

Vaccination With Different Doses of *Dermatophagoides farinae* in a Mouse Model of Allergic Asthma

Jie Han, Ying Huang, Xue Jun Wu and Mu-Kui Wang

Abstract: *Background:* The efficacy of vaccination with different doses of a vaccine has not been clarified. The purpose of this study was to investigate the relationship between the doses of purified house dust mite allergen, and allergic airway inflammation and airway reactivity in a mouse model of allergic asthma. *Methods:* C57BL/6 mice were sensitized with *Dermatophagoides farinae* (Der f) and randomly treated with saline, 0.1, 1, or 2 mg Der f vaccine, respectively. The airway hyperresponsiveness, the numbers of total cells and eosinophils and the levels of interferon- γ and interleukin (IL)-4 in the bronchoalveolar lavage fluid (BALF) from individual mice were measured. The percentages of CD11c⁺ CD86⁺ and CD11c⁺ MHC II⁺ dendritic cells were characterized, and the levels of IL-12 in the supernatants of cultured dendritic cells were measured. *Results:* The airway hyperresponsiveness, the numbers of total cells and eosinophils in the BALF and the percentages of CD11c⁺ CD86⁺ and CD11c⁺ MHC II⁺ dendritic cells were associated with immunologic downregulation. The levels of IFN- γ in the BALF and the levels of IL-12 in the supernatants of cultured dendritic cells were associated with immunologic upregulation. It was found that vaccination with the medium dose of Der f showed a stronger inhibitory effect. *Conclusions:* There seems to be a dose-dependent response between allergen and both airway inflammation and hyperreactivity in the mouse model of allergic asthma.

Key Indexing Terms: *Dermatophagoides farinae* vaccine; Allergic asthma; Immunotherapy; Dendritic cell. [Am J Med Sci 2015;350(2):116–120.]

Asthma is a serious global public health problem. Asthma affects approximately 300 million individuals in the world. Allergic asthma (AS) is a chronic airway inflammatory disease, which is characterized by inflammatory infiltrates in the lung that secrete many inflammatory mediators, leading to airway hyperresponsiveness (AHR) and airway epithelial damage. Airway inflammation promotes recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the morning.¹ Allergens can be one of the most important environmental factors, which initiate the development and progression of AS. Recent clinical investigations have indicated that sensitization to house dust mite allergens, such as

Dermatophagoides farinae (Der f), is a popular causative factor for AS in China.²

Currently, pharmacologic therapy is a primary treatment of asthma, and environmental control precautions are also important. However, allergen immunotherapy has been shown to be effective in decreasing the severity of asthma in house dust AS.³ A previous clinical trial has demonstrated that an allergen-specific vaccine can effectively inhibit allergic inflammation and benefit patients with AS.⁴ The therapeutic and potential adverse effects of allergen-specific vaccine are dependent on the dose of vaccines.⁵ Subcutaneous vaccination with a low dose of Der f1 effectively inhibits the onset asthma,⁶ although another study shows that the efficacy of vaccination with a high dose of Der f1 is better than that with a lower dose of allergen in mouse model of asthma.⁷ However, the optimal dose with which vaccination results in effective therapy and the potential mechanisms underlying the action of allergen vaccine have not been clarified. Notably, vaccination with a high dose of allergen-specific vaccine did not increase the therapeutic efficacy, but it did promote adverse effects.⁸ Hence, it is critical to determine the effects of vaccination with varying doses of allergen-specific vaccines and to investigate the potential therapeutic mechanisms and adverse effects.

In this study, the authors used a mouse model of asthma to test the effects of vaccination with different doses of Der f and the potential mechanisms underlying the action of allergen vaccines. The indicators used in this study included the AHR, the numbers of total cells and eosinophils and the levels of interferon (IFN)- γ and interleukin (IL)-4 in the bronchoalveolar lavage fluid (BALF), which were standard biomarkers for allergic airway inflammation. The percentages of CD11c⁺ CD86⁺ and CD11c⁺ MHC II⁺ dendritic cells and IL-12 in the supernatant of cultured dendritic cells may be associated with the regulation of airway inflammation. The authors found that vaccination with a high dose of Der f induced Th1 responses and decreased airway resistance and inflammatory infiltration. However, vaccination with an increased dose of Der f did not improve the therapeutic efficacy. The authors discussed the implications of these findings.

MATERIAL AND METHODS

Animals

Female 3-to-4 week old C57BL/6 mice were purchased from the Animal Experimental Center of Chongqing Medical University and housed in a specific pathogen-free facility at the Animal Experimental Center of Children's Hospital, and these animals had free access of normal mouse chow and water ad libitum. The experimental protocol was approved by the Animal Research and Care Committee of Chongqing Medical University.

Sensitization and Challenge

Individual mice were sensitized by intraperitoneal injection with 100 μ L of a mixture containing 50 μ g Der f extracts (Skin prick *Dermatophagoides Farinae* extracts, YBS0049208;

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Wowu BioTech, Zhejiang, China) and 2 mg $\text{Al}(\text{OH})_3$ or alum alone on days 0, 7 and 14. Two weeks after the last sensitization, the mice were randomized and treated intraperitoneally with 100 μL of vehicle saline (saline control) or saline containing different doses (0.1 mg, low dose; 1 mg, medium dose; or 2 mg, high dose) of Der f extracts on days 28, 35, 42, and 49, which were designated as the saline control, LDF, MDF, and HDF groups ($n = 8$ per group), respectively. Ten days after the last treatment, all mice were challenged by exposure to aerosolized Der f extracts (300 $\mu\text{g}/\text{mL}$) for 30 minutes daily for 7 consecutive days^{9,10} using an ultrasonic nebulizer (Ultraschall-vernebler Sirius Nova, Bad Ems, Germany). The mice were subjected to AHR test, BAL, and sacrificed at 24 hours after the last challenge.

Assessment of Airway Hyperresponsiveness

The authors have used AHR in this mouse model as a surrogate to evaluate the mechanisms of airway inflammation in human asthma. The AHR to increased concentrations (3.125–50 mg/mL) of aerosolized methacholine (Mch) in individual unrestrained and conscious mice was measured by whole-body plethysmograph (Buxco Electronics, Troy, NY) to determine changes in enhanced respiratory pause (in Penh). Briefly, in 24 hours after the last challenge, individual mice were acclimated in the main chamber for 5 minutes and nebulized with 250 μL of aerosolized saline. Subsequently, the mice were nebulized with increasing concentrations (3.125, 6.25, 12.5, 25, and 50 mg/mL) of aerosolized Mch for 3 minutes, and their Penh responses were recorded for 5 minutes. Data are expressed as the Penh after challenge with each concentration of Mch, and the baseline Penh was designated as 100%.

Bronchoalveolar Lavage

One day after the final challenge, individual mice were subjected to BAL with 3 aliquots of cold phosphate-buffered saline (500 μL each) through a tracheal cannula. The collected BALF samples were centrifuged at 4°C, $\times 600g$ for 10 minutes, and the supernatants were stored at -70°C . The cells in individual BALF samples were stained with Wright-Giemsa to determine the number of viable cells. The numbers of eosinophils in BALF samples were counted in a blinded manner. The concentrations of IL-4 and IFN- γ in BALF samples were determined by enzyme-linked immunosorbent assay (ELISA).

Cell Culture

The femur and tibia of individual animals were dissected immediately after sacrifice. After removing the muscles and connective tissues, each bone was aseptically flushed with Dulbecco's modified eagle medium (DMEM) to harvest the bone marrow cells. After lysis of red blood cells by hypotonic ammonium chloride buffer (0.155 M NH_4Cl , 10 mM KHCO_3 and 0.01 mM Na_2EDTA), mouse bone marrow mononuclear cells were isolated by Ficoll (1.082 g) gradient centrifugation at $\times 1000g$ 15 minutes. The mononuclear cells were harvested and washed with DMEM. Mouse bone marrow mononuclear cells ($1 \times 10^6/\text{mL}$) were incubated in 10% Fetal Calf Serum–Roswell Park Memorial Institute 1640 in 6-well plates in the presence of 10 $\mu\text{g}/\text{L}$ of Granulocyte-Macrophage Colony Stimulating Factor and 5 $\mu\text{g}/\text{L}$ of IL-4 and were exposed to newly prepared medium every other day for 7 days to expand dendritic cells *in vitro*. The supernatants of cultured dendritic cells were harvested, and the concentrations of IL-12 in the supernatants were determined by ELISA. The cells were harvested for flow cytometric analysis.

Flow Cytometry

The harvested cells ($1 \times 10^6/\text{tube}$) were stained in duplicate with PE-Cy5-anti-CD11c and APC-anti-CD86, or APC-anti-MHC II and isotype controls. The frequency of CD11c⁺ CD86⁺ and CD11c⁺ MHC II⁺ dendritic cells was determined by flow cytometric analysis.

Enzyme-Linked Immunosorbent Assay

The levels of IFN- γ in the supernatants of BALF and the concentrations of IL-12 in the supernatants of cultured bone marrow dendritic cells were measured by ELISA using specific kits, according to the manufacturer's instruction (R&D Systems; Changning, Shanghai, China).

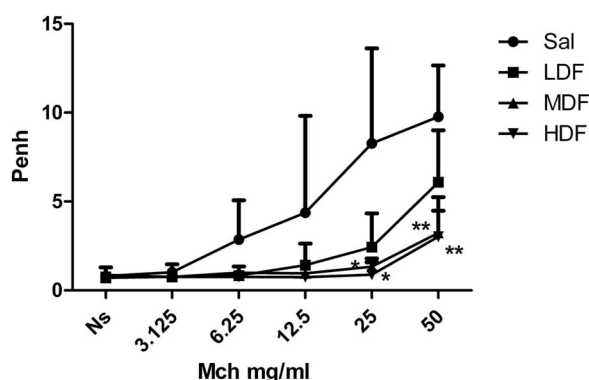
Statistics

Data are expressed as the mean $\pm$ standard deviation. The difference among the groups of animals was analyzed by analysis of variance and post hoc Tukey's test, and the difference between 2 groups was analyzed by Student's *t* test using SPSS 13.0. A *P* value of <0.05 was considered statistically significant.

RESULTS

Treatment With Medium or High Dose of Der f Significantly Mitigates Allergic Airway Resistance in Mice

To determine the effects of vaccination with different doses of Der f extracts, mice were sensitized with Der f extracts and treated with different doses of Der f. Following challenge with aerosolized Der f extracts, the Penh responses to increasing doses of Mch of individual mice were tested (Figure 1B). Following challenge with increasing doses of Mch, the saline control mice developed dose-dependent responses to Mch. In contrast, the strength of responses to Mch in all the Der f extract-treated groups of mice was significantly reduced, and the inhibitory effects of treatment with different doses of Der f extracts appeared to be dose dependent. These data suggest that vaccination with Der f extracts significantly mitigated allergic AHR in mice.



Variation of Penh value. *: $P < 0.05$; **: $P < 0.01$, compared with Sal

FIGURE 1. Vaccination with Der f reduces airway hyperresponsiveness in a mouse model of asthma. Female C57BL/6 mice were sensitized by Der f/alum and randomly treated with saline as the control (Sal) or vaccinated with low (LDF), medium (MDF), and high (HDF) dose of Der f. After continual challenge with allergen, the airway hyperresponsiveness to the indicated concentrations of aerosolized Mch in individual unrestrained and conscious mice was measured by Penh. Data are expressed as the mean $\pm$ standard deviation of individual groups ($n = 8$). * $P < 0.05$; ** $P < 0.01$ versus the controls.

Vaccination With Der f Reduces the Numbers of BALF Cells

During the inflammatory allergic process, inflammatory cells infiltrate into the lungs. To test the effects of vaccination with different doses of Der f on inflammatory infiltrates in the lungs, the numbers of total cells (TCS) and eosinophils (EOS) in the BALF from the different groups of mice were determined (Figure 2). In comparison with that in the saline asthmatic mice, the numbers of total cells and eosinophils in the Der f-vaccinated mice were significantly reduced, particularly in the mice vaccinated with the medium dose of Der f. Interestingly, the levels of IFN- γ in the BALF samples from the group of mice vaccinated with medium dose were significantly higher than that in other groups. These data suggest that vaccination with a medium dose of Der f may induce potent Th1 responses, which inhibit allergic eosinophil responses in the lungs of mice.

Vaccination With Der F Inhibits Bone Marrow Dendritic Cells but Increases the Levels of IL-12 in the Supernatants of Cultured Dendritic Cells

Dendritic cells are potent antigen-presenting cells and are crucial for initiating immune responses.^{11,12} The numbers and maturation of dendritic cells are crucial for inducing potent antigen-specific T-cell immunity.¹³ To understand the potential mechanisms underlying the action of the Der f vaccine, the authors isolated bone marrow cells from individual mice and stimulated them with IL-4 and Granulocyte-Macrophage Colony Stimulating Factor for 7 days, a protocol for promoting immature dendritic cell expansion *in vitro*.¹⁴ Subsequently, the cells were characterized by flow cytometric analysis. The authors found that the percentages of

CD11c⁺ MHC II⁺ and CD11c⁺ CD86⁺ dendritic cells in the mice treated with medium and high dose of Der f were significantly lower than those in the saline control mice (Figure 2B). The inhibitory effects of Der f vaccines on the percentages of CD11c⁺ CD86⁺ dendritic cells appeared to be dose dependent. These data indicated that vaccination with Der f modulated the development of dendritic cells in mice.

Further analysis revealed that the levels of IL-12 in the supernatants of cultured dendritic cells from the mice treated with a medium dose of Der f were significantly higher than those in the saline control mice (Figure 2D). However, vaccination with a lower or higher dose of Der f did not significantly modulate the levels of IL-12 in the supernatants of cultured dendritic cells, as compared with that in the saline control mice. These data further support the notion that vaccination with an optimal dose of Der f effectively modulates the function of dendritic cells in mice.

DISCUSSION

Asthma is an inflammatory disorder of the airways, which involves several types of inflammatory cells and multiple mediators that result in characteristic pathophysiological changes.^{15,16} The inflammatory process was thought to be mediated by antigen-specific Th2 and Th17 cells. Currently, allergen-specific immunotherapy is an accepted treatment of asthma, particularly for those with house dust mite allergens.³ However, the dose effects of allergen vaccines remain controversial. In this study, the authors tested the dose effect of Der f in a mouse model of asthma. They found that vaccination with different doses of Der f inhibited AHR in a dose-dependent

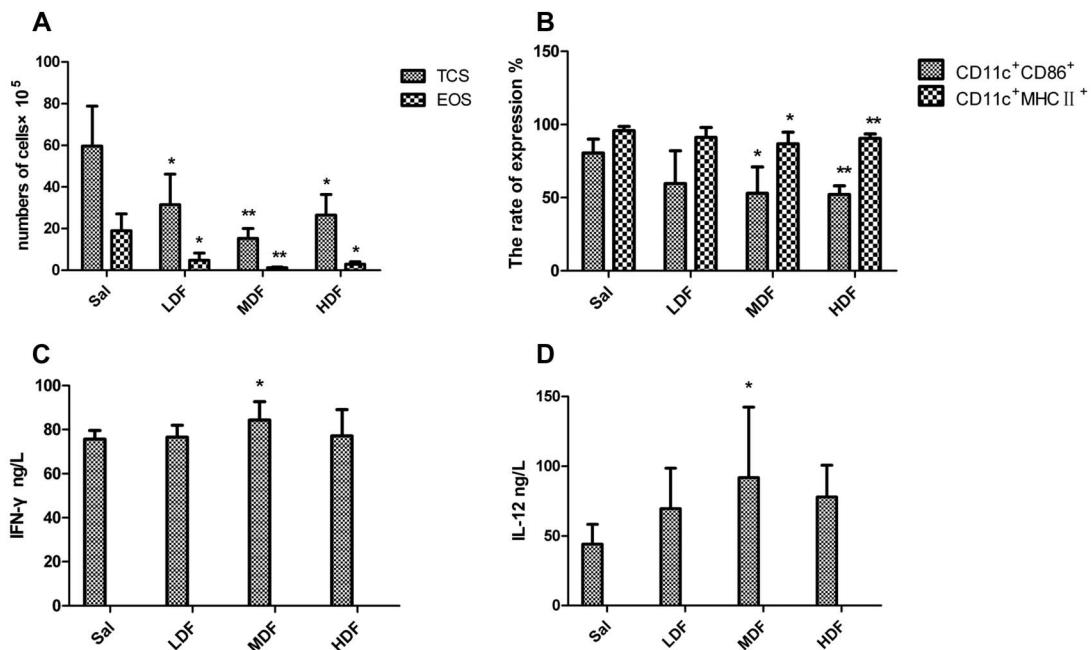


FIGURE 2. Vaccination with a medium dose of Der f reduces inflammatory infiltrates in the lung and bone marrow dendritic cell maturation but enhances Th1 responses in asthmatic mice. The numbers of total cells and eosinophils and the levels of interferon- γ in the BALF from individual mice were examined by cell counting and enzyme-linked immunosorbent assay, respectively. The bone marrow dendritic cells were prepared and expanded *in vitro* for 7 days. The frequency of CD11c⁺ CD86⁺ and CD11c⁺ MHC II⁺ dendritic cells was characterized by flow cytometric analysis. The levels of interleukin-12 in the supernatants of cultured dendritic cells were measured by enzyme-linked immunosorbent assay. Data are expressed as the means $\pm$ standard deviation of individual groups of mice ($n = 8$ per group) from at least 3 separate experiments. (A) The numbers of total cells in the BALF. (B) The concentrations of interferon- γ in the BALF. (C) The frequency of dendritic cells. (D) The concentrations of IL-12 in the supernatants of cultured dendritic cells. * $P < 0.05$; ** $P < 0.01$ versus the controls.

manner. However, vaccination with a medium dose of Der f achieved the best effect on inhibiting eosinophil infiltration in the lungs and on inducing potent IFN- γ responses in a mouse model of asthma. The findings suggest that allergen-induced Th1 responses were associated with the inhibition of airway inflammation and support the notion that vaccination with an optimal dose of allergen effectively modulates airway resistance and inflammation in mice.

Allergen-specific vaccine has been thought to induce immune deviation of antigen-specific T-cell responses. Although Th2 and Th17 responses are associated with the development and progression of allergic inflammation, previous studies have shown that induction of Th1 responses can inhibit Th2 and Th17 responses, thereby mitigating allergic inflammation.¹³ Furthermore, vaccination with allergen may induce regulatory T-cell (Treg) responses, which secrete inhibitory IL-10 and transforming growth factor- β 1.^{17–19} The authors found that vaccination with a medium dose of Der f induced potent IFN- γ responses in mice. Although Tregs can also inhibit Th1 responses in many inflammatory diseases, the potent Th1 responses in the Der f-vaccinated mice suggest that vaccination with Der f may not effectively induce Treg responses in the mouse model of asthma.

During the process of functional T-cell differentiation, IL-12 secreted by antigen-presenting cells, such as dendritic cells, can induce T-bet expression and Th1-cell differentiation,²⁰ and Th1 cells secrete IFN- γ , which inhibits the GATA3 and ROR γ T expression and Th2 and Th17 differentiation. Indeed, dendritic cells are crucial for inducing antigen-specific T-cell immunity.²¹ The maturity of dendritic cells, which are marked by the frequency of CD11c⁺ CD86⁺ and CD11c⁺ MHC II⁺, can affect the regulation of inflammation in mice. In this study, the authors found that vaccination with medium and high doses of Der f significantly reduced the frequency of CD11c⁺ CD86⁺ and CD11c⁺ MHC II⁺ dendritic cells in mice. However, vaccination with a medium dose of Der f significantly increased CD11c⁺ CD86⁺ and CD11c⁺ MHC II⁺ dendritic cell maturation *in vitro*. The significantly increased dendritic cells should promote antigen-specific Th1 responses in mice. The authors detected significantly higher levels of IL-12 in the cultured dendritic cells from the mice that had been vaccinated with a medium dose of Der f. The significantly higher levels of IL-12 secreted by dendritic cells were consistent with potent Th1 responses in those mice. However, the potent dendritic cell responses were not detected in the mice that received a high dose of Der f. It is possible that vaccination with an optimal dose of allergen may modulate the development of dendritic cells in the bone marrow, but allergen-induced IFN- γ responses may preferably direct the maturation of IL-12-secreting dendritic cells in mice. The authors are interested in further investigating how vaccination with Der f modulates dendritic cell development in the bone marrow.

A study has shown that the therapeutic efficacy of allergen vaccines is dose dependent in mouse model of asthma. The authors found that vaccination with a medium dose of allergen achieved better therapeutic effect on inhibiting airway eosinophil infiltration and inducing IFN- γ responses, as well as enhancing IL-12 production by dendritic cells. However, the authors observed that vaccination with a higher dose of Der f did not enhance therapeutic effect, rather than diminished its effect in mice. Therefore, vaccination with allergens should optimize the dose to achieve therapeutic effect in the clinic.

In summary, the data indicated that although vaccination with Der f inhibited airway resistance in a dose-dependent

manner, vaccination with a medium dose of Der f may achieve therapeutic effect on inhibiting airway inflammation and modulating dendritic cell maturation in mice. Therefore, optimization of vaccine doses positively affects the airway inflammatory response and AHR in this mouse model of asthma.

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