



Inhibition of IFN- γ promotes anti-asthma effect of *Mycobacterium bovis* Bacillus Calmette-Guerin neonatal vaccination: A murine asthma model



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ABSTRACT

Objective: The *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG) neonatal vaccination inhibits allergy-induced pathologic changes. However, the mechanisms underlying this process are unclear. This study aimed to investigate the role of interferon (IFN)- γ and interleukin (IL)-17 in the protective effects of the BCG neonatal vaccination on allergic pulmonary inflammation and airway hyperresponsiveness (AHR). **Methods:** Wild type (WT)-neonate and IL-17 knock out (KO) neonate mice were vaccinated with BCG. A murine asthma model was developed by sensitization and then challenging with ovalbumin (OVA). Recombinant IL-17 or recombinant IFN- γ was delivered to the airway to overexpress IL-17 or IFN- γ . An anti-IFN- γ neutralizing antibody was used to block the effects of IFN- γ .

Results: We found exogenous IL-17 delivered to the airway reversed the anti-asthma effects of the neonatal BCG vaccination. BCG neonatal vaccination further reduced OVA-induced inflammation and AHR in IL-17 KO mice. Inhibition of IFN- γ in BCG neonatal vaccinated OVA-induced asthma model mice led to a further reduction in airway inflammation and AHR. In addition, airway inflammation and AHR were robust following treatment with exogenous IFN- γ . Neutralizing IL-17 was not sufficient to block OVA-induced airway inflammation and AHR. In IL-17 KO mice, airway inflammation and AHR did not occur following treatment with an anti-IFN- γ neutralizing antibody.

Conclusions: In an OVA-induced murine asthma model, inhibition of IFN- γ enhanced the anti-asthma effects of BCG neonatal vaccination.

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1. Introduction

Asthma is a complex disorder characterized by inflammation with eosinophil, lymphocyte and mast cell infiltration and clinically manifests as recurrent episodes of wheezing and airway hyperresponsiveness (AHR) [1]. Asthma is described as a Th2-mediated inflammatory response, and the balance between the Th2 response and Th1 response has been thought to

determine allergic airway inflammation. The “hygiene hypothesis” confirms that the Bacillus Calmette-Guerin (BCG) neonatal vaccination alleviates the symptoms of asthma in both animal models and human beings by increasing the secretion of Th1 cytokines [2–4].

However, there is increasing evidence of heterogeneity in asthma phenotypes, with a significant proportion of asthmatic patients demonstrating a low or non-Th2-mediated phenotype, which may be less sensitive to glucocorticoid treatment [5,6]. Some asthma patients have been characterized by the presence of high levels of IFN- γ , IL-17, and neutrophils in the lungs. These forms of asthma respond poorly to treatment with

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corticosteroids. Therefore, it is important to determine whether BCG vaccination could prevent asthma, as well as determine the underlying mechanisms with particular reference to IFN- γ or IL-17.

In a murine asthma model, neonatal BCG vaccination suppresses the symptoms of asthma by down-regulating IL-17 [7]. Involvement of IL-17 in AHR elevation and neutrophilia has been reported [8,9]. In addition, a high concentration of IL-17 in biopsies from neutrophilic asthma patients [9,10] and a correlation between IL-17 levels with the incidence of AHR and the severity of disease [11,12] have been reported. However, how BCG inhibits allergy-induced pathologic changes remains controversial. In this study, we aimed to investigate the role of IL-17 and IFN- γ on the anti-asthma effect of neonatal BCG vaccination and the OVA-challenged asthmatic symptoms.

2. Material and methods

2.1. Animals

Wild-type (WT) mice were purchased from the Laboratory Animal Center of Chongqing Medical University. Professor Bin Li of the Third Military Medical University in Chongqing, China provided IL-17 KO mice with a C57BL/6 background. These mice were housed in a pathogen-free facility. We used mice neonates, and the experimental protocols were in accordance with the guidelines issued by the Chinese Council on Animal Care. The Ethics Committee of the Chongqing Medical University of Medical Sciences in China approved all of the animal procedures (ethics approval number: 2013004).

2.2. Immunization and treatment

Mice in the OVA group were sensitized, and their airways were challenged with OVA. Mice in the control group were sensitized and their airways were challenged with phosphate buffer solution (PBS). Mice in the BCG+OVA group received the BCG neonate vaccination, were sensitized, and their airways were challenged with OVA. Mice in the BCG+OVA+anti-IFN- γ monoclonal antibody (mAb) group were vaccinated with BCG, were sensitized, their airways were challenged with OVA, and then they received anti-IFN- γ mAb injections. Mice in the OVA+anti-IFN- γ mAb group were sensitized, their airways were challenged with OVA, and then they received anti-IFN- γ mAb injections. Mice in the BCG+OVA+recombinant IL-17 (rIL-17) group and BCG+OVA+recombinant IFN- γ (rIFN- γ) group were vaccinated with BCG, were sensitized, their airways were challenged with OVA, and then they received rIL-17 or rIFN- γ . The number of experimental and control groups were at least 6 mice.

On day 1, mice in the BCG neonatal vaccination groups were subcutaneously injected in the back with 20 μ L of BCG [1×10^5 colony-forming units (CFU)] using a 30-gauge needle and a tuberculin syringe. On day 28 and day 42, mice were sensitized by intraperitoneal injection (i.p.) with 100 μ g OVA (Sigma) diluted in 50% aluminum hydroxide (Pierce) to a total volume of 200 μ L. To induce asthma, mice were challenged from day 49 to day 55 by exposure to 1% OVA aerosols in a Plexiglas exposure chamber for 30 min/day. Mice treated with the IFN- γ neutralizing mAb were injected i.p. with an anti-IFN- γ mAb (10 μ g aIFN- γ ; R&D Systems) in 200 μ L PBS on days 48, 51, and 55 during the OVA challenge. One day after the final OVA challenge, mice were euthanized. rIL-17 (15 μ g in 60 μ L ABS; R&D Systems) or rIFN- γ was delivered to the airway by tracheal instillation 12 h after the last day of

OVA challenge (day 55). Then mice were euthanized on the next day.

2.3. Bronchoalveolar lavage (BALF) and cell counting

BALF was collected 24 h after the final OVA challenge (day 55). The total cell number in BALF was counted immediately upon collection. The cell pellet was used to prepare slides for differential cell counting. The number of monocytes, lymphocytes, neutrophils, and eosinophils were counted using Wright's-Giemsa stain in a blinded fashion. Each slide had at least 200 cells.

2.4. Determination of AHR

AHR to methacholine (MCh) in conscious, spontaneously breathing animals was measured by single-chamber, whole-body plethysmography (Buxco Electronics Inc, Troy, NY, USA) as described previously [13]. Briefly, mice were exposed to aerosolized PBS (for the baseline measurement) or MCh (3125–50 mg/mL) for 3 min, and readings were taken and averaged for 3 min after each nebulization. Data are presented as the Penh (enhanced pauses) vs. the MCh concentration (mg/mL) used to generate the aerosol. Penh is a dimensionless value that represents a function of the ratio of peak expiratory flow to peak inspiratory flow and a function of the timing of expiration. It correlates with pulmonary airflow resistance.

2.5. Histopathology

Formalin-fixed lungs were embedded in paraffin, sectioned in 6 μ m thick slices, and stained with hematoxylin and eosin for routine histology. Lung lesions were scored semi-quantitatively as described by other researchers [10].

2.6. Cytokine analysis

Cytokine concentrations in BALF were measured with commercial enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Murine IFN- γ ELISA kits, IL-10 ELISA kits and IL-12 ELISA kits were purchased from Bender MedSystems (Vienna, Austria). Murine IL-13 ELISA kits and IL-23 ELISA kits were purchased from R&D Systems (Minneapolis, MN USA).

2.7. Statistical analysis

All results are expressed as mean $\pm$ SEM. Analysis of variance (ANOVA) was used to determine significant differences between groups. Groups were compared with unpaired *t*-tests. *P* < 0.05 was considered statistically significant.

3. Results

3.1. Exogenous rIL-17 reversed the anti-asthma effects following neonatal BCG vaccination

We first investigated the anti-asthma effect of BCG neonatal vaccination in an OVA-induced asthma model treated with rIL-17. Compared with the control group, more inflammatory cells were observed in the BALF of mice in the WT BCG+OVA+rIL-17 group (Fig. 1A). Histologic analyses demonstrated remarkable differences in cellular infiltration into the lung between the two groups. Mice in the WT BCG+OVA+rIL-17 group displayed significantly more

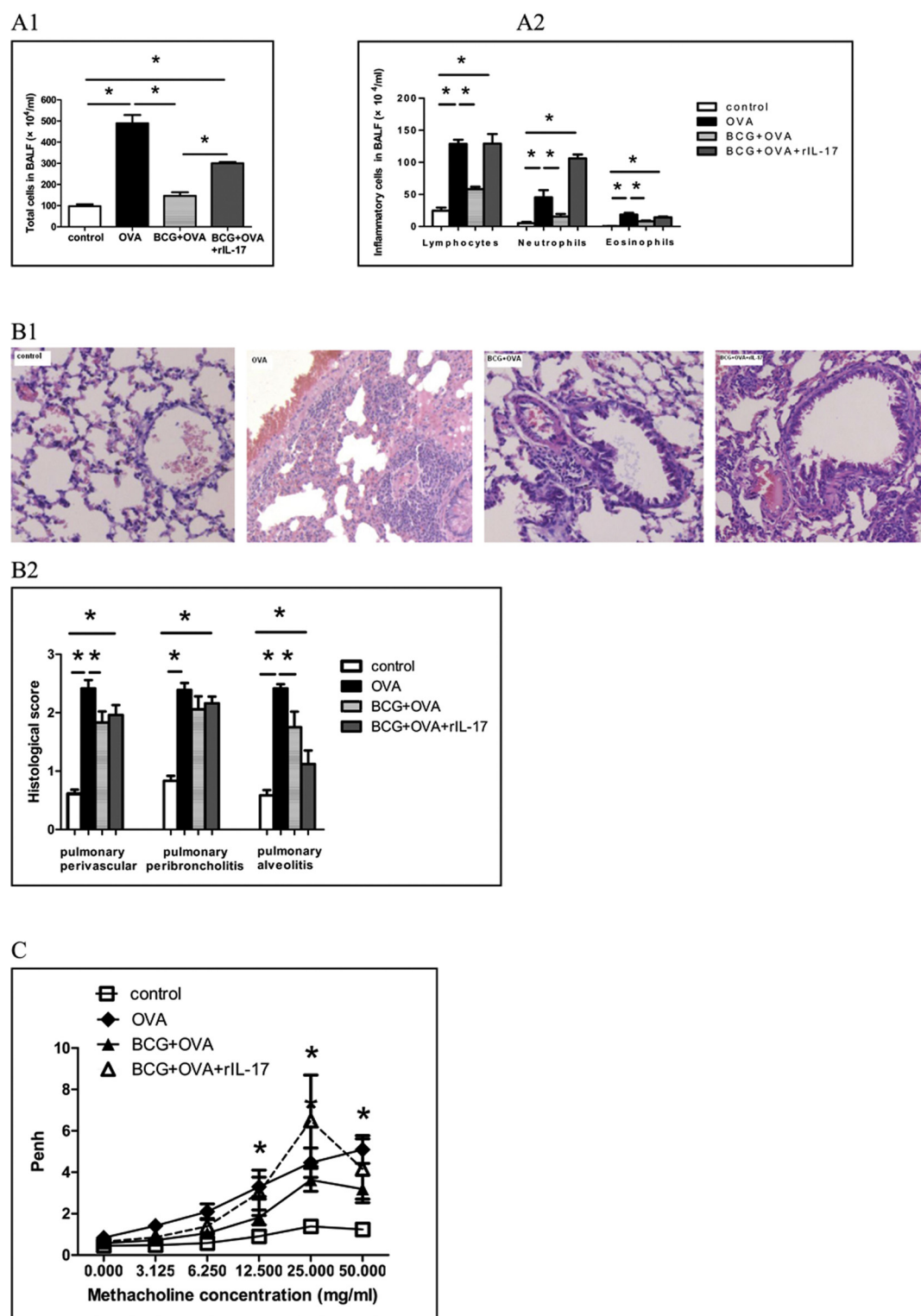


Fig. 1. Exogenous rIL-17 reversed the anti-asthma effects of neonatal BCG vaccination. (A) The number of total cells and inflammatory cells were dramatically elevated in OVA sensitized and challenged mice that received BCG neonatal vaccination and rIL-17 delivered to the airway than that in the control mice. BALF samples were collected 24 h after the final challenge, and then inflammatory cells were counted using Wright's-Giemsa staining. (A1) Total cells in BALF; (A2) lymphocytes, neutrophils and eosinophils in BALF. Data are expressed as the mean $\pm$ SEM of the number of cells in each group of mice. * $P < 0.05$ vs. each other. (B) Tissue pathology in the four groups of mice. Twenty-four hours after the final challenge, mouse lung tissue sections were stained with hematoxylin and eosin (HE). Data are representative of each group of mice. (B1) Representative photomicrographs of lung stained with HE (magnification: 100 $\times$); (B2) histological scores of pulmonary peribronchiolitis, perivascularitis and alveolitis. Data are expressed as mean score $\pm$ SEM of each group. * $P < 0.05$ vs. each other. (C) Exogenous rIL-17 reversed the suppression of AHR by neonatal BCG vaccination. Twenty-four hours after the final challenge, AHR (Penh) in response to increased doses of nebulized MCh was assessed by a whole-body plethysmograph in the experimental groups and negative control mice injected with saline. Data are presented as the mean $\pm$ SEM. * $P < 0.05$ control group vs. BCG + OVA + rIL-17 group. The number of mice in control group was 8. The number of mice in OVA group was 8. The number of mice in BCG + OVA group was 8. The number of mice in BCG + OVA + rIL-17 group was 6.

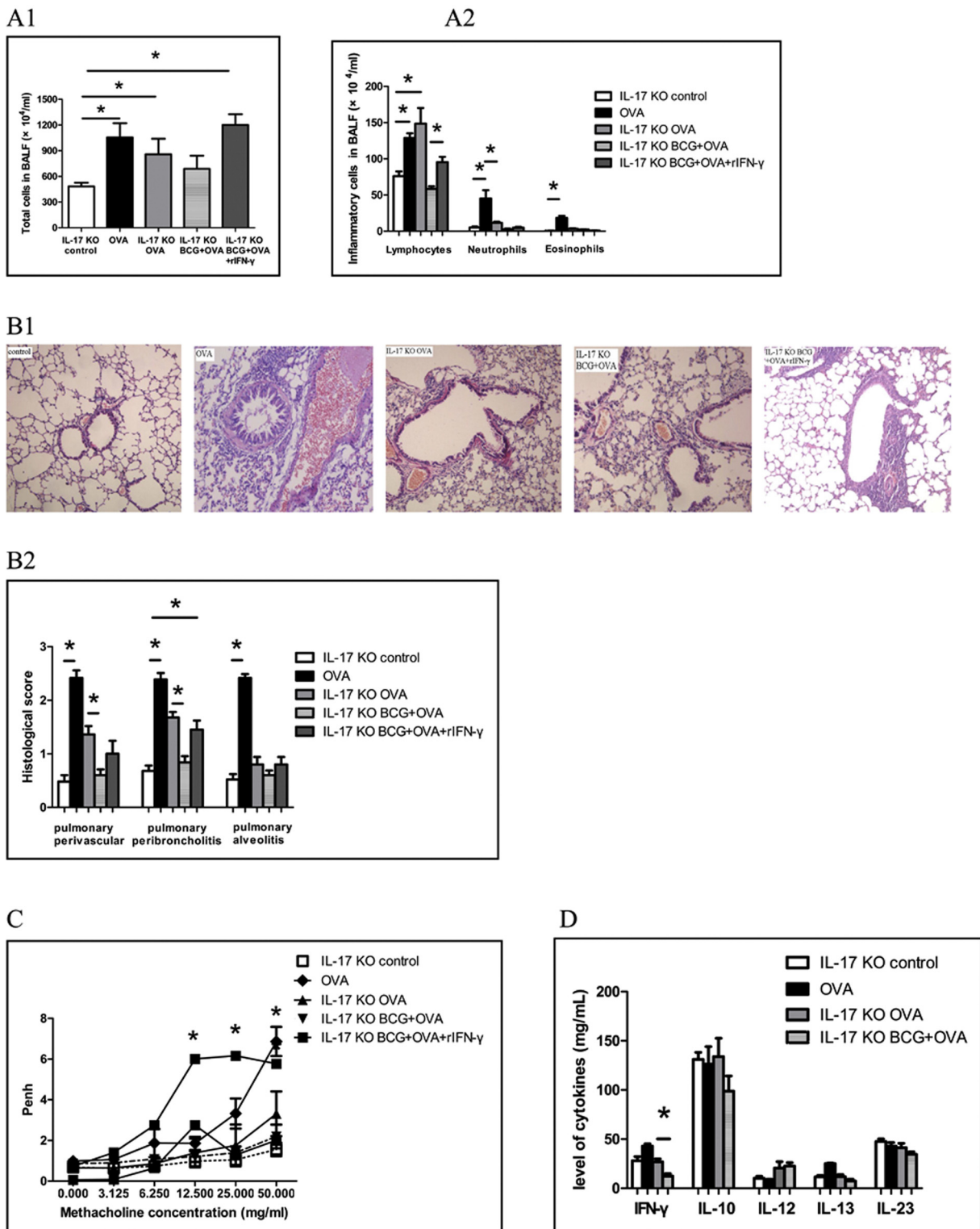


Fig. 2. BCG neonatal vaccination further reduced OVA-induced inflammation and AHR in IL-17 KO mice asthma model. (A) Neonatal BCG vaccination further reduced OVA-induced inflammation in BALF in IL-17 KO mice, while exogenous rIFN- γ reversed the effect. (A1) Total cells in BALF; (A2) lymphocytes, neutrophils and eosinophils in BALF. Data are expressed as the mean $\pm$ SEM of the number of cells in each group of mice. * P < 0.05 vs. each other. (B) Neonatal BCG vaccination further reduced OVA-induced tissue inflammation in IL-17 KO mice, while exogenous rIFN- γ reversed the effect. (B1) Representative photomicrographs of lung stained with HE (magnification 100 $\times$); (B2) histological scores of pulmonary perivascularitis, peribronchitis, alveolitis. * P < 0.05 vs. each other. (C) Neonatal BCG vaccination further reduced OVA-induced AHR in IL-17 KO mice, while exogenous rIFN- γ reversed the effect. Data are presented as the mean $\pm$ SEM. * P < 0.05 IL-17 KO BCG + OVA group vs. IL-17 KO BCG + OVA + rIFN- γ group. (D) IFN- γ levels were significantly decreased in IL-17 KO mice that received neonatal BCG vaccination when compared to IL-17 KO mice. Cytokine levels were measured by ELISA. The data represent means $\pm$ SEM. * P < 0.05 vs. each other. The number of mice in control group was 8. The number of mice in OVA group was 8. The number of mice in IL-17 KO OVA group was 8. The number of mice in IL-17 KO BCG + OVA group was 8. The number of mice in IL-17 KO BCG + OVA + rIFN- γ group was 6.

alveolar, peribronchial and perivascular cellular infiltration than that in the control group (Fig. 1B). In addition, Penh values in mice in the WT BCG + OVA + rIL-17 group were significantly higher than that of the control group in response to MCh exposure (125–50 mg/mL) (Fig. 1C). These findings indicated that exogenous IL-17 reversed the anti-asthma effects of neonatal BCG vaccination OVA-induced asthmatic mice.

3.2. BCG neonatal vaccination further reduced OVA-induced inflammation and AHR in IL-17 KO mice through decreasing IFN- γ

Although neonatal BCG vaccination had anti-asthma effects in OVA-induced asthmatic mice, it was not clear whether the effects were dependent on IL-17 down-regulation. Compared with the control group, inflammatory cells were increased in mice in the WT OVA group and the IL-17 KO OVA group following OVA treatment (Fig. 2A) while the number of inflammatory cells was decreased in BALF of mice in the IL-17 KO BCG + OVA group when compared to mice in the IL-17 KO OVA group. In the WT OVA group and IL-17 KO OVA group, airway sensitization and challenge led to dense peribronchiolar and perivascular infiltration of inflammatory cells (Fig. 2B). In the IL-17 KO BCG + OVA group, tissue inflammation was greatly reduced, with significantly less peribronchial and perivascular cellular infiltration, than that in the IL-17 KO OVA group (Fig. 2B). Fig. 2C demonstrates the response to different doses of aerosolized MCh. The Penh values of the WT OVA group gradually increased with elevated concentrations of MCh, whereas the Penh values of mice in the IL-17 KO OVA group increased at a concentration of 50 mg/mL. The Penh values in the IL-17 KO BCG + OVA group were significantly lower than that of the mice in the IL-17 KO OVA group in response to a MCh concentration of 50 mg/mL (Fig. 2C). These results demonstrated that without IL-17, OVA still induced airway inflammation and AHR in mice, but BCG neonatal vaccination still results in reduced airway inflammation and AHR in OVA-induced asthmatic mice.

Further, to investigate the factors contributing to the anti-asthma effects of BCG neonatal vaccination in IL-17 KO mice, we evaluated the levels of proinflammatory cytokines, including IFN- γ , IL-12, IL-13, IL-23 and IL-10. Interestingly, only the levels of IFN- γ were significantly decreased in the IL-17 KO BCG + OVA group when compared with mice in the IL-17 KO OVA group (Fig. 2D). This finding indicated that BCG neonatal vaccination suppressed AHR by down-regulating IFN- γ levels.

To further confirm this finding, exogenous rIFN- γ was delivered to the airway of mice in the IL-17 KO BCG + OVA group. Compared with the control group, the number of inflammatory cells was increased in BALF of mice in the IL-17 KO BCG + OVA + rIFN- γ group (Fig. 2A). Consistently, in the IL-17 KO BCG + OVA + rIFN- γ group, tissue inflammation was greatly elevated, with significantly more peribronchial cellular infiltration, than that in the control group (Fig. 2B). Similarly, the Penh values in the IL-17 KO BCG + OVA + rIFN- γ group were significantly higher than that of the IL-17 KO BCG + OVA group in response to MCh (125–50 mg/mL) (Fig. 2C). These findings suggested that without IL-17, exogenous rIFN- γ induced airway inflammation and AHR in neonatal BCG vaccinated mice.

3.3. Neutralization of IFN- γ increases the anti-asthma effects of BCG neonatal vaccination

Next, we investigated whether the inhibition of IFN- γ in BCG neonatal vaccinated asthmatic mice could eliminate

airway inflammation and AHR more effectively. Compared with the BALF of the WT OVA group, the number of inflammatory cells was dramatically reduced in the WT BCG + OVA + anti-IFN- γ mAb group (Fig. 3A). In the WT OVA group, airway sensitization and challenge led to a dense peribronchiolar and perivascular inflammatory infiltrate while in the WT BCG + OVA + anti-IFN- γ mAb group, tissue inflammation was greatly reduced (Fig. 3B), with significantly less alveolar, peribronchiolar and perivascular cellular infiltration.

Fig. 3C shows the response to different doses of aerosolized MCh (25–50 mg/mL). The Penh values in the WT BCG + OVA + anti-IFN- γ mAb group were significantly lower than that of the WT BCG + OVA group.

Taken together, these data indicated that neutralization of IFN- γ increased the anti-asthma effects of BCG neonatal vaccination.

3.4. Inhibition of IFN- γ reduced airway inflammation and AHR in IL-17 KO asthmatic mice

To further confirm the role of IFN- γ in OVA-induced airway inflammation and AHR, we evaluated the airway inflammation and AHR in IL-17 KO asthmatic mice after neutralization of IFN- γ with an anti-IFN- γ mAb.

Compared with the control group, the number of inflammatory cells was increased in BALF of mice in the IL-17 KO OVA group (Fig. 4A). In contrast, there were less inflammatory cells in BALF of mice in the IL-17 KO OVA + anti-IFN- γ mAb group (Fig. 4A). In the IL-17 KO OVA group, airway sensitization and challenge led to a dense peribronchiolar and perivascular inflammatory infiltrate (Fig. 4B). In the IL-17 KO OVA + anti-IFN- γ mAb group, tissue inflammation was not observed (Fig. 4B).

Fig. 4C demonstrates the response to different doses of aerosolized MCh. The Penh values of mice in the IL-17 KO OVA group gradually increased with elevated concentrations of MCh, whereas the Penh values of mice in the IL-17 KO OVA + anti-IFN- γ mAb group were not significantly increased. These findings demonstrated that IFN- γ played an important role in OVA-induced airway inflammation and AHR.

4. Discussion

IL-17 plays a critical role in inducing airway inflammation. In asthmatic patients, IL-17 expression is increased in sputum, lung cells, BALF, and peripheral blood [14,15]. The up-regulation of IL-17 mRNA levels in the airways of asthmatic mice further provides evidence for the involvement of IL-17 in the pathogenesis of asthma [16]. It has been previously shown that neonatal BCG vaccination suppresses the symptoms of asthma and is associated with reduced levels of IL-17 in BALF [7]. In addition, it has been shown that extended freeze-drying cycles of BCG significantly decreased the levels of neutrophils, IL-17, and retinoic acid receptor-related orphan receptor- γ t [17]. Consistent with these findings, our present study showed that knocking out IL-17 reduced AHR and significantly suppressed airway inflammation. In contrast, when exogenous IL-17 was delivered to the airway, the anti-asthma effects following neonatal BCG vaccination were reversed. These findings suggest that IL-17 plays a pivotal role in airway inflammation and AHR and that the anti-asthma effects of BCG neonatal vaccination occur via the down-regulation of IL-17.

Using an OVA-induced animal model, previous studies have shown that IL-17 deficient mice or mice treated with an IL-17 neutralization antibody still exhibited AHR [15,18,19]. Consistently, in our model, following OVA sensitization and challenge, IL-17 KO

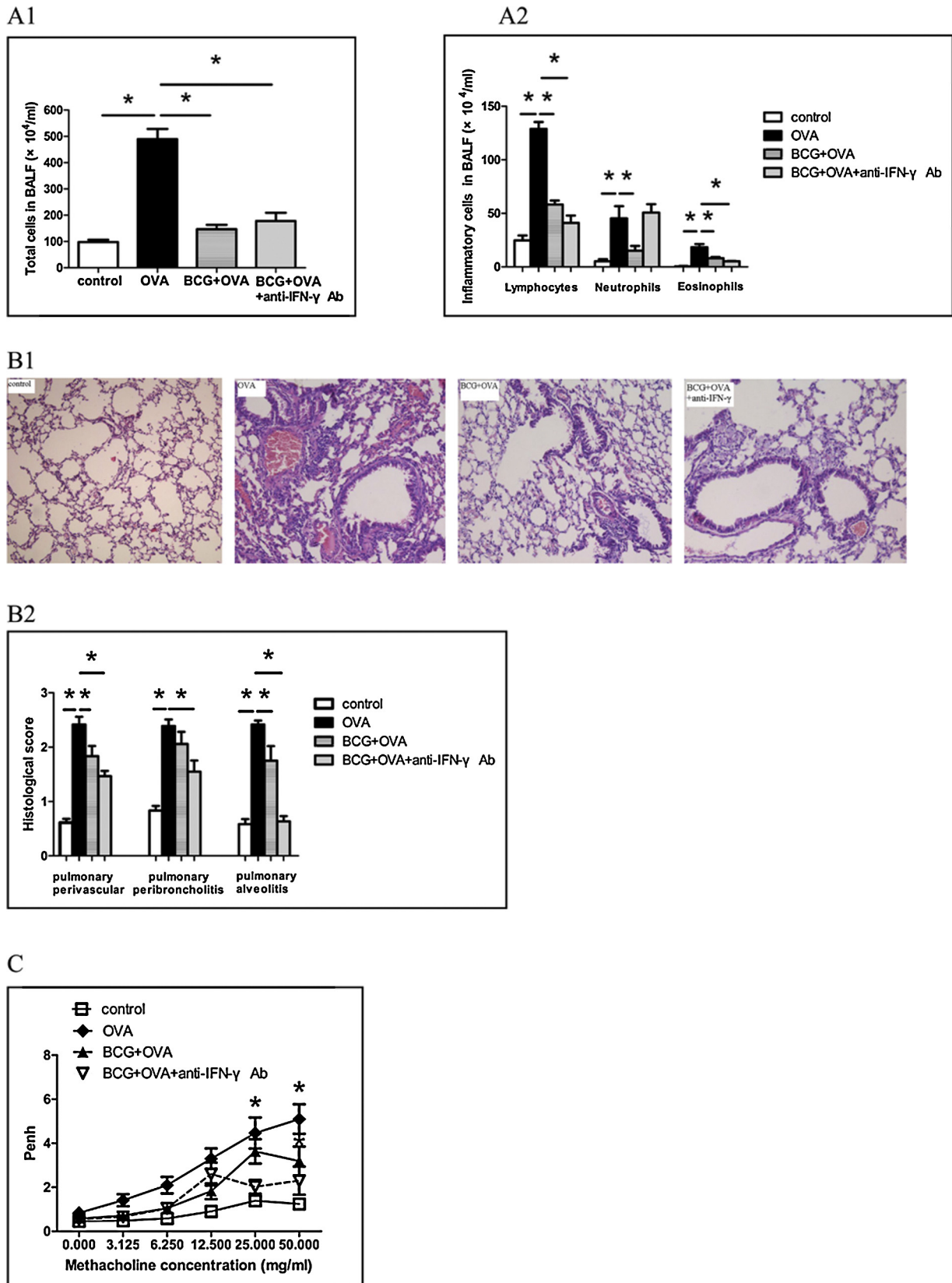


Fig. 3. Neutralization of IFN- γ improved the anti-asthma effects following BCG neonatal vaccination. (A) The number of total cells and inflammatory cells were reduced in BCG neonatal vaccinated asthmatic mice treated with an anti-IFN- γ mAb. (A1) Total cells in BALF; (A2) lymphocytes, neutrophils and eosinophils in BALF. Data are expressed as the mean $\pm$ SEM. $^*P < 0.05$ vs. each other. (B) Tissue inflammation was suppressed in BCG neonatal vaccinated asthmatic mice treated with an anti-IFN- γ mAb. (B1) Representative photomicrographs of lung stained with HE (magnification: 100 $\times$); (B2) histological scores of pulmonary peribronchiolitis, perivascularitis and alveolitis. Data are expressed as mean score $\pm$ SEM. $^*P < 0.05$ vs. each other. (C) OVA-induced AHR was further suppressed in BCG neonatal vaccinated mice treated with an anti-IFN- γ mAb. Data are presented as the mean $\pm$ SEM. $^*P < 0.05$ BCG + OVA group + anti-IFN- γ Ab vs. BCG + OVA group and control group vs. BCG + OVA group. The number of mice in control group was 8. The number of mice in OVA group was 8. The number of mice in BCG + OVA group was 8. The number of mice in BCG + OVA + anti-IFN- γ Ab group was 6.

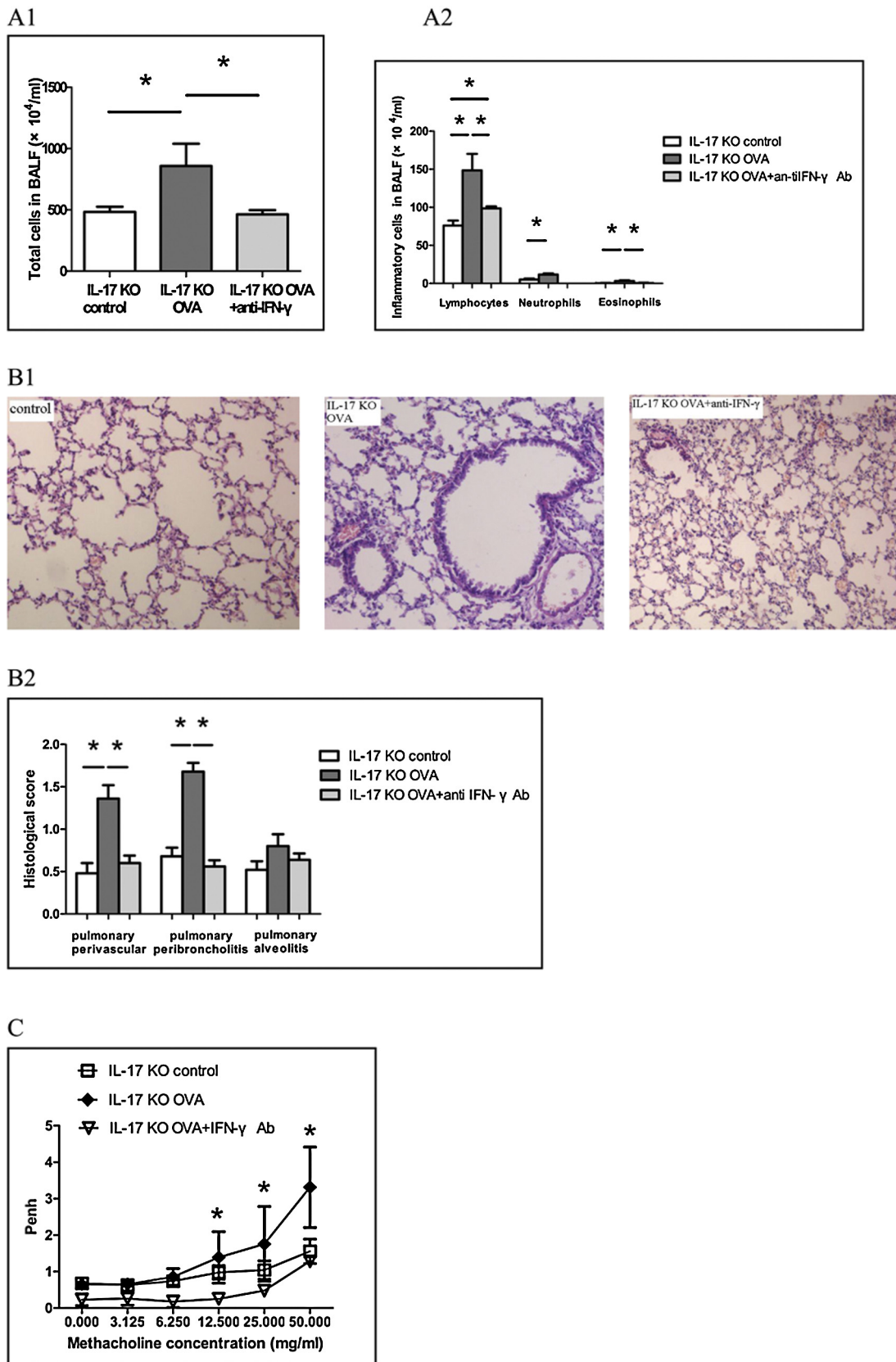


Fig. 4. Inhibition of IFN- γ reduced airway inflammation and AHR in IL-17 KO asthmatic mice. (A) The number of total cells and inflammatory cells were not increased in IL-17 KO asthmatic mice treated with an IFN- γ mAb. (A1) Total cells in BALF; (A2) lymphocytes, neutrophils and eosinophils in BALF. Data are expressed as the mean $\pm$ SEM. * P < 0.05 vs. each other. (B) Tissue inflammation was not observed in IL-17 KO asthmatic mice treated with an IFN- γ mAb. (B1) Representative photomicrographs of lung stained with HE (magnification: 100 $\times$); (B2) histological scores of pulmonary peribronchiolitis, perivascularitis and alveolitis. BCG vaccination decreased peribronchial, perivascular and alveolitis inflammation. Data are expressed as mean score $\pm$ SEM. * P < 0.05 vs. each other. (C) AHR was not observed in IL-17 KO asthmatic mice treated with an anti-IFN- γ mAb. Data are presented as the mean $\pm$ SEM. * P < 0.05 IL-17 KO OVA + anti-IFN- γ Ab group vs. IL-17 KO OVA group. The number of mice in control group was 8. The number of mice in IL-17 KO OVA group was 8. The number of mice in IL-17 KO OVA + anti-IFN- γ Ab group was 6.

mice exhibited AHR. So some additional factors contribute to OVA induced airway inflammation and AHR. In this study, we found that following inhibition of IL-17, IFN- γ presented as a mediator in inducing airway inflammation and AHR in OVA-induced asthmatic mice. Although cellular responses mediated by Th2-type cytokines or Th17-type cytokines are generally attenuated by Th1-type cytokines, studies have suggested that IFN- γ is not always protective but rather pathogenic for the development of asthma in some cases [20,21]. In addition, several studies have shown that increased IFN- γ levels are involved in the inflammatory process of asthma [20,22,23]. In a study by Kim, lung-targeted IFN- γ over-expression contributed to AHR and inflammation, while AHR significantly decreased in OVA-challenged IFN- γ -deficient mice [24]. Furthermore, Haapakoski et al. demonstrated that IFN- γ -mediated AHR was diminished following treatment with a soluble IFN- γ neutralizing mAb in a murine model [25]. We also found IFN- γ KO mice could not develop asthma model by OVA sensitization and challenge (data not shown). So it is possible that IFN- γ presents both pro-asthma and anti-asthma effects in different situations. In this study, inhibition of IFN- γ in IL-17 KO asthmatic mice or neonatal BCG vaccinated asthmatic mice did not induce airway inflammation and AHR. These results indicated that when IL-17-mediated airway inflammation and AHR was inhibited by neonatal BCG vaccination in OVA-induced asthmatic mice, IFN- γ played a pathogenic role in the development of asthma. In addition, our data indicated that in a low-level IL-17 environment, neonatal BCG vaccination had potent suppressive effects on IFN- γ -mediated airway inflammation and AHR. Our data provide explanations for why anti-asthma effect of neonatal BCG vaccination are not always complete as observed in a previous study [7]. Firstly neonatal BCG vaccination inhibited IL-17 level while IFN- γ level was not influenced and then IFN- γ played a pathogenic role in the development of asthma instead.

5. Conclusions

In conclusion, inhibition of IFN- γ , enhanced the anti-asthma effects of BCG neonatal vaccination in an OVA-induced murine asthma model. IL-17 was important for the development of airway inflammation and AHR. However, in the absence of IL-17, IFN- γ plays an important role in the development of airway inflammation and AHR. New treatments for asthma are highly specific and may only influence a single pathogenetic aspect of the disease. Thus, these treatments may have a minor clinical impact [26]. In contrast, neonatal BCG vaccination exerts its effects through combined mechanisms simultaneously: (1) an immunoregulatory effect on IL-17-mediated airway inflammation and AHR; and (2) modifying IFN- γ in the absence of IL-17. The neonatal BCG vaccination appears to be an effective immunotherapeutic strategy for asthma in humans.

Conflict of interest statement

The authors declare that they have no conflict of interests.

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References

- [1] Umetsu DT, McIntire JJ, Akbari O, Macaubas C, DeKruyff RH. Asthma: an epidemic of dysregulated immunity. *Nat Immunol* 2002;3:715–20.
- [2] von Mutius E. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: farm lifestyles and the hygiene hypothesis. *Clin Exp Immunol* 2010;160:130–5.
- [3] Lagranderie M, Nahori MA, Balazuc AM, Kiefer-Biasizzo H, Lapa e Silva JR, Milon G, Marchal G, Vargaftig BB. Dendritic cells recruited to the lung shortly after intranasal delivery of *Mycobacterium bovis* BCG drive the primary immune response towards a type 1 cytokine production. *Immunology* 2003;108:352–64.
- [4] Oliveira FH, Sarinho SW, Montenegro S, Neuenschwander C, Queiroz R, Medeiros D, Schor D, Sarinho E. Production of interferon gamma in asthmatic patients with small bacille Calmette-Guérin scars: a pilot study. *Allergy Asthma Proc* 2006;27:16–22.
- [5] Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 2012;18:716–25.
- [6] Bhakta NR, Woodruff PG. Human asthma phenotypes: from the clinic, to cytokines, and back again. *Immunol Rev* 2011;242:220–32.
- [7] Deng Y, Chen W, Zang N, Li S, Luo Y, Ni K, Wang L, Xie X, Liu W, Yang X, Fu Z, Liu E. The antiasthma effect of neonatal BCG vaccination does not depend on the Th17/Th1 but IL-17/IFN- γ balance in a BALB/c mouse asthma model. *J Clin Immunol* 2011;31:419–29.
- [8] Lindén A, Laan M, Anderson GP. Neutrophils, interleukin-17A and lung disease. *Eur Respir J* 2005;25:159–72.
- [9] Pène J, Chevalier S, Preisser L, Vénéreau E, Guilleux MH, Ghannam S, Molès JP, Danger Y, Ravon E, Lesaux S, Yssel H, Gascan H. Chronically inflamed human tissues are infiltrated by highly differentiated Th17 lymphocytes. *J Immunol* 2008;180:7423–30.
- [10] Sun YC, Zhou QT, Yao WZ. Sputum interleukin-17 is increased and associated with airway neutrophilia in patients with severe asthma. *Chin Med J (Engl)* 2005;118:953–6.
- [11] Barczyk A, Pierzchala W, Sozańska E. Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine. *Respir Med* 2003;97:726–33.
- [12] Chakir J, Shannon J, Molet S, Fukakusa M, Elias J, Laviolette M, Boulet LP, Hamid Q. Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF- β , IL-11, IL-17, and type I and type III collagen expression. *J Allergy Clin Immunol* 2003;111:1293–8.
- [13] Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, Gelfand EW. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Respir Crit Care Med* 1997;156:766–75.
- [14] Molet S, Hamid Q, Davoine F, Nutku E, Taha R, Pagé N, Olivenstein R, Elias J, Chakir J. IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. *J Allergy Clin Immunol* 2001;108:430–8.
- [15] Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, Sekikawa K, Asano M, Iwakura Y. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity* 2002;17:375–87.
- [16] Park SJ, Lee KS, Kim SR, Min KH, Choe YH, Moon H, Chae HJ, Yoo WH, Lee YC. Peroxisome proliferator-activated receptor gamma agonist down-regulates IL-17 expression in a murine model of allergic airway inflammation. *J Immunol* 2009;183:3259–67.
- [17] Lagranderie M, Abolhassani M, Vanoirbeek JA, Lima C, Balazuc AM, Vargaftig BB, Marchal G. *Mycobacterium bovis* Bacillus Calmette-Guérin killed by extended freeze-drying targets plasmacytoid dendritic cells to regulate lung inflammation. *J Immunol* 2010;184:1062–70.
- [18] Hellings PW, Kasran A, Liu Z, Vandekerckhove P, Wuyts A, Overbergh L, Mathieu C, Ceuppens JL. Interleukin-17 orchestrates the granulocyte influx into airways after allergen inhalation in a mouse model of allergic asthma. *Am J Respir Cell Mol Biol* 2003;28:42–50.
- [19] He R, Oyoshi MK, Jin H, Geha RS. Epicutaneous antigen exposure induces a Th17 response that drives airway inflammation after inhalation challenge. *Proc Natl Acad Sci USA* 2007;104:15817–22.
- [20] Trautmann A, Schmid-Grendelmeier P, Krüger K, Cramer R, Akdis M, Akkaya A, Bröcker EB, Blaser K, Akdis CA. T cells and eosinophils cooperate in the induction of bronchial epithelial cell apoptosis in asthma. *J Allergy Clin Immunol* 2002;109:329–37.
- [21] Hansen G, Berry G, DeKruyff RH, Umetsu DT. Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyperactivity but cause severe airway inflammation. *J Clin Invest* 1999;103:175–83.
- [22] Cohn L, Homer RJ, Niu N, Bottomly K. T helper 1 cells and interferon γ regulate allergic airway inflammation and mucus production. *J Exp Med* 1999;190:1309–18.
- [23] Cembrzynska-Nowak M, Szklarz E, Inglot AD, Teodorczyk-Injeyan JA. Elevated release of tumor necrosis factor-alpha and interferon-gamma by bronchoalveolar leukocytes from patients with bronchial asthma. *Am Rev Respir Dis* 1993;147:291–5.

- [24] Kim YS, Choi SJ, Choi JP, Jeon SG, Oh S-, Lee BJ, Gho YS, Lee CG, Zhu Z, Elias JA, Kim YK. IL-12-STAT4-IFN- γ axis is a key downstream pathway in the development of IL-13-mediated asthma phenotypes in a Th2 type asthma model. *Exp Mol Med* 2010;42:533–46.
- [25] Haapakoski R, Karisola P, Fyhrquist N, Savinko T, Wolff H, Turjanmaa K, Palosuo T, Reunala T, Lauerma A, Alenius H. Intradermal cytosine-phosphate-guanosine treatment reduces lung inflammation but induces IFN- γ -mediated airway hyperreactivity in a murine model of natural rubber latex allergy. *Am J Respir Cell Mol Biol* 2011;44:639–47.
- [26] Adcock IM, Caramori G, Chung KF. New targets for drug development in asthma. *Lancet* 2008;372:1073–87.