CD4⁺CD25⁺ Cells Reduced GATA3 mRNA Expression and CD8⁺IL-4⁺ Cell Frequencies in the Lungs

T-bet and GATA3 were the major transcription factors for regulating IFN- γ and IL-13 expression in T cells, respectively.^{22,23} T-bet and GATA3 mRNA expression in the lungs was measured by qPCR. The frequencies of CD3⁺CD8⁺IL-4⁺ (Tc2) and CD3⁺CD8⁺IFN- γ ⁺ cells (Tc1) were determined by flow cytometry. GATA3 mRNA expression and Tc2 frequencies in OVA-sensitized mice were significantly higher than those in PBS-sensitized mice. T-bet mRNA expression and Tc1 frequencies in OVA-sensitized mice were significantly lower than those in PBS-sensitized mice. Increased GATA3 mRNA expression and Tc2 frequencies were down-regulated when OVA-

sensitized mice were treated with CD4⁺CD25⁺ cells. Meanwhile, reduced T-bet mRNA expression and Tc1 frequencies were up-regulated when OVA-sensitized mice were treated with CD4⁺CD25⁺ cells (Figure 6).

The Correlations between IL-13 and IFN- γ or Between IL-13 and Tc1 in OVA-induced Asthma Model of Mice

Next, we analyzed the correlations between IL-13 and IFN- γ levels or between IL-13 and Tc1 frequencies in OVA-induced asthma model of mice. As shown in Figure 7, IL-13 expression levels in BALF were negatively correlated with Tc1 frequencies (*r*=-0.890, *p*=0.001) and with BALF IFN- γ levels (*r*=-0.905, *p*=0.000).

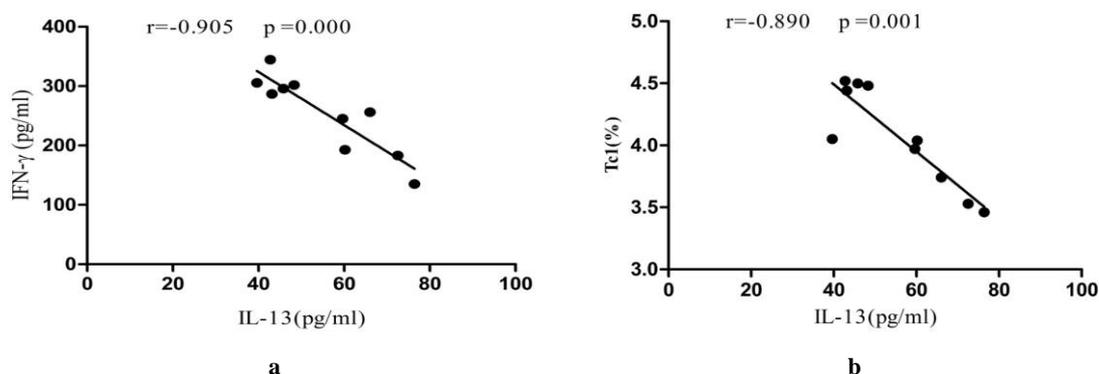


Figure 7. The correlations between IL-13 and IFN- γ or between IL-13 and Tc1 in experimental mice

The correlation between IL-13 and IFN- γ or Tc1 in OVA-induced asthma model of mice was displayed. (n=5-6 mice per group). a: Correlation between IL-13 and IFN- γ levels in BALF. b: Correlation between IL-13 levels in BALF and the percentage of Tc1 in the lung mononuclear cells.

DISCUSSION

The present study demonstrated that Tc2 frequencies were increased whereas Tc1 frequencies were decreased in the OVA (ovalbumin)-induced asthma model of mice. CD4⁺CD25⁺ cells (Tregs) reduced Tc2 frequencies but increased Tc1 frequencies. In addition, IL-13 levels in BALF were negatively correlated with Tc1 frequencies in lung mononuclear cells and IL-13 levels were negatively correlated with IFN- γ levels in BALF.

Th2-primed allergic asthma has been well clarified and been a classical asthma phenotype. However, increasing evidence supported CD8⁺ T cells involved in the development of Th2-driven asthma.²⁴⁻²⁷ The numbers of CD3⁺CD8⁺ T cells from the lung mononuclear cells were increased in our present study (data not shown), which was consistent with this report that bronchial CD8⁺ T cells were also elevated in asthma patients.²⁸ In vitro experiment displayed Tc1 cells were prone to conversion into Tc2 cells in the presence of IL-4.⁸ Meanwhile, in vivo experiment demonstrated IL-13 generation from antigen-activated effector CD8⁺ T cells was required for the intact development of airway inflammation and AHR.^{5,29} The dialog between CD8⁺ T and Th2 cells is indispensable for Th2-mediated airway allergic inflammation and AHR.³⁰ We displayed that Tc2 cells were markedly elevated in OVA-induced asthma model of mice, accompanied by higher levels of Th2-like cytokines. In addition to classical Th2, other immune cells could also

produce Th2-like cytokines, such as B effector cells,³¹ natural killer cells³² and innate nuocytes³³ Th2-like cytokines, including IL-4, IL-5, and IL-13, exerted critical roles in allergen-induced airway inflammation and airway hyper-responsiveness. Therefore, we speculated that Th2 cytokine-producing cells contributed to the generation of Th2-driven asthma phenotype.

In generally, Tc1 cells mainly exert anti-inflammatory roles by producing IFN- γ . Where as IFN- γ -producing CD8⁺ T cells were skewed to polarize into IL-13-producing CD8⁺ T cells under conditions rich in IL-4. Our results showed IL-13 levels and Tc2 frequencies were increased, but IFN- γ levels and Tc1 frequencies were decreased in OVA-induced asthma model, which was possibly related to Tc1 polarization into Tc2 in the atopic environment. In addition, data demonstrated IFN- γ secreted from activated effector CD8⁺ T cells could modify the shift from mature dendritic cells (mDCs) to tolerogenic DCs (tDCs) properties and then attenuated Th2-mediated allergic response, conversely, IFN- γ -null CD8⁺ T cells could generate IL-13 and enhance allergic airway inflammation and AHR,^{34,35} which supported the role of CD8⁺IFN- γ ⁺ cells in allergic asthma.

Tregs could prevent excessive inflammation and maintain tolerance to a specific allergen. In allergic patients, Tregs could not effectively inhibit cell proliferation and pro-inflammatory cytokines generation from allergen-driven effector CD4⁺ T cells.^{36,37} Our previous study also demonstrated that

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Tregs were decreased in number and impaired in function.^{14,15} Meanwhile supplement with Tregs could attenuate airway inflammation and AHR through decreasing the levels of cytokines from effector CD4⁺ T cells. In the present study, we found the adoptive transfer of Tregs not only decreased IL-4, IL-5, and IL-13 levels and increased IFN- γ levels but also altered the expression for GATA3 and T-bet mRNA. These changes in the microenvironment possibly contributed to the phenotype conversion of CD8⁺ T cells and DCs.

Our present study demonstrated that decreasing Tc2 numbers and altering cytokine levels in local microenvironment were also implicated in the protective effects of Tregs in OVA-induced murine asthma model. However, the detailed process between Tregs and phenotype conversion of CD8⁺ T cells were not explored in our present study. No positive control group was set up to compare the effect of Tregs on airway inflammation and AHR, which was also the limitation of the current study. In a further study, we will investigate how Tregs influence Tc cell phenotype conversion. And dexamethasone will be used to evaluate the role of Tregs in a future study.

CD4⁺CD25⁺ cells prevented airway inflammation and AHR by decreasing Tc2 frequencies and cytokine levels in OVA-induced asthma model of mice, supporting Tregs could be used as a potent target to alleviate airway inflammation and AHR in asthma.

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