

## Original Article

# Dust mite vaccine enhances airway antibacterial host defense by up-regulating beta defensins in asthmatic mice

Xiao-Meng Yang<sup>1,3</sup>, Jun-Yi Wang<sup>1,4</sup>, Xiao-Jun Xiao<sup>1</sup>, Xie-Fang Yuan<sup>1</sup>, Xiao-Yu Liu<sup>1</sup>, Ping-Chang Yang<sup>1</sup>, Guo-Ping Li<sup>1,4</sup>, Pei-Xin Ran<sup>2</sup>, Zhi-Gang Liu<sup>1,2,4</sup>

<sup>1</sup>The State Key Laboratory of Respiratory Disease for Allergy at Shenzhen University, Shenzhen University School of Medicine, 3688 Nanhai Blvd, Shenzhen 518060, China; <sup>2</sup>State Key Laboratory of Respiratory Disease, Guangzhou Medical University, Guangzhou 510006, China; <sup>3</sup>Shenzhen Luohu Maternity and Child Healthcare Hospital, 2013 Taibai Road, Shenzhen 518019, China; <sup>4</sup>Allergy & Inflammation Research Unit, Department of Respiratory Medicine, Chengdu Institute of Respiratory Health, The Third People's Hospital of Chengdu, Chengdu 610031, Sichuan, China

Received April 24, 2018; Accepted September 13, 2018; Epub December 15, 2018; Published December 30, 2018

**Abstract:** The airway is always exposed to the microbial environment. It is accepted that allergic airway inflammation inhibits airway antibacterial host defense. This study aimed to test a hypothesis that allergen-specific immunotherapy (ASIT) with a dust mite vaccine could enhance antibacterial host defense in the allergic airway. An asthmatic mouse model was exposed to *P. aeruginosa* and treated by ASIT with a dust mite vaccine in our study. ASIT with dust mite vaccine could decrease the severity of *P. aeruginosa* infection in the airway of asthmatic mice, and enhance production of mouse beta defensin 3 (MBD-3) in airway epithelial cells and decrease IL-4 levels in asthmatic mice. Furthermore, IL-4 could down-regulate production of MBD-3 in lung epithelial MLE-12 cells. The results indicate that ASIT with dust mite vaccine can enhance airway antibacterial host defense by up-regulating beta defensin through decreasing IL-4 production in asthmatic mice.

**Keywords:** Allergen specific immunotherapy, dust mite vaccine, antibacterial host defense, beta defensin 3

## Introduction

Allergic asthma is an IgE-mediated chronic inflammatory disease with characteristics of pulmonary eosinophilic granulocyte infiltration and airway hyperreactivity [1, 2]. The airway is always exposed to the microbial environment [3]. Respiratory tract infections are the most common diseases. Previous investigations have indicated that refractory asthma is a risk factor for respiratory tract infection and allergic airway inflammation inhibits airway antibacterial host defense [4, 5].

The innate immune system is the first line of host defense, which consists of naïve immune barrier, immune cells and immune molecules [6]. Antimicrobial peptide (AMP) is the most important immune molecules, which has a broad spectrum effect on preventing microbial invasion [7, 8]. Airway epithelial cells are the

active part of the innate pulmonary immune system. The cells secrete host defense molecules, such as beta defensins, which is a significant kind of AMP [9]. Low levels of AMP expression can increase susceptibility to microbial infections [10]. Treatment with beta defensins in severe combined immunodeficiency mice can decrease bacterial levels in subcutaneous tissue [11]. Th2-type cytokines can inhibit antimicrobial host defense in individuals with allergic diseases [12, 13]. Allergen specific immunotherapy (ASIT) is the only effective approach to cure allergic asthma [14]. Treg cells are important immune regulatory cells in ASIT for asthmatic patients [15]. Active Treg cells can directly inhibit the Th2 responses and the activation of mast cells, basophile granulocytes and eosinophilic granulocytes [16, 17]. However, it is unclear how ASIT with dust mite vaccine affects airway antibacterial host defense.

## Dust mite vaccine enhances antibacterial host defense

Therefore, we hypothesize that ASIT with dust mite vaccine can enhance antibacterial host defense in airway epithelial cells by up-regulating AMP through inhibit Th2 immunoreactions. To test this hypothesis, the ability of dust mite vaccine to enhance airway antibacterial host defense was examined in asthmatic mice exposed to *P. aeruginosa*.

### Materials and methods

#### Materials

Female BALB/c mice (Body weight 16 to 22 g, 6 to 8 weeks old) were purchased from the Animal Center of Guangdong Province and maintained in a pathogen-free environment. All experiments were approved by the Animal Ethic Committee at Shenzhen University. The experiments were performed in accordance with the Institutional Guidelines for the Care and Use of Laboratory Animals. *P. aeruginosa* was provided by the clinical laboratory of Shenzhen Luohu Maternity and Child Healthcare Hospital.

#### Sensitization and challenge protocol

BALB/c mice were randomly divided into 4 groups (n = 4); the PBS group and PBS/*P.aer* group were treated with phosphate buffered solution (PBS). The other two groups were sensitized intraperitoneally with 50 µg HDM extract absorbed to 2 mg alum on days 0, 7, and 14. From day 28 (every 2 days, 3 administrations in total), mice were sublingually treated with PBS (Der f/*P.aer* group) or 0.1 g HDM extract (Der f/ASIT/*P.aer* group) respectively. Seven days after final immunization, mice were intra-nasally challenged with 50 µg HDM extract daily for 1 week.

#### Acute *P. aeruginosa* pneumonia model

PBS/*P.aer*, Der f/*P.aer* and Der f/ASIT/*P.aer* group mice were anesthetized using diethyl ether. They were then infected intra-nasally with  $1 \times 10^7$  CFUs *P. aeruginosa*. The PBS group of mice received equivalent doses of PBS. Mice were euthanized 24 hours after infection.

#### Histopathological analysis and Immunofluorescence histochemistry

Lung tissue was fixed in 4% cold formalin solution for 24 hours at room temperature and

embedded in paraffin. Five µm serial sections were cut. One part of the sections was stained with hematoxylin-eosin (HE) to examine the histological changes using light microscopy. Then the inflammation index was carried out using color segmentation (ImageJ 1.48v, National Institutes of Health, USA). The other part of the sections was deparaffinized and rehydrated. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide and the sections were incubated at room temperature for 10 minutes. Antigen retrieval was performed with citrate (pH = 6) at 95°C in an aqueous bath. The process lasted 40 minutes. The sections were incubated with rabbit polyclonal antibody against MBD-3 (Abcam, Cambridge, MA, USA) at 37°C for 45 min (1:300). The secondary antibody (Envision™, DAKO, Denmark) was applied (1:500) and incubated at 37°C for 45 minutes. Finally, the slides were visualized using DAB immunostaining under a light microscope (Leica, Solms, Germany). The intensity of fluorescence staining of MBD-3 was carried out using color segmentation (ImageJ 1.48v, Wayne Rasband National Institutes of Health, USA).

#### Wright's stain and gram stain

The BALF was collected and centrifuged at 400 g at 4°C. Then the supernatant was removed and 100 µL PBS was added to resuspend the sample. One part of the smear of the suspension was measured by Wrights' stain. The number of inflammatory cells in the smear under high power field was counted using light microscopy. The other part of the smear was measured by gram stain. The number of gram-negative bacilli in the smear under oil immersion field was counted using light microscopy.

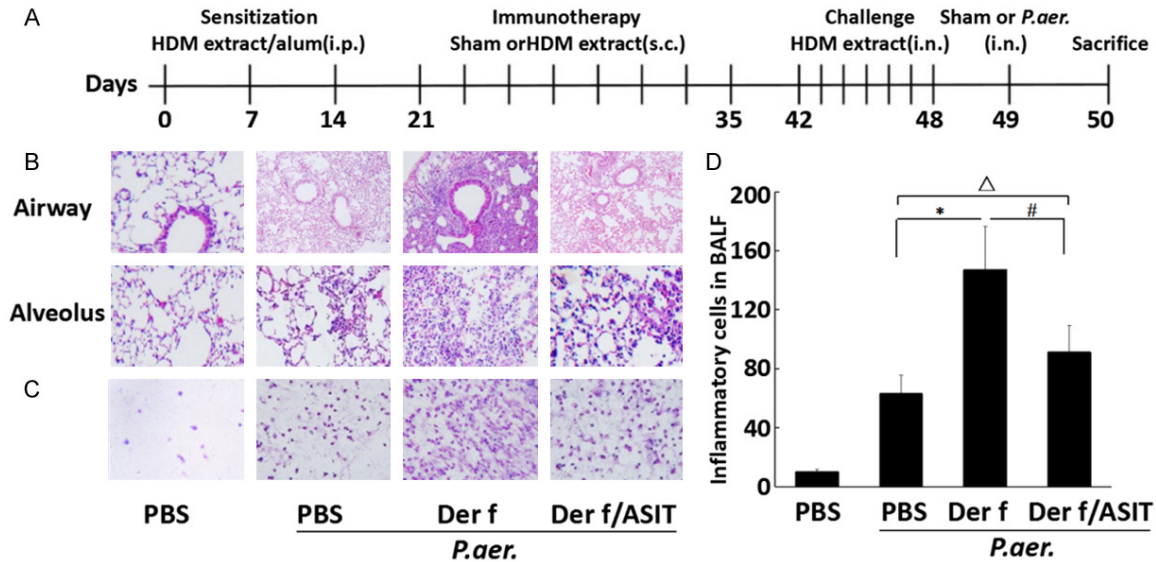
#### Quantitation of bacteria

The lungs were removed, weighed, and homogenized in Roswell Park Memorial Institute 1640 medium (RPMI 1640), and the suspension were inoculated on *P. aeruginosa*-selective plates. Bacterial colonies were counted after incubation at 37°C for 24 hours.

#### Detection of IL-4 and IFN-γ in BALF

The BALF was collected and centrifuged at 400 g at 4°C. Then the supernatant was lyophilized and stored at -20°C until they were used for

## Dust mite vaccine enhances antibacterial host defense



**Figure 1.** Effects of dust mite vaccine on lung inflammation in asthmatic mice exposed to *P. aeruginosa*. **A.** Animal model. Mice were sensitized by intraperitoneally with HDM extract in aluminum hydroxide on days 0, 7, and 14. From days 21 to day 35, mice were sublingually treated with HDM extract. From day 42, mice were intra-nasally challenged with HDM extract daily for 1 week. On day 49, mice were infected intra-nasally with  $1 \times 10^7$  CFUs  $\text{ml}^{-1}$  *P. aeruginosa*. Mice were killed after 24 hours of *P. aeruginosa* infection. **B.** Lung samples were stained with hematoxylin and eosin (HE) (original magnification  $\times 200$ ). There was less inflammatory cells infiltration in the airway of Der f/ASIT/*P.aer* mice than Der f/*P.aer* mice. **C.** Bronchial alveolar lavage fluid (BALF) was stained with Wright's staining (original magnification  $\times 400$ ). There were less inflammatory cells in the BALF of Der f/ASIT/*P.aer* than Der f/*P.aer* mice. **D.** Count of inflammatory cells in the BALF. Inflammatory cells in Der f/*P.aer* mice were higher than in PBS/*P.aer* mice and Der f/ASIT/*P.aer* mice ( $^*P < 0.01$ ,  $^{\#}P < 0.05$ ). Inflammatory cells in Der f/ASIT/*P.aer* mice were higher than in PBS/*P.aer* mice ( $^{\Delta}P < 0.05$ ). Inflammatory cells in PBS were lower than in PBS/*P.aer* mice, Der f/*P.aer* mice and Der f/ASIT/*P.aer* mice ( $P < 0.01$ ).

cytokine assay. The levels of BALF IL-4 and IFN- $\gamma$  were evaluated by sandwich ELISA (R&D Systems, Minneapolis, MN, USA) according to the manufacturers' instructions.

### Cell culture and infection experiments

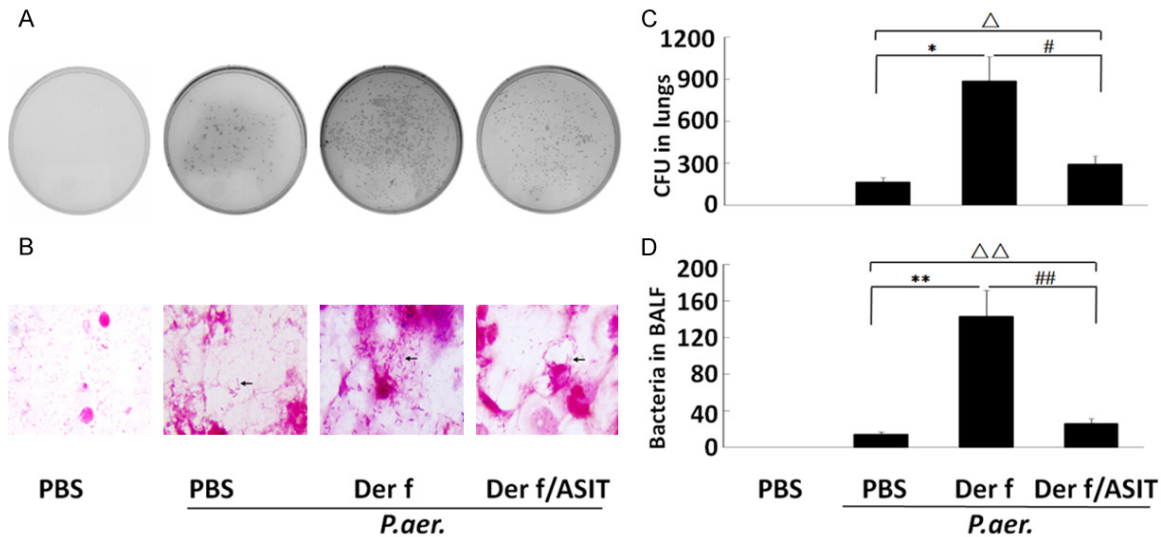
MLE-12 cells were incubated in 6-well cell culture plates at  $37^{\circ}\text{C}$  and 5%  $\text{CO}_2$  in DMEM/F12 culture medium. The cells were pretreated for 48 hours at  $37^{\circ}\text{C}$  until they reached 80% confluence. The medium were removed, then the cells were incubated in serum-free DMEM/F12 culture medium and stimulated by  $50 \text{ ng ml}^{-1}$  IL-4 or PBS for 48 hours. Then  $10^7 \text{ CFU ml}^{-1}$  *P. aeruginosa* was added to MLE-12 cells. After incubation for 1 hour, the medium were removed, the cells were washed with PBS and incubated with serum-free DMEM/F12 culture medium containing polymyxin  $100 \mu\text{g ml}^{-1}$  for 1 hour to kill extracellular bacteria. The culture media was drew off and plated in LB solid culture medium to confirm that the extracellular bacteria had been killed. The cells were then

suspended with 1 ml sterile PBS.  $100 \mu\text{l}$  cell suspensions were spread on LB plates to determine levels of intracellular bacteria. The plates were cultured at  $37^{\circ}\text{C}$  for 24 hours, and colonies were counted. Duplicates were made for each sample and control.

### Quantitative real-time PCR

Total RNA was extracted from mouse lung tissue or *P. aeruginosa* infection MLE-12 cells with Trizol (Invitrogen, Carls-bad, California) according to the manufacturer's instructions. The cDNA was prepared by using the iScript cDNA Synthesis kit (Bio-Rad Laboratories, Hercules, California). The PCR primers (GeneRay Biotechnology, Shanghai, China) used for RT-PCR were as follows: for MBD-3, sense: 5'-GCATTGGCAACTCGTCAG-3' and antisense: 5'-TGGAGGAGCAAATTCTGGTGT-3'; for  $\beta$ -actin, sense: 5'-CCTGACTGACTACCTCATGAAG-3' and antisense: 5'-CGCGACCATCCTCTTCTTAG-3'. RT-PCR amplification reaction was prepared with the SYBR Green PCR kit (Bio-Rad) and

## Dust mite vaccine enhances antibacterial host defense



**Figure 2.** Measurement of *P. aeruginosa* in lung homogenates by quantitative culture on blood agar plates and in BLAF by gram stain. A. Represents the bacterial colonies on blood agar plates among PBS mice, PBS/*P.aer* mice, Der f/*P.aer* mice and Der f/ASIT/*P.aer* mice. C. Represents CFUs, shown in column configuration. There were significantly more CFUs in Der f/*P.aer* mice than in PBS/*P.aer* mice and Der f/ASIT/*P.aer* mice (\* $P < 0.01$ , # $P < 0.01$ ). There were obviously more CFUs in Der f/ASIT/*P.aer* mice than in PBS/*P.aer* mice ( $\Delta P < 0.05$ ). PBS/mice had no observable CFUs. B. Represents bacterial number in BLAF among PBS mice, PBS/*P.aer* mice, Der f/*P.aer* mice and Der f/ASIT/*P.aer* mice. D. Represents bacterial number, shown in column configuration. There were sharply more bacteria in Der f/*P.aer* mice than in PBS/*P.aer* mice and Der f/ASIT/*P.aer* mice (\*\* $P < 0.01$ , ## $P < 0.01$ ). There were more bacteria in Der f/ASIT/*P.aer* mice than in PBS/*P.aer* mice ( $\Delta P < 0.05$ ). PBS/mice had no observable bacteria.

performed using the ABI 7300 Real Time PCR System (Applied Biosystems, Carlsbad, California). PCR products were verified by melting curve analysis. Relative mRNA levels of target genes were calculated by the  $2^{-\Delta\Delta Ct}$  method.

### Statistical analysis

Data are represented as mean  $\pm$  SD and analyzed by ANOVA (S-N-K's post hoc test) or Student's *t*-test. *P* value less than 0.05 was considered significant. The data were analyzed by SPSS 17.0 statistical software.

### Results

#### Effects of dust mite vaccine on lung inflammation in asthmatic mice exposed to *P. aeruginosa*

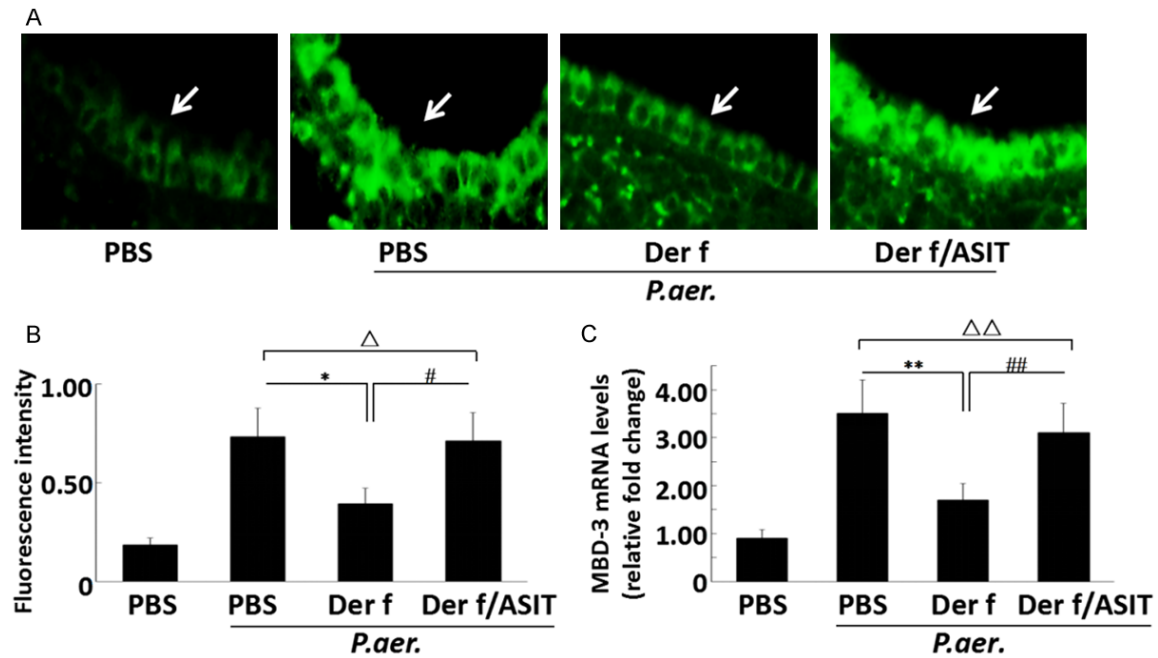
The mechanism of increased lung infection risk in asthmatic patients exposed to bacteria remains unclear. Twenty-four hours after exposure to *P. aeruginosa*, histological analysis showed there was less inflammatory infiltration in asthmatic mice treated with dust mite vaccine (Der f/ASIT/*P.aer*) than that in untreated

asthmatic mice (Der f/*P.aer*) ( $P < 0.01$ ) (Figure 1B). Meanwhile, Wright's staining showed there was less inflammatory cells in the bronchial alveolar lavage fluid (BALF) of asthmatic mice exposed to *P. aeruginosa* treated with dust mite vaccine (Der f/ASIT/*P.aer*) than that in untreated asthmatic mice (Der f/*P.aer*) ( $P < 0.01$ ) (Figure 1C, 1D). This indicated that *P. aeruginosa* infection increased lung inflammation in asthmatic mice. However, ASIT with dust mite vaccine could inhibit inflammation in asthmatic mice exposed to *P. aeruginosa*.

#### Effects of dust mite vaccine on bacterial levels in asthmatic mice exposed to *P. aeruginosa*

To determine whether the dust mite vaccine could decrease the risk of pulmonary infection, bacteria levels in the lungs of the mice infection with *P. aeruginosa* were determined. Lower numbers of bacterial colony forming units (CFU) were observed in Der f/ASIT/*P.aer* mice than Der f/*P.aer* mice ( $P < 0.01$ ) (Figure 2A, 2C). The number of CFU in control mice, which received phosphate-buffered saline (PBS) instead of active bacteria, was zero. Lower numbers of bacteria were observed in Der f/ASIT/*P.aer*





**Figure 3.** MBD-3 expression in lungs by immunofluorescence histochemistry and qPCR. A. MBD-3 expression and localization in lung tissues indicated by the white arrow were detected by immunofluorescence histochemistry (original magnification  $\times 200$ ). B. Fluorescence intensity of MBD-3 in lung tissues was analyzed by ImageJ 1.48v soft. Fluorescence intensity of MBD-3 in Der f/*P.aer* mice was significantly lower than PBS/*P.aer* mice and Der f/ASIT/*P.aer* mice ( $*P < 0.01$ ,  $\#P < 0.05$ ). There was no significant difference of fluorescence intensity of MBD-3 between Der f/ASIT/*P.aer* mice and PBS/mice ( $\Delta P > 0.05$ ). C. MBD-3 expression in lung tissue was measured by qPCR shown in column configuration. C. Showed that MBD-3 expression in Der f/*P.aer* mice was sharply lower than in PBS/*P.aer* mice and Der f/ASIT/*P.aer* mice ( $**P < 0.01$ ,  $##P < 0.05$ ). There was no significant difference of MBD-3 expression between Der f/ASIT/*P.aer* mice and PBS/mice ( $\Delta\Delta P > 0.05$ ).

mice than Der f/*P.aer* mice ( $P < 0.01$ ) (Figure 2B, 2D). These data show that ASIT with dust mite vaccine can induce the clearance of *P. aeruginosa* and decrease pulmonary infection in asthmatic mice.

#### Effects of dust mite vaccine on MBD-3 in asthmatic mice exposed to *P. aeruginosa*

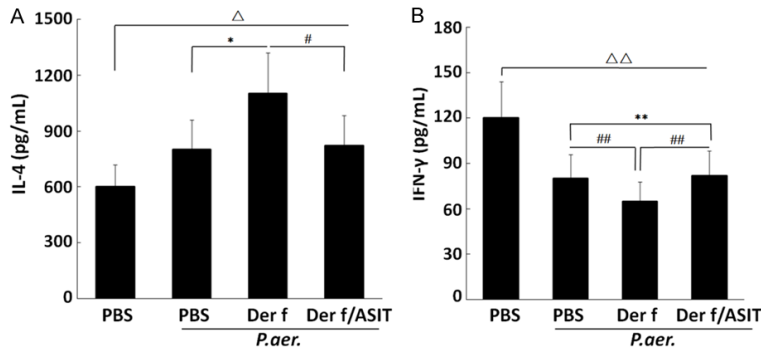
Airway epithelial cells can be induced to express mouse beta defensin 3 (MBD-3). Whether the dust mite vaccine can enhance the expression of MBD-3 remains unclear. In our study, MBD-3 was expressed in epithelial cells of lungs in normal mice or asthmatic mice ASIT with dust mite vaccine when the mice were exposed to *P. aeruginosa* (Figure 3A, 3B). MBD-3 expression in Der f/*P.aer* mice was significantly lower than in PBS/*P.aer* mice and Der f/ASIT/*P.aer* mice ( $P < 0.01$ ) (Figure 3C). However, there was no significant difference of MBD-3 expression between in Der f/ASIT/*P.aer* mice and PBS/mice ( $P > 0.05$ ) (Figure 3C). Thus, ASIT with dust mite vaccine enhanced produc-

tion of MBD-3 during antibacterial immune response to asthma.

#### Effects of dust mite vaccine on IL-4 and IFN- $\gamma$ in asthmatic mice exposed to *P. aeruginosa*

Levels of IL-4 and IFN- $\gamma$  in the bronchial alveolar lavage fluid (BALF) of mice exposed to *P. aeruginosa* were tested to determine the relationship between ASIT with dust mite vaccine and Th1/Th2 immunoreactions for antibacterial host defense during asthma. The levels of IL-4 in BALF were higher in Der f/*P.aer* mice than those in PBS/*P.aer* mice ( $P < 0.01$ ). However, the levels of IL-4 in Der f/ASIT/*P.aer* mice were lower than those in Der f/*P.aer* mice ( $P < 0.01$ ). IL-4 levels were lower in PBS control mice (PBS) than the other mice (PBS/*P.aer*, Der f/*P.aer* and Der f/ASIT/*P.aer* mice) ( $P < 0.01$ ) (Figure 4). The level of IFN- $\gamma$  in PBS/*P.aer* mice was not significantly different from that of Der f/ASIT/*P.aer* mice ( $P > 0.05$ ). IFN- $\gamma$  levels were lower in Der f/*P.aer* mice than those in PBS/*P.aer* and Der f/

## Dust mite vaccine enhances antibacterial host defense



**Figure 4.** BLAF levels of IL-4 and IFN- $\gamma$ . Cytokines in BALF were detected by ELISA after 24 hours of *P.aer* infection. IL-4 was higher in Der f/*P.aer* mice than PBS/*P.aer* mice (\* $P$ <0.01). IL-4 in Der f/ASIT/*P.aer* mice were lower than Der f/*P.aer* mice (# $P$ <0.01). IL-4 was lower in PBS mice than the other mice (PBS/*P.aer*, Der f/*P.aer* and Der f/ASIT/*P.aer* mice) ( $\Delta P$ <0.01). IFN- $\gamma$  in PBS/*P.aer* mice was not significantly different from that of Der f/ASIT/*P.aer* mice (\*\* $P$ >0.05). IFN- $\gamma$  was lower in Der f/*P.aer* mice than PBS/*P.aer* and Der f/ASIT/*P.aer* mice (### $P$ <0.01). IFN- $\gamma$  was higher in PBS mice than the other mice (PBS/*P.aer*, Der f/*P.aer* and Der f/ASIT/*P.aer* mice) ( $\Delta\Delta P$ <0.01).

ASIT/*P.aer* mice ( $P$ <0.01). IFN- $\gamma$  levels were higher in PBS control mice (PBS) than the other mice (PBS/*P.aer*, Der f/*P.aer* and Der f/ASIT/*P.aer* mice) ( $P$ <0.01) (**Figure 4**). These data demonstrate that ASIT with dust mite vaccine could decrease IL-4 levels and increase IFN- $\gamma$  levels in asthmatic mice exposed to *P. aeruginosa*.

### Effects of IL-4 on the antibacterial host defense in MLE-12 cells

Allergic airway inflammation inhibits airway antibacterial host defense [4, 5]. However, whether IL-4 can inhibit the antibacterial host defense in airway epithelial cells remains unclear. In our present studies, MLE-12 cells were pretreated with IL-4 or PBS and then infected with *P. aeruginosa*. The infected MLE-12 cells were homogenized with sterile PBS and spread on LB plates to determine levels of intracellular bacteria. IL-4 increased the levels of *P. aeruginosa* in MLE-12 cells (**Figure 5A**). The total bacterial CFUs were significantly higher in MLE-12 cells pretreated with IL-4 than those pretreated with PBS ( $P$ <0.01) (**Figure 5B**). Infected MLE-12 cells were collected and MBD-3 mRNA expression was analyzed by qPCR. IL-4 was found to be decreased in MBD-3 mRNA expression in MLE-12 cells. MBD-3 mRNA expression was significantly lower in MLE-12 cells pretreated with IL-4 than those pretreated with PBS ( $P$ <0.01) (**Figure 5C**). This

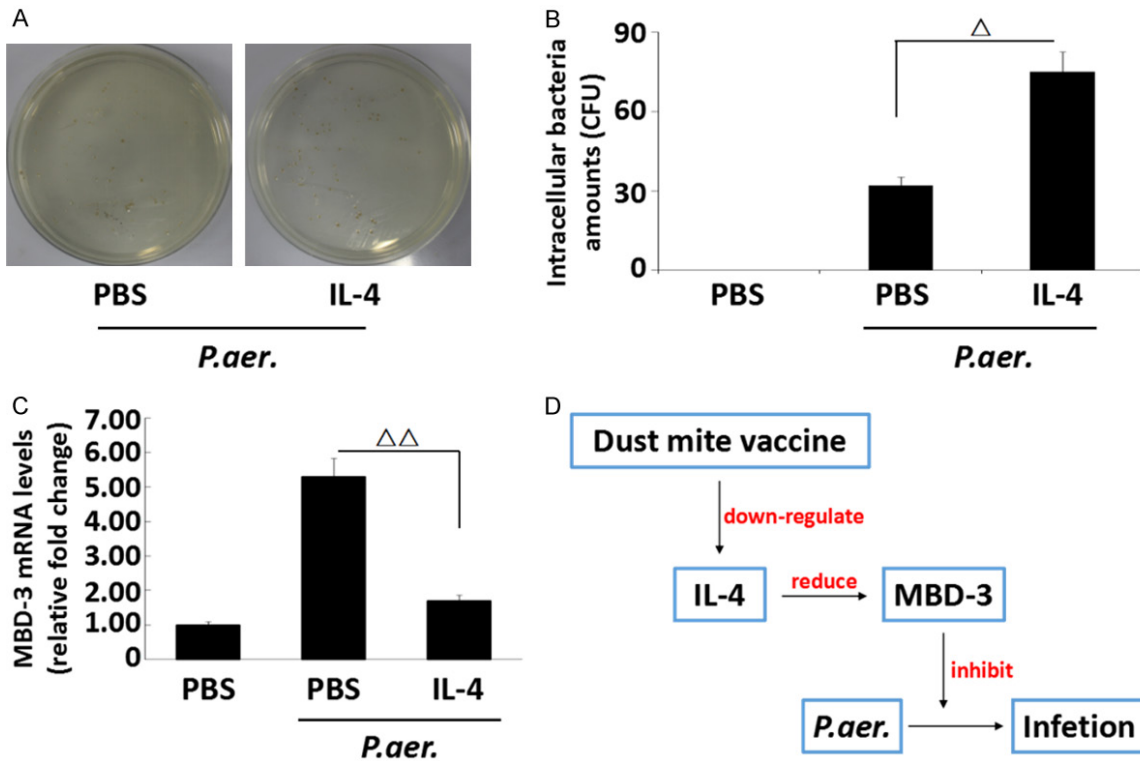
indicated that IL-4 might weaken the antibacterial host defense of airway epithelium cells by downregulating MBD-3 (**Figure 5D**).

### Discussion

Allergic asthma is the most common allergic disease in China, which is a complex chronic inflammatory airway disease. Previous research has indicated that allergic airway inflammation inhibits airway antibacterial host defense, meanwhile, respiratory tract infections and bacterial colonization play an important role in refractory asthma [4, 5]. The airway is always exposed to the microbial environment [18]. Respiratory tract infections are the most common diseases. *P. aeruginosa* is the risk cause of detrimental chronic lung infections, which is also a major determinant of morbidity and mortality [19].

Allergen specific immunotherapy (ASIT) is the only effective approach to cure allergic asthma [14]; it is recommended by world health organization (WHO) as a specific therapy for asthma [20]. Treg cells are important immune regulatory cells in ASIT for asthmatic patients. Insufficient quantity and hypofunction of Treg are the important factors of occurrence of mite allergic asthma [15]. Treg cells can inhibit Th2 responses and activation of mast cells, basophil, granulocytes, and eosinophilic granulocytes directly [16, 17]. ASIT with dust mite vaccine can inhibit accentuated Th2-type immune response and up-regulate quantity and function of Treg, which can lessen or release the severity of asthma [21]. However, whether ASIT with dust mite vaccine can affect antimicrobial host defense among asthmatic patients remains indistinct. In our study, lung inflammation and bacterial levels in asthmatic mice exposed to *P. aeruginosa* were found to die more than those in normal mice exposed to *P. aeruginosa*. However, when ASIT with dust mite vaccine, lung inflammation in asthmatic mice exposed to *P. aeruginosa* was found to be less-

## Dust mite vaccine enhances antibacterial host defense



**Figure 5.** In vitro effects of IL-4 on the antibacterial host defense in airway epithelium cells. A. MLE-12 cells were pretreated with PBS or IL-4, infected with *P. aeruginosa*. Levels of intracellular bacteria were determined by culturing infected MLE-12 cells on LB plates. B. Bacteria were counted using drop plates. Intracellular bacteria in MLE-12 cells pretreated with PBS was sharply lower than MLE-12 cells pretreated with IL-4 ( $^{\Delta}P < 0.01$ ). C. MBD-3 expression in MLE-12 cells was determined by qPCR. MBD-3 expression in MLE-12 cells pretreated with PBS was obviously higher than in MLE-12 cells pretreated with IL-4 ( $^{\Delta\Delta}P < 0.01$ ). D. Effects of dust mite vaccine on respiratory infection due to *P. aeruginosa*.

ened, and the clearance of *P. aeruginosa* was found to be increased. These results indicated that *P. aeruginosa* infection increases lung inflammation in asthmatic mice. However, ASIT with dust mite vaccine enhances antimicrobial host defense among asthmatic mice.

Innate immunity is a natural immune defense system formed during the development and evolution of the body, which is the first line of host defense against microbial invasion. It consists of naïve immune barrier, immune cells and immune molecular [6]. Antimicrobial peptide (AMP) is the most important naïve immune molecular, which has a broad spectrum bactericidal action [7, 8]. Defensins and cathelicidins are primary AMP factors expressed in the lung and secreted by airway epithelial cells, macrophages, neutrophils, and other classical host defense cells [22]. Defensin is an important family of endogenous antimicrobial peptide, which can kill the intruded pathogens quickly

and nonspecifically [23]. As multifunctional effector molecules of innate immunity, defensins are the key components in immune system, which can kill or inhibit most microbes such as bacteria, fungus and envelope virus *in vitro* [24]. Human beta defensin 2 (HBD-2) is the chief component of airway innate immunity, which is the first human defensins inducible expression by inflammation and cytokines. HBD-2 plays a significant role in anti-infection immunity in skin, mucous membrane, and airway mucous membrane especially [25]. In our study, expression of mouse beta defensin 3 (MBD-3) (MBD-3 is a defensin in mice which is the homolog of HBD-2.) was found to be reduced in asthmatic mice exposed to *P. aeruginosa* compared to normal mice exposed to *P. aeruginosa*. Nevertheless, expression of MBD-3 was found to be recovered in lung of mice accepted ASIT with dust mite vaccine. Thus, our study indicates that *P. aeruginosa*

infection inhibits lung MBD-3 expression in asthmatic mice. However, ASIT with dust mite vaccine induced lung MBD-3 expression among asthmatic mice.

Previous research has suggested that the imbalance in Th1/Th2 immunity plays an important role in the pathogenesis of allergic asthma. Increasing quantity and hypofunction of Th2 are the important causes of allergic asthma [26]. Th1 cells produce Th1-type cytokines such as IFN- $\gamma$ , IL-2, and IL-12, which induce cellular immune response to defense against viruses and bacteria. Th2 cells produce Th2-type cytokines such as IL-4, IL-5, IL-6, IL-9, and IL-13, which induce humoral immune response to participate in allergic inflammation and defense against parasites. Allergic mice showed more viable bacteria in their lungs after infection significantly. Th2-type cytokines can inhibit antimicrobial host defense in individuals with allergic diseases [11, 12]. In our study, compared to normal mice exposed to *P. aeruginosa*, BALF IL-4 levels of asthmatic mice exposed to *P. aeruginosa* were found to be increased and BALF IFN- $\gamma$  levels decreased. However, when ASIT with dust mite vaccine was used, BALF IL-4 levels decreased and BALF IFN- $\gamma$  levels increased. Th2-induced inflammation was found to down-regulate host defense and reduce AMP expression in the skin [27, 28]. When incubated with IL-4 or IL-13, airway epithelial cells couldn't kill bacteria efficiently, which indicated that these cytokines inhibit antimicrobial activity of the airway epithelium [29]. Thus, our study indicates that ASIT with dust mite vaccine increased lung IFN- $\gamma$  levels and decreased IL-4 levels in mice with allergic inflammation exposed to *P. aeruginosa*. The function of down-regulation IL-4 levels induced by ASIT with dust mite vaccine could be related to the function of up-regulation antimicrobial host defense induced by ASIT with dust mite vaccine.

### Conclusions

We show that ASIT with dust mite vaccine could decrease the severity of *P. aeruginosa* infection in the airway of asthmatic mice and enhance antibacterial host defense in airway epithelial cells by up-regulating MBD-3 through down-regulating IL-4 levels. These findings may suggest that ASIT with dust mite vaccine may enhance airway antibacterial host defense in asthma patients.

### Acknowledgements

Supported by grants from the National Natural Science Foundation of China (No. 91442118, 91542104), Shenzhen Scientific Technology Basic Research Projects (No. JCYJ2012083-0091748817, JCYJ20160328144536436, JCYJ20150525092941036).

### Disclosure of conflict of interest

None.

**Address correspondence to:** Drs. Pei-Xin Ran and Zhi-Gang Liu, State Key Laboratory of Respiratory Disease, Guangzhou Medical University, Guangzhou 510006, China. Tel: 86-755-86671907; Fax: 86-755-86671907; E-mail: pxran@gzhmc.edu.cn (PXR); lzg@szu.edu.cn (ZGL)

### References

- [1] Li Y, Hua S. Mechanisms of pathogenesis in allergic asthma: role of interleukin-23. *Respirology* 2014; 19: 663-669.
- [2] Murdoch JR, Lloyd CM. Chronic inflammation and asthma. *Mutat Res* 2010; 690: 24-39.
- [3] von Mutius E. The microbial environment and its influence on asthma prevention in early life. *J Allergy Clin Immunol* 2016; 137: 680-689.
- [4] Wood PR, Hill VL, Burks ML, Peters JI, Singh H, Kannan TR, Vale S, Cagle MP, Principe MF, Baseman JB, Brooks EG. *Mycoplasma pneumoniae* in children with acute and refractory asthma. *Ann Allergy Asthma Immunol* 2013; 110: 328-334.
- [5] Beisswenger C, Kandler K, Hess C, Garn H, Felgentreff K, Wegmann M, Renz H, Vogelmeier C, Bals R. Allergic airway inflammation inhibits pulmonary antibacterial host defense. *J Immunol* 2006; 177: 1833-1837.
- [6] Wood KJ, Goto R. Mechanisms of rejection: current perspectives. *Transplantation* 2012; 93: 1-10.
- [7] Peters BM, Shirtliff ME, Jabra-Rizk MA. Antimicrobial peptides: primeval molecules or future drugs? *PLoS Pathog* 2010; 6: e1001067.
- [8] Diamond G, Beckloff N, Weinberg A, Kisich KO. The roles of antimicrobial peptides in innate host defense. *Curr Pharm Des* 2009; 15: 2377-2392.
- [9] McCormick TS, Weinberg A. Epithelial cell-derived antimicrobial peptides are multifunctional agents that bridge innate and adaptive immunity. *Periodontol* 2000 2010; 54: 195-206.
- [10] Bardan A, Nizet V, Gallo RL. Antimicrobial peptides and the skin. *Expert Opin Biol Ther* 2004; 4: 543-549.



## Dust mite vaccine enhances antibacterial host defense

- [11] Guaní-Guerra E, Negrete-García MC, Montes-Vizuet R, Asbun-Bojalil J, Terán LM. Human  $\beta$ -defensin-2 induction in nasal mucosa after administration of bacterial lysates. *Arch Med Res* 2011; 42: 189-194.
- [12] Beisswenger C, Kandler K, Hess C, Garn H, Felgentreff K, Wegmann M, Renz H, Vogelmeier C, Bals R. Allergic airway inflammation inhibits pulmonary antibacterial host defense. *J Immunol* 2006; 177: 1833-1837.
- [13] Jensen JM, Ahrens K, Meingassner J, Scherer A, Bräutigam M, Stütz A, Schwarz T, Fölster-Holst R, Harder J, Gläser R, Proksch E. Differential suppression of epidermal antimicrobial protein expression in atopic dermatitis and in EFAD mice by pimecrolimus compared to corticosteroids. *Exp Dermatol* 2011; 20: 783-788.
- [14] Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998; 102: 558-562.
- [15] Wambre E, Bonvalet M, Bodo VB, Maillère B, Leclert G, Moussu H, Von Hofe E, Louise A, Balazuc AM, Ebo D, Hoarau C, Garcia G, Van Overtvelt L, Moingeon P. Distinct characteristics of seasonal (Bet v 1) vs. perennial (Der p 1/Der p 2) allergen-specific CD4(+) T cell responses. *Clin Exp Allergy* 2011; 41: 192-203.
- [16] Eifan AO, Akkoc T, Yildiz A, Keles S, Ozdemir C, Bahceciler NN, Barlan IB. Clinical efficacy and immunological mechanisms of sublingual and subcutaneous immunotherapy in asthmatic/rhinitis children sensitized to house dust mite: an open randomized controlled trial. *Clin Exp Allergy* 2010; 40: 922-932.
- [17] Eiwegger T, Gruber S, Szépfalusi Z, Akdis CA. Novel developments in the mechanisms of immune tolerance to allergens. *Hum Vaccin Immunother* 2012; 8: 1485-1491.
- [18] Durack J, Boushey HA, Lynch SV. Airway microbiota and the implications of dysbiosis in asthma. *Curr Allergy Asthma Rep* 2016; 16: 52.
- [19] Cullen L, McClean S. Bacterial adaptation during chronic respiratory infections. *Pathogens* 2015; 4: 66-89.
- [20] Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy and immune tolerance to allergens. *World Allergy Organ J* 2015; 8: 17.
- [21] Genc S, Eroglu H, Kucuksezer UC, Aktas-Cetin E, Gelincik A, Ustyol-Aycan E, Buyukozturk S, Deniz G. The decreased CD4+CD25+FoxP3+ T cells in nonstimulated allergic rhinitis patients sensitized to house dust mites. *J Asthma* 2012; 49: 569-574.
- [22] Tecle T, Tripathi S, Hartshorn KL. Review: defensins and cathelicidins in lung immunity. *Innate Immun* 2010; 16: 151-159.
- [23] Verma C, Seebah S, Low SM, Zhou L, Liu SP, Li J, Beuerman RW. Defensins: antimicrobial peptides for therapeutic development. *Biotechnol J* 2007; 2: 1353-1359.
- [24] Pevec B, Radulovic Pevec M, Stipic Markovic A, Batista I, Rijavec M, Silar M, Kosnik M, Korosec P. House dust mite-specific immunotherapy alters the basal expression of T regulatory and *fc̳ri* pathway genes. *Int Arch Allergy Immunol* 2012; 159: 287-296.
- [25] Hiemstra PS, Fernie-King BA, McMichael J, Lachmann PJ, Sallenave JM. Antimicrobial peptides: mediators of innate immunity as templates for the development of novel anti-infective and immune therapeutics. *Curr Pharm Des* 2004; 10: 2891-2905.
- [26] Ngoc PL, Gold DR, Tzianabos AO, Weiss ST, Celedón JC. Cytokines, allergy, and asthma. *Curr Opin Allergy Clin Immunol* 2005; 5: 161-166.
- [27] Britto CJ, Liu Q, Curran DR, Patham B, Dela Cruz CS, Cohn L. Short palate, lung, and nasal epithelial clone-1 is a tightly regulated airway sensor in innate and adaptive immunity. *Am J Respir Cell Mol Biol* 2013; 48: 717-724.
- [28] Nomura I, Goleva E, Howell MD, Hamid QA, Ong PY, Hall CF, Darst MA, Gao B, Boguniewicz M, Travers JB, Leung DY. Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes. *J Immunol* 2003; 171: 3262-3269.
- [29] Zanetti M. The role of cathelicidins in the innate host defenses of mammals. *Curr Issues Mol Biol* 2005; 7: 179-96.