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Mycobacterium Vaccae Regulate γδT17 and γδTreg Cells in Mice Asthmatic Lung

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ABSTRACT

Background Dysregulation of the balance between different T cell populations is believed to be an important basis for asthma.

Objective To observe the changes in γδT subtypes in transgenic asthmatic mice after aerosol inhalation of *Mycobacterium vaccae*, and to further investigate the mechanism of *M. vaccae* in asthmatic mice and its relationship with γδT cells.

Methods TCR-$\beta^{-/-}$ mice were exposed to atomized normal saline or *M. vaccae* for 5 days and the γδT cells from the lung tissues were isolated. Changes in γδT17 and γδTreg populations were detected. Asthma was induced in BALB/c mice using ovalbumin, which was then transplanted with control or *M. vaccae*-primed γδT cells. First we analyzed the content of γδT cells that secrete IL-17 (IL-17 γδT cells) and $Foxp3^+$ γδT cells in lung tissues and then measured the content of IL-17 in the bronchoalveolar lavage fluid (BALF) by ELISA.

Results Exposure to *M. vaccae* increased and decreased the relative proportions of $Foxp3^+$ γδT cells and IL-17^+ γδT cells, respectively, thereby decreasing airway reactivity and inflammation levels in asthmatic mice, and significantly decreasing IL-17 levels in BALF. Furthermore, mice treated with these primed T cells showed a decrease in IL-17^+ γδT cells, and a concomitant increase in $Foxp3^+$ γδT cells in their lung tissues. Furthermore, adoptive transfer of *M. vaccae*-primed γδT cells decreased GATA3 and NICD and increased T-bet in lung.

Conclusions The *M. vaccae*-primed γδT cells alleviated the symptoms of asthma by reversing Th2 polarization in the lungs and inhibiting the Notch/GATA3 pathway.

Keywords: Asthma, *Mycobacterium vaccae*, γδT cells

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INTRODUCTION

The regulatory imbalance between Th1/Th2 cells, such as the lack of Th1 cells and/or the appearance of overactive Th2 cells, is the most fundamental immunological mechanism in the pathogenesis of asthma (1, 2). Recent studies have shown that imbalances in other T cell populations, such as Th17/Treg cells are also involved in the development and progression of asthma (3, 4). In addition, the γδT cell subsets are also involved in the pathogenesis of asthma, which can promote airway remodeling by increasing the production and releasing multiple inflammatory factors and cytokines, ultimately leading to the development of asthma (5, 6). γδT cells release large amounts of Th2 cytokines such as IL-17, which recruits other granulocytes and exacerbates the inflammatory process (7, 8).

High levels of IL-17 are strongly associated with airway inflammation, reactivity, and airway remodeling in asthmatics (9-11). The γδT17 cells are directly involved in the pathogenesis of ovalbumin (OVA)-induced asthma (12), which show an aberrant γδT17/γδTreg ratio (4). The γδTregs is an immunosuppressive γδT cell subset of T cells manifested by γδ-T cell receptors (TCRs) and Foxp3 expression (13).

The T-bet/Stat-3 ratio is a decisive factor in Th1/Th2 polarization and stability (14), and an indicator of immune imbalance in asthmatic patients (15). A recent study showed that Shengjiang powder biased T cell polarization towards the Th2 phenotype in patients with sepsis patients by regulating T-bet and GATA3 (16). Furthermore, ectopic expression of T-bet and Foxp3 accompanied by GATA-3 and RORγT knockdown inhibited the production of IL-17, IL-13, IL-5, and OVA-specific IgE in asthmatic mice, which inhibited eosinophil infiltration and alleviated airway inflammation (17). Consistent with this, Ginsenoside Rb alleviated allergic symptoms in OVA-induced asthmatic mice GATA3 and upregulated T-bet (18). Similarly, oral feeding of *Lactobacillus bulgaricus* N45.10 reduced allergic asthma-triggered inflammation and airway remodeling in mice by upregulating T-bet and reducing GATA3 levels, which altered the proportion of Th1/Th2 cytokines (19). A recently concluded clinical trial reduced the severity of asthma by altering the T-bet/GATA3 ratio in the mononuclear cells of the airways involved in the inflammatory response (20). Finally, there is evidence that the Notch/Hes1 signaling pathway and IL-17 cytokines are closely associated with the development of γδT cells (21). Th2 cell differentiation in asthma patients is mainly regulated through the Notch signaling (22, 23), while Treg cells can restore the balance between Th1 and Th2 cells through the Dll4/Notch signaling pathway and thus reverse the pathophysiological process of asthma (24). We have previously reviewed the role of the Notch signaling pathway in asthma and Th1/Th2 regulation (25).

Mycobacterium infection can inhibit airway inflammation and hyperresponsiveness in asthma (26). Inactivated *M. phlei* in BCG inhibits the Th2 subset and relieves the symptoms of asthma (27). Studies show that the inhalation of inactivated *M. phlei* aerosol can restore the Th1/ Th2 balance in the murine asthma model and reduce the inflammatory lesions in the lungs (28-30). At the molecular level, inactivated *M. phlei* restored the T-bet/GATA-3 balance and inhibited Th2 cytokine secretion (31). Inactivated *M. phlei* reduced the immune response mediated by IL-17^{+} γδT cells, thus attenuating the pathophysiological changes in asthmatic mice (32). *Mycobacterium vaccae* and inactivated Mycobacterium phlei have similar functions, because they are both of the genus *Mycobacterium*, and have the same immunomodulatory effect as other T cells to regulate the immune response of the body, aerosol bacteria can stimulate the body's immune response through mucosal immunity, cellular immune response regulates airway inflammation and airway hyperresponsiveness. A recent

study showed that *M. vaccae* atomization can reduce the level of hyperresponsiveness and inflammatory factors in the airways and thus achieve the prevention of allergic bronchial asthma (33). To further elucidate the immunomodulatory mechanism of asthma, we exposed TCR-β$^{-/-}$ mice to atomized *M. vaccae* and transplanted the primed γδT cells into OVA-induced asthmatic mice, and we wanted to observe the pathogenesis of asthma by analyzing changes in various immunological and molecular indices, and also research the functions of inactivated *M. vaccae* in asthmatic mice. *Mycobacterium* has a regulatory effect on γδT. This article focuses on its regulatory effect on T-bet/GATA3 pathway and γδT17/γδTreg.

MATERIALS AND METHODS

Male TCR-β$^{-/-}$ mice（Mice with the αβT gene removed and γδT cells present in mice) (Beijing Baiaosaitu Company) and male BALB/c mice (Hunan Slake Jingda Experimental Animal Co). All animals (4-6 weeks, weighing 18-22 g) grew in a specific pathogen-free (SPF) environment and survived according to the feeding requirements of the Animal Welfare Act. Ethical approval for this study was obtained from the Animal Ethics Committee of Guangxi Medical University (IACUC: 201711033.)

The TCR-β$^{-/-}$ mice were randomized into the control and *M. vaccae*-infected groups (n=20 each). Therefore, the mice were exposed to *M. vaccae* (22.5 μg *M. vaccae* in 10 ml normal saline) or the same volume of normal saline for 20 min per day for 5 consecutive days. The lung tissue was collected to isolate the γδT cells. The isolated γδT cells were stored in PBS solution and placed on ice at 4℃ to detect the activity of γδT cells to ensure that the γδT cells were active. The removed γδT cells were immediately transfused into BALB/c mice (Not TCR-β-/-). The BALB/c mice were randomly divided into the normal control, asthma model, and the γδT cell control groups (TCR-β-/- mice were exposed to 10ml normal saline for 5 consecutive days, 20 min daily, and γδT cells were isolated from lung tissue, injected to the BALB/c mice of OVA-induced asthma) and sensitized γδT cell adoptive transfer groups (TCR-β-/- mice were exposed to M. vaccae (22.5 μg M. vaccae in 10 ml normal saline) for 5 consecutive days for 20 min every day, and lung tissues were isolated from γδT cells, injected to the BALB/c mice of OVA-induced asthma) 38 (n=8 each). To construct the asthma model, we injected 25 mg OVA (Sigma-Aldrich, USA) intraperitoneally on days 0, 7, and 14, respectively. From day 22, OVA at a concentration of 2% (mg/mL) was released in a closed chamber for 7 consecutive days. The mice in adoptive transfer groups T cells were injected intravenously with 10^6 γδT cells extracted from the M. vaccae or saline-treated TCR-β$^{-/-}$ mice before the first intraperitoneal injection and on the first day of atomization. Lung tissues were harvested for testing (Figure 1). Therefore, lung tissues, branchio-alveolar lavage fluid (BALF), and lung tissue were harvested for various tests.

Airway Reactivity

The mice in each experimental group were placed on specific test devices to detect specific airway resistance (sRaw) after stimulation with 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, and 50 mg/ml of methacholine and PBS, respectively.

Enzyme-linked Immunosorbent Assay (ELISA)

After 40 minutes of airway reactivity test, the animals were anesthetized with 10% (300 mg/kg) chloral hydrate intraperitoneal injection, no symptoms of peritoneal inflammation in mice were observed, when mouse movement decreased, and suffering was considered humane endpoints that required immediate intervention and sudden death of cervical dislocation. No symptoms of peritoneal inflammation in mice were seen. The animals were intubated and 500 μM of

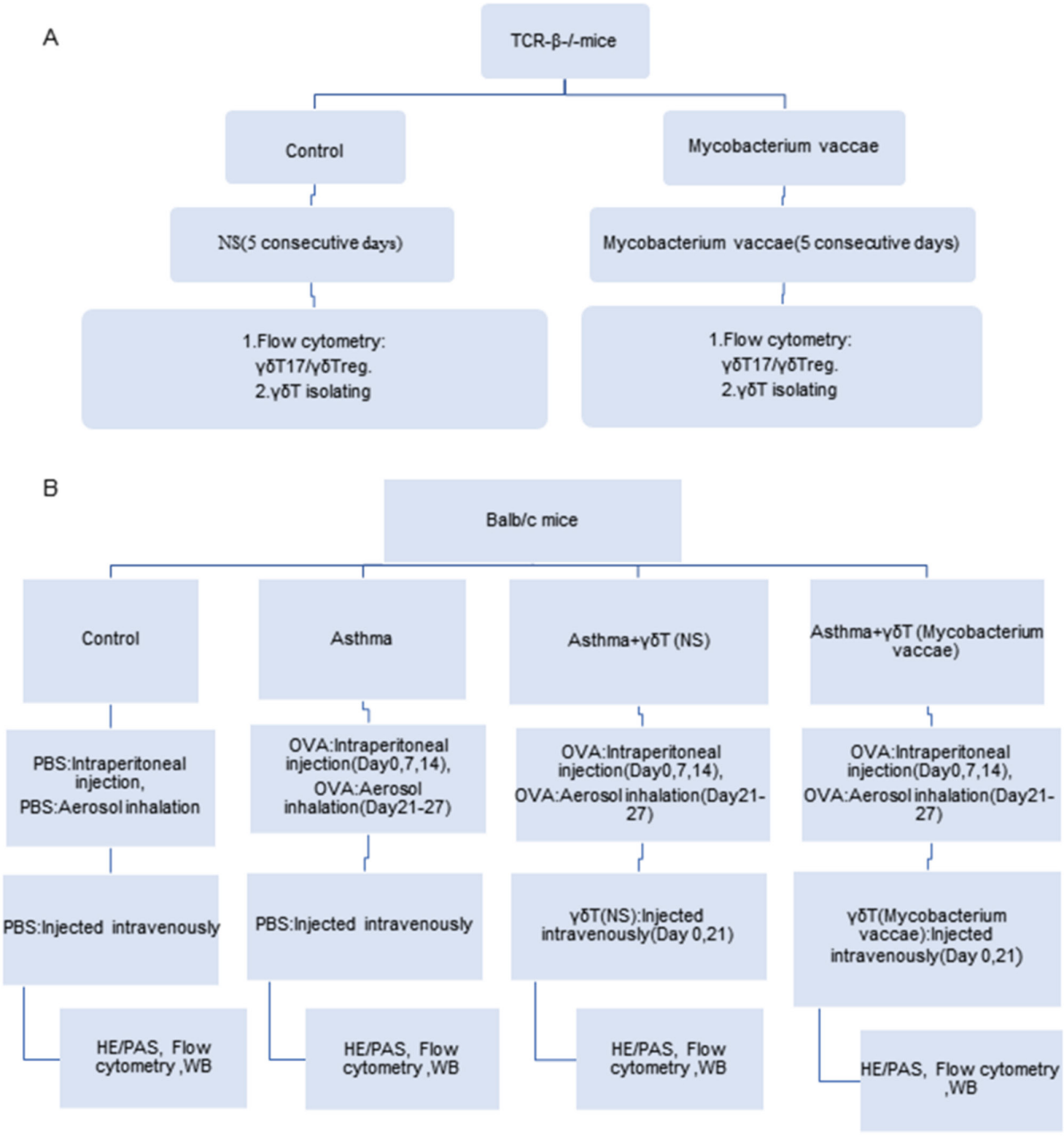

Figure 1. The flow chart of TCR-β-/- mice.The mice were randonly divided into 2 groups and intervene shown above.In the *Mycobacterium vaccae* group, mice were Aerosol bacteria with *Mycobacterium vaccae* over 5 consecutive days,then, flow cytometry was performed to detect γδT17/γδTreg and γδT isolating.The contol group was intervened with NS.Figure1-1.The flow chart of OVA senstization model of the asthmatic BALB/C micc.The mice were randonly divided into 4 groups and treated shown above.In the asthmatic group, mice were senstized with 25μg of OVA emulsified in 1mg of alummum hydoxid in a total volume of 200μl on days 0.7 and14 by intraperitoneal adnmistaton Begmming om the 21st day,the mice were callcnged with 2% OVA for 30 min per day by an ultrasmic nebulizer in a closed chamber far 7d to establist models. The group C was intervene with γδT cell(NS) for injected intravenously on day 0,21.The group D was intervene with γδT cell(*Mycobacterium vaccae*) for injected intravenously on day 0,21.The contol group was treated with PBS mstad of OVA at the senstization and challenge stages.

precooled PBS was slowly injected into the trachea before BALF was removed and stored in liquid nitrogen. IL-17 levels were measured using an ELISA kit (Multi-Sciences: Wuhan Magee Biotechnology Co., Ltd.) according to the instructions.

Histopathology

4% paraformaldehyde was used to fix lung tissue, followed by paraffin embedding and sectioning. The 4 μm-thick sections were stained with hematoxylin-eosin (HE) and periodic acid-Schiff (PAS) according to standard protocols. The extent of pulmonary inflammation was observed under a light microscope and classified according to the semiquantitative scoring method (34). The sections were sequentially placed in xylene and gradient alcohol, followed by HE and immunofluorescence staining. To perform immunofluorescence staining, the corresponding primary antibodies were added: polyclonal goat anti-mouse T-bet antibody (1:1000; ab91109, Abcam), GATA3 antibody (1:1500; ab106625, Abcam), incubated overnight in a wet box, and the next day horseradish peroxidase-labeled secondary antibody (1: 800; ab205719, Abcam) was added, incubated for 2 h at room temperature. After being washed, they were observed under a fluorescent microscope and photographed.

Flow Cytometry

The lung cell precipitate was resuspended in serum-supplemented RPMI 1640 medium and diluted to a density of 109/ml, and incubated with 25ng/ml PMA, 1ng/ml ionomycin, and monensin at room temperature for 4 hours. The cells were harvested, washed with PBS, and stained with PerCP-Cy5-5 anti-CD3 and APC anti-γδT antibodies at 4°C for 30 minutes, fixed and permeabilized at 4°C for 20 minutes, stained with PE anti-IL-17 or PE anti-Foxp3 antibody at 4°C in the dark for 30 min, resuspended in 200 μl PBS, and analyzed by flow cytometry with FlowJo 7.6 software.

Western Blotting

The prepared cold RIPA lysate containing PMSF was added to the lung tissue, followed by homogenization. The precipitate was discarded after centrifugation and the supernatant was taken for determination of the protein concentration while boiling with the addition of 5XLoading buffer. A 10% separation gel was used for SDS-PAGE, followed by the transfer of protein bands to PVDF membranes. 5% nonfat milk was used to block and primary antibodies (anti-GAPDH (1:5000), anti-NICD (1:2500), anti-GATA3 (1:5000), and anti-T-bet (1:5000) were added after completion, and incubated at 4°C. overnight, TBST was washed 3 times and then incubated for 2 hours at room temperature by adding the corresponding labeled secondary antibody (1:1000). Licor Odyssey software was used for the analysis of positive bands.

Statistical Analysis

SPSS software (version 22.0) was used to perform a statistical analysis of the data. The data were expressed as mean±standard deviation (mean±SD). All experiments were carried out independently at least three times. The unpaired t-test was used for the comparison between the two data groups. One-way analysis of variance (ANOVA) was used to analyze the differences between three and more groups, and the Bonferroni test was used for pairwise comparisons between the groups. Pearson's correlation was used to measure the correlation between the samples. P value<0.05 was considered statistically significant.

RESULTS

Percentage of γδTreg and γδT17 Cells

The TCR-$\beta^{-/-}$ mice exposed to *M. vaccae* had fewer γδT17 and more γδTreg cells in their lung tissue compared with the control group (Figure 2), indicating that the bacteria can skew the γδT17/γδTreg ratio.

Airway Reactivity

As shown in Figure 3-A, the asthmatic mice had significantly higher airway responsiveness after MCH stimulation compared with the control animals (P<0.05), which was markedly decreased by *M.*

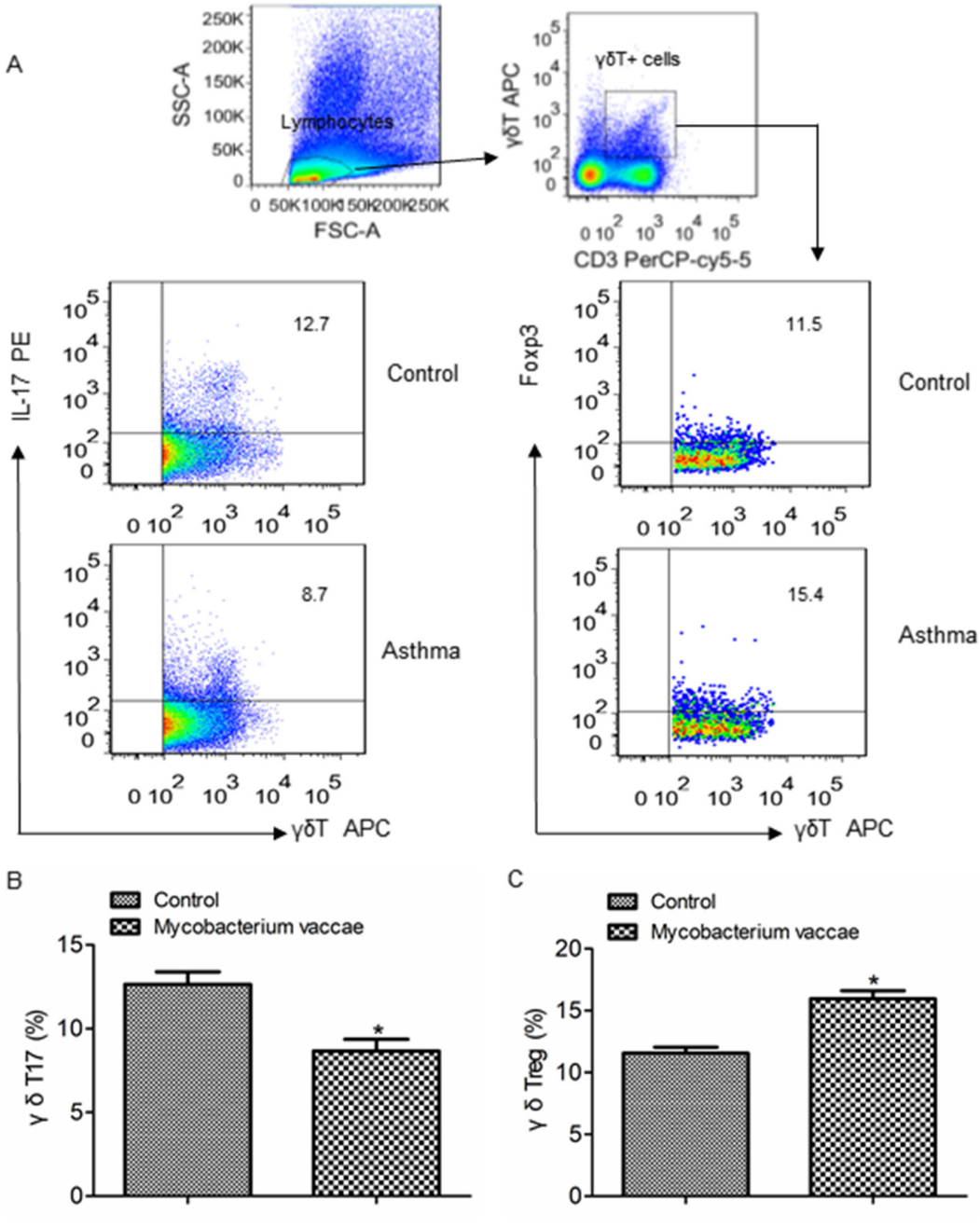

Figure 2. Flow cytometry plots showing relative percentages of lung tissues IL-17+ cell percentages and Treg+γδT cell percentages in the control and infected mice. n = 20, **p*<0.05 vs control.

vaccae-primed γδT cells (P<0.05). On the contrary, non-primed control γδT cells did not affect the response to MCH (P>0.05). *M. vaccae*-sensitized γδT cells alleviated airway reactivity and inflammation in OVA-exposed mice.

Lung Histopathology

The control mice had regular bronchial morphology with normal alveolar interval and little infiltration of inflammatory cells around the bronchus and blood vessels, no epithelial hyperplasia or thickening of the bronchial wall was observed. Asthmatic stimulation increased the infiltration of inflammatory cells, which narrowed the bronchial lumen and thickened its wall. On the contrary, adoptive transfer of naive T cells (NS) or M. vaccae-primed γδT cells mitigated the above patho-inflammatory changes, and the

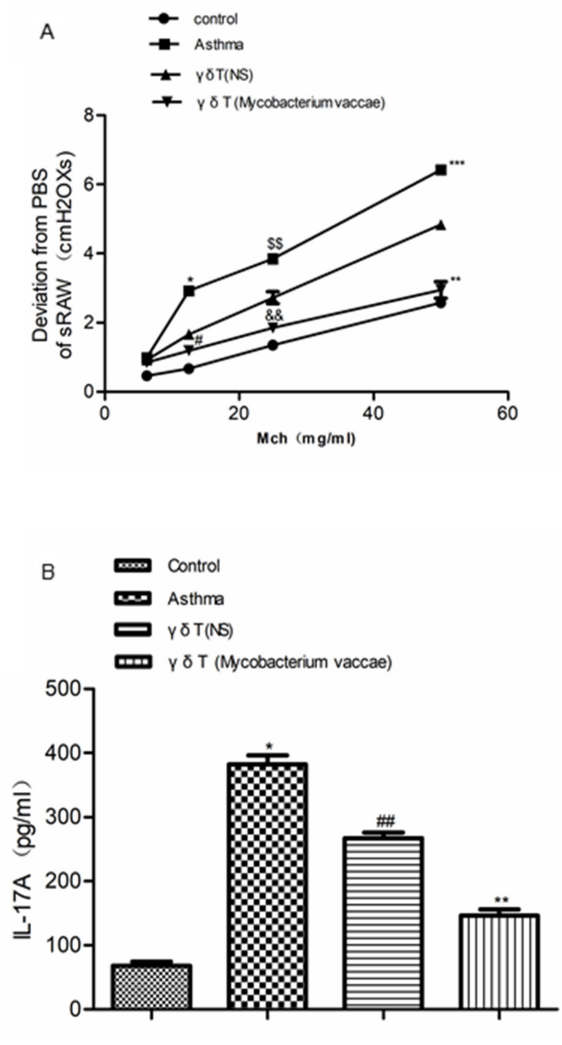

Figure 3. SRaw growth curve showing the airway responsiveness of OVA-stimulated mice after stimulation with 12.5mg/ml, 25mg/ml and 50mg/ml MCH, n=8, *P<0.05, #P<0.05, $$P<0.01, ***P<0.001, vs control, #P<0.05, &&P<0.01, **P<0.01 vs asthma (Figure 3-A). Effect of γδT cells on BALF IL-17 level in asthmatic mice. Results of ELISA indicating the amount of IL-17A released in BALF in (a) control, (b) asthma, (c) non-primed γδT cells groups and (d) M. vaccae-primed γδT cells. N=8, *P<0.05 vs control, ** P<0.01 vs asthma, ## P>0.05 vs asthma (Figure 3-B).

primed cells resulted in a more obvious effect. Furthermore, PAS staining showed excessive mucus exudation and goblet cell hyperplasia in asthmatic mice compared with only a few intraluminal bronchial goblet cells and a lack of mucus leakage in the healthy controls. T cells primed with nave and M. vaccae-primed γδT cells decreased the amount of mucous in the bronchi and perivascular space, and the change was more obvious with the primed cells (Figure 4, Table 1).

IL-17 Levels

Consistent with histopathological findings, IL-17A levels were significantly higher in BALF of asthmatic mice compared with the normal controls and were restored to almost baseline levels by adoptive transfer of *M. vaccae*-primed γδT cells (Figure 4, P<0.05). On the contrary, non-primed γδT cells had no ameliorative effect on BALF IL-17 levels (P<0.05, (Figure 3).

M. Vaccae-primed γδT Cells Reversed Th2 Polarization in Asthmatic Mice

Compared with the control mice, the in situ expression of T-bet was significantly lower and that of GATA-3and NICD was significantly higher in the lung tissues of asthmatic mice (P<0.05 for both). Consistent with the findings so far, T cells treated with *M. vaccae*-primed γδT cells reversed the levels of both in these mice (P<0.05), whereas the control γδT cells had no significant effect (P>0.05; Figure 4).

Expression of IL-17 + γδT and Foxp3 + γδT

Asthmatic stimulation significantly increased the number of IL-17$^+$ γδ T cells in lung tissues compared with the control mice (23±3.1 vs 10±3.7; P<0.05), which was decreased by *M. vaccae*-primed γδT cells (13±2.8; P<0.05). The Foxp3$^+$ γδT cell count was lower in asthmatic mice compared with the healthy mice (2.4±0.6 vs 5.4±05; P<0.05), and restored after adoptive transfer of *M. vaccae*-primed γδT cells (4.5±1.4 vs 2.4±0.6; P<0.05) but not the control γδT cells (2.7±0.6 vs 2.4±0.6; P>0.05). Then, a positive correlation was established between the relative levels of NICD protein and the percentage of IL-17+ γδT cells (r=0.56, P<0.001). This strongly indicates that the γδT17/γδ Treg ratio alters upon asthma induction to a more Th2 phenotype, and *M. vaccae*-primed γδT cells restore this balance and reverse Th2 polarization. Furthermore, we also observed

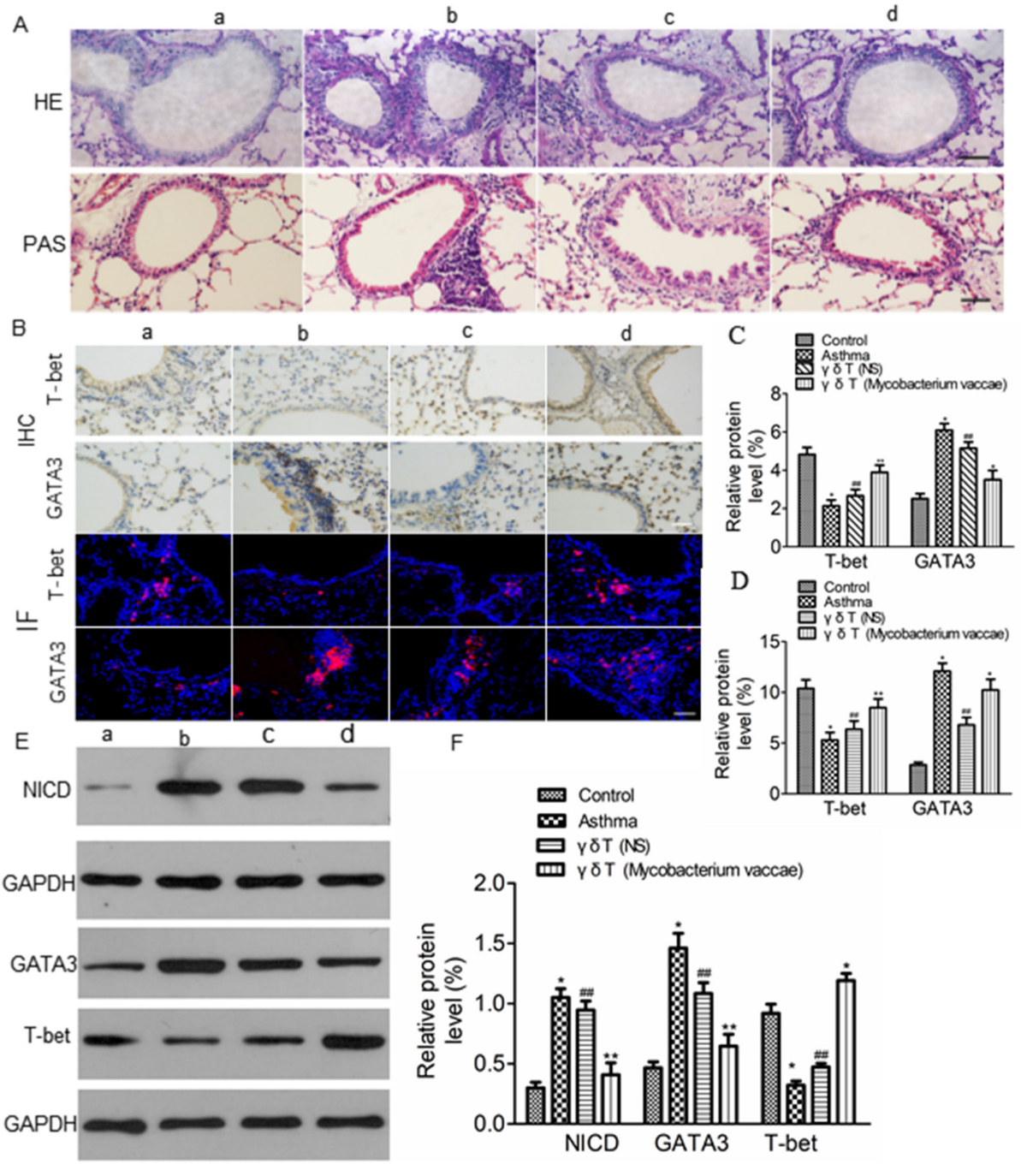

Figure 4. Effect of γδT cells on the lung tissues of asthmatic mice. Representative images of HE-stained lung tissues showing the bronchio-alveolar architecture of the different animal groups. Original magnification – 400x, scale bar - 25μm). Representative images of PAS-stained tissues showing goblet cells and mucus exudation in the airway epithelium of different groups. Original magnification – 200x, scale bar - 50μm), n=8 （Figure 4-A). Representative IHC images (Figure 4-B,C) and IF images(Figure 4-B,D) showing the in situ expression intensity of T-bet and GATA3 and WB images(E,F) showing T-bet ,GATA3 and NICD protein levels in the lung tissues of the different groups. *P<0.05 vs control, **P<0.01 vs asthma, **P>0.05 vs asthma. Original magnification – 200x, scale bar - 50μm), n=8.

a significant negative correlation between the relative levels of NICD protein and the percentage of Foxp3^{+} γδT cells (r=-0.74, P<0.01). Taken together, *M. vaccae* alleviates the symptoms of OVA-induced asthma by lowering the balance towards the γδTreg cells through the inhibition of the Notch pathway. (Figure 5).

Table 1. Airway inflammation scores and mucus scores

Group	Inflammation scores					Mucus scores				
	0	1	2	3	4	0	1	2	3	4
a	8	0	0	0	0	7	1	0	0	0
b	0	0	0	3	5	0	0	0	3	5
c	0	0	2	3	3	0	0	2	2	4
d	0	4	2	2	0	0	4	3	1	0

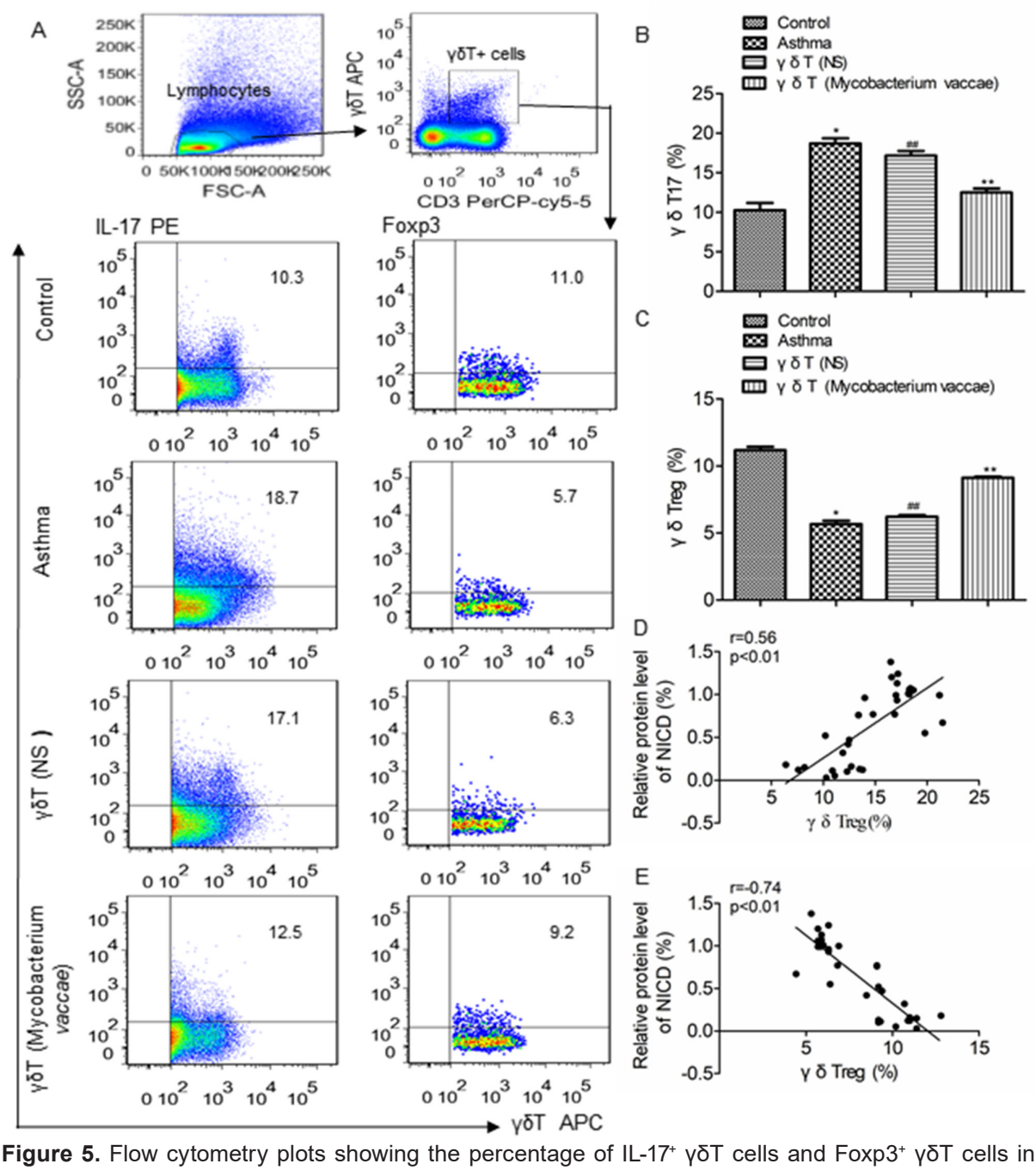

Figure 5. Flow cytometry plots showing the percentage of IL-17⁺ γδT cells and Foxp3⁺ γδT cells in the lung tissues of different groups. Foxp3+ γδT cells in the lung tissues of different groups (Figure 5-A,B,C). Correlation between NICD protein levels in lung tissue and the percentage of IL-17+ γδT cells (r=0.56, P<0.001) is shown （Figure 5-D）. Correlation between NICD protein levels in lung tissue and the percentage of Foxp3⁺ γδT cells is also shown (r=-0.74, P<0.01) （Figure 5-E）. *P<0.05 vs control, **P<0.01 vs asthma, **P>0.05 vs asthma. N=8.

DISCUSSION

The pathogenesis of asthma is still not fully understood, and current studies suggest that immune T cells are closely associated with it. Among them, the Th1/Th2 imbalance of γδ T cells is widely accepted to be involved in the development and progression of asthma (34). Not only in asthma disease but also other allergic and autoimmune diseases, IL-17+ γδT and Treg subsets are involved in varying degrees (35). The γδT17 cells, in particular, drive airway remodeling and hyperresponsiveness in asthma by secreting inflammatory cytokines and activating neutrophils. GATA3 is the key Th2 transcription factor (36) that promotes the synthesis of Th2 cytokines (37). On the other hand, the T-bet triggers Th1 differentiation by upregulating IFN-γ. The Th1/Th2 imbalance in asthma is the result of an aberrant T-bet/GATA3 ratio, which causes hypersecretion of Th2-type cytokines and triggers airway inflammation.

We primed the γδT cells in TCR-$\beta^{-/-}$ mice with *M. vaccae* and transplanted these cells into an OVA-induced asthma model. T cells treated with *M. vaccae*-primed γδT cells not only alleviated airway hyperresponsiveness and inflammatory damage in lung tissues of asthmatic mice but also decreased IL-17 levels in BALF. Furthermore, the mice treated with these primed γδT cells also showed a higher proportion of $Foxp3^+$ relative to IL-17^+ T cells in their lung tissues, which corresponded to a higher ratio of T-bet / GATA3. In addition, T cells treated with *M. vaccae*-primed γδT cells inhibited the Notch pathway in inflamed lungs, as indicated by the decreased levels of NICD. These results are consistent with the antiasthmatic effects of *Mycobacteria* and its culture supernatants, as well as those of the BCG vaccine, in mice models (38, 39). We hypothesize that the improvement effect of primed T cells was directly due to the cells injected themselves, which is supported by the involvement of γδT17/γδTreg cells in the occurrence and development of asthma (40), or indirectly via inhibition of the Notch/GATA3 pathway and Th2 polarization. The specific regulatory mechanism will have to be verified in subsequent studies.

M. vaccae and *M. phlei* have an immunomodulatory effect on their hosts. Aerosol bacteria can stimulate mucosal immunity, which triggers airway inflammation and hyperresponsiveness. A previous meta-analysis showed that *M. vaccae* immunotherapy can effectively treat pulmonary TB (41). Another study showed that *M. vaccae* administered before chronic subordinate colony housing (CSC) strongly promoted active stress coping (42). These experimental data indicate the clinical application of *M. vaccae.* The imbalance of γδT17/γδTregs is correlated with the pathogenesis of OVA-induced asthma (4). Inhalation of inactivated *M. phlei* can correct the Th1/Th2 γδT cell balance in asthma (43), thereby reducing lung inflammatory lesions. Consistent with this, aerosol bacteria from inactivated *M. phlei* reduced airway inflammation in the asthmatic mice, upregulated T-bet and GATA-3 mRNA levels, and inhibited Th2 cytokine secretion (31). In this study, aerosolized *M. vaccae* decreased the proportion of γδT17 cells and increased that of γδTregs, which reduced AHR, inflammation, and IL-17 levels in BALF. The mechanism was first the direct effect of injected γδT cells on asthma. Second, the effect of infused Treg on the infused γδTreg in the mouse body can inhibit the Notch pathway, regulate Th1/Th2 differentiation, and then regulate the proportion of T-bet / GATA3. Our findings provide the theoretical basis for new therapeutic approaches against asthma.

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Conflict of Interest: None declared.

REFERENCES

1. Al-Daghri N M, Alokail M S, Draz H M, et al. Th1/Th2 cytokine pattern in Arab children with severe asthma [J]. International journal of clinical and experimental medicine, 2014, 7(8): 2286-91.
2. Youssef D M, Elbehidy R M, Shokry D M, et al. The influence of leptin on Th1/Th2 balance in obese children with asthma [J]. Jornal brasileiro de pneumologia : publicacao oficial da Sociedade Brasileira de Pneumologia e Tisilogia, 2013, 39(5): 562-8.
3. Ming M, Luo Z, Lv S, et al. Inhalation of inactivated-Mycobacterium phlei prevents asthma-mediated airway hyperresponsiveness and airway eosinophilia in mice by reducing IL-5 and IL-13 levels [J]. Molecular medicine reports, 2016, 14(6): 5343-9.
4. Yang X, Zhang J H, Deng W S, et al. Imbalance of γδT17/γδTreg cells in the pathogenesis of allergic asthma induced by ovalbumin [J]. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas, 2018, 51(9): e7127.
5. Glanville N, Message S D, Walton R P, et al. γδT cells suppress inflammation and disease during rhinovirus-induced asthma exacerbations [J]. Mucosal immunology, 2013, 6(6): 1091-100.
6. Hahn Y S, Taube C, Jin N, et al. Different potentials of gamma delta T cell subsets in regulating airway responsiveness: V gamma 1+ cells, but not V gamma 4+ cells, promote airway hyperreactivity, Th2 cytokines, and airway inflammation [J]. Journal of immunology (Baltimore, Md. : 1950), 2004, 172(5): 2894-902.
7. Nakada E M, Shan J, Kinyanjui M W, et al. Adjuvant-dependent regulation of interleukin-17 expressing γδ T cells and inhibition of Th2 responses in allergic airways disease [J]. Respiratory research, 2014, 15(1): 90.
8. Zhong F, Cui D, Tao H, et al. IL-17A-producing T cells and associated cytokines are involved in the progression of gastric cancer [J]. Oncology reports, 2015, 34(5): 2365-74.
9. Silva M J, De Santana M B R, Tosta B R, et al. Variants in the IL17 pathway genes are associated with atopic asthma and atopy makers in a South American population [J]. Allergy, asthma, and clinical immunology : official journal of the Canadian Society of Allergy and Clinical Immunology, 2019, 15(28.
10. Chiba Y, Tanoue G, Suto R, et al. Interleukin-17A directly acts on bronchial smooth muscle cells and augments the contractility [J]. Pharmacological reports : PR, 2017, 69(3): 377-85.
11. Whitehead G S, Kang H S, Thomas S Y, et al. Therapeutic suppression of pulmonary neutrophilia and allergic airway hyperresponsiveness by a RORγt inverse agonist [J]. JCI insight, 2019, 5(14):
12. Papotto P H, Ribot J C, Silva-Santos B. IL-17(+) γδ T cells as kick-starters of inflammation [J]. Nature immunology, 2017, 18(6): 604-11.
13. Kang N, Tang L, Li X, et al. Identification and characterization of Foxp3(+) gammadelta T cells in mouse and human [J]. Immunology letters, 2009, 125(2): 105-13.
14. Lazarevic V, Glimcher L H, Lord G M. T-bet: a bridge between innate and adaptive immunity [J]. Nature reviews. Immunology, 2013, 13(11): 777-89.
15. Yong J, Chen G Q, Huang B, et al. Correlation between the ratio of T-bet/GATA-3 and the levels of IL-4 and IFN-γ in patients with allergic asthma [J]. Molecular medicine reports, 2011, 4(4): 663-6.
16. Lee G R. The Balance of Th17 versus Treg Cells in Autoimmunity [J]. International journal of molecular sciences, 2018, 19(3):
17. Kim S H, Hong J H, Lee Y C. Oleanolic acid suppresses ovalbumin-induced airway inflammation and Th2-mediated allergic asthma by modulating the transcription factors T-bet, GATA-3, RORγt and Foxp3 in asthmatic mice [J]. International immunopharmacology, 2014, 18(2): 311-24.
18. Chen T, Xiao L, Zhu L, et al. Anti-Asthmatic Effects of Ginsenoside Rb1 in a Mouse Model of Allergic Asthma Through Relegating Th1/Th2 [J]. Inflammation, 2015, 38(5): 1814-22.
19. Anatriello E, Cunha M, Nogueira J, et al. Oral feeding of Lactobacillus bulgaricus N45.10 inhibits the lung inflammation and airway remodeling in murine allergic asthma: Relevance to the Th1/Th2 cytokines and STAT6/T-bet [J]. Cellular immunology, 2019, 341(103928.
20. Kardan M, Rafiei A, Ghaffari J, et al. Effect of ginger extract on expression of GATA3, T-bet and ROR-γt in peripheral blood mononuclear cells of patients with Allergic Asthma [J]. Allergologia et immunopathologia, 2019, 47(4): 378-85.
21. Shibata K, Yamada H, Sato T, et al. Notch-Hes1 pathway is required for the development of IL-17–producing γδ T cells [J]. Blood, 2011, 118(3): 586-93.
22. Nieuwenhuis M A, Vonk J M, Himes B E, et al. PTTG1IP and MAML3, novel genomewide association study genes for severity of hyperresponsiveness in adult asthma [J]. Allergy, 2017, 72(5): 792-801.
23. Zhang W, Nie Y, Chong L, et al. PI3K and Notch signal pathways coordinately regulate the

activation and proliferation of T lymphocytes in asthma [J]. Life sciences, 2013, 92(17-19): 890-5.
24. Huang M T, Dai Y S, Chou Y B, et al. Regulatory T cells negatively regulate neovasculature of airway remodeling via DLL4-Notch signaling [J]. Journal of immunology (Baltimore, Md. : 1950), 2009, 183(7): 4745-54.
25. Yao Y E, Li C Q. Notch signaling pathway and its relationship with bronchial asthma [J]. Journal of Practical Medicine, 2019, 035(002): 315-7.
26. Zuany-Amorim C, Sawicka E, Manlius C, et al. Suppression of airway eosinophilia by killed Mycobacterium vaccae-induced allergen-specific regulatory T-cells [J]. Nature medicine, 2002, 8(6): 625-9.
27. Ahrens B, Gruber C, Rha R D, et al. BCG priming of dendritic cells enhances T regulatory and Th1 function and suppresses allergen-induced Th2 function in vitro and in vivo [J]. International archives of allergy and immunology, 2009, 150(3): 210-20.
28. Li C Q, XU Y J, Yang D L, et al. Study on Th1/Th2 immune response pattern of γδT cells in rat bronchial asthma model [J]. Chinese Journal of Internal Medicine, 2004, 043(005): 342-4.
29. Zhang J, Li C, Guo S. Effects of inhaled inactivated Mycobacterium phlei on airway inflammation in mouse asthmatic models [J]. Journal of aerosol medicine and pulmonary drug delivery, 2012, 25(2): 96-103.
30. Zhang J, Guo S, Li C, et al. Therapeutic effects of inhaled inactivated Mycobacterium phlei in adult patients with moderate persistent asthma [J]. Immunotherapy, 2012, 4(4): 383-7.
31. Liu W W, SUN Q X, Zhang J H, et al. Effects of aerosol inhalation on t-BET /GATA-3 expression in mice with asthma [J]. Chinese Bulletin of Pharmacology,2018, v.34(8): 71-6.
32. Ming M, Li C, Luo Z, et al. The effect of inhaled inactived Mycobacterium phlei as a treatment for asthma [J]. Molecular medicine reports, 2017, 15(2): 777-83.
33. Jiang X H, Li C Q, Feng G Y, et al. Inhalation of nebulized Mycobacterium vaccae can protect against allergic bronchial asthma in mice by regulating the TGF-β/Smad signal transduction pathway [J]. Allergy, asthma, and clinical immunology : official journal of the Canadian Society of Allergy and Clinical Immunology, 2020, 16(59.
34. Murdoch J R, Gregory L G, Lloyd C M. γδT cells regulate chronic airway inflammation and development of airway remodelling [J]. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, 2014, 44(11): 1386-98.
35. Roark C L, Simonian P L, Fontenot A P, et al. gammadelta T cells: an important source of IL-17 [J]. Current opinion in immunology, 2008, 20(3): 353-7.
36. Grieger J A, Wood L G, Clifton V L. Improving asthma during pregnancy with dietary antioxidants: the current evidence [J]. Nutrients, 2013, 5(8): 3212-34.
37. Chakir H, Wang H, Lefebvre D E, et al. T-bet/GATA-3 ratio as a measure of the Th1/Th2 cytokine profile in mixed cell populations: predominant role of GATA-3 [J]. Journal of immunological methods, 2003, 278(1-2): 157-69.
38. Han E R, Choi I S, Eom S H, et al. Preventive effects of mycobacteria and their culture supernatants against asthma development in BALB/c mice [J]. Allergy, asthma & immunology research, 2010, 2(1): 34-40.
39. Deng Y, Li W, Luo Y, et al. Inhibition of IFN-γ promotes anti-asthma effect of Mycobacterium bovis Bacillus Calmette-Guerin neonatal vaccination: a murine asthma model [J]. Vaccine, 2014, 32(18): 2070-8.
40. Zhao J, Lloyd C M, Noble A. Th17 responses in chronic allergic airway inflammation abrogate regulatory T-cell-mediated tolerance and contribute to airway remodeling [J]. Mucosal immunology, 2013, 6(2): 335-46.
41. Huang C Y, Hsieh W Y. Efficacy of Mycobacterium vaccae immunotherapy for patients with tuberculosis: A systematic review and meta-analysis [J]. Human vaccines & immunotherapeutics, 2017, 13(9): 1960-71.
42. Amoroso M, Böttcher A, Lowry C A, et al. Subcutaneous Mycobacterium vaccae promotes resilience in a mouse model of chronic psychosocial stress when administered prior to or during psychosocial stress [J]. Brain, behavior, and immunity, 2019,
43. Kinyanjui M W, Shan J, Nakada E M, et al. Dose-dependent effects of IL-17 on IL-13-induced airway inflammatory responses and airway hyperresponsiveness [J]. Journal of immunology (Baltimore, Md. : 1950), 2013, 190(8): 3859-68.