

Article

Therapeutic Effects of DNA Vaccine on Allergen-Induced Allergic Airway Inflammation in Mouse Model

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Vaccination with DNA encoding *Dermatophagoides pteronyssinus* group 2 (Der p 2) allergen previously showed its effects of immunologic protection on Der p 2 allergen-induced allergic airway inflammation in mice. In present study, we investigated whether DNA vaccine encoding Der p 2 could exert therapeutic role on allergen-induced allergic airway inflammation in mouse model and explored the mechanism of DNA vaccination in asthma specific-allergen immunotherapy. After sensitized and challenged by Der p 2, the BALB/c mice were immunized with DNA vaccine. The degrees of cellular infiltration were scored. IgE levels in serum and IL-4/IL-13 levels in BALF were determined by ELISA. The lung tissues were assessed by histological examinations. Expressions of STAT6 and NF- κ B in lung were determined by immunohistochemistry staining. Vaccination of mice with DNA vaccine inhibited the development of airway inflammation and the production of mucin induced by allergen, and reduced the level of Der p 2-specific IgE level. Significant reductions of eosinophil infiltration and levels of IL-4 and IL-13 in BALF were observed after vaccination. Further more, DNA vaccination inhibited STAT6 and NF- κ B expression in lung tissue in Der p 2-immunized mice. These results indicated that DNA vaccine encoding Der p 2 allergen could be used for therapy of allergen-induced allergic airway inflammation in our mouse model. *Cellular & Molecular Immunology*. 2006;3(5):379-384.

Key Words: DNA vaccine, *Dermatophagoides pteronyssinus* group 2, allergic airway inflammation

Introduction

Allergic asthma is currently considered a chronic airway inflammatory disorder associated with the presence of activated CD4⁺ Th2-type lymphocytes, eosinophils, and mast cells. Immunotherapy with protein allergens is effective in reducing symptoms in patients with allergic rhinitis and asthma (1). Unfortunately, the dose of allergen that can be administered to patients with allergic asthma is frequently limited because of reduced FEV 1 (Forced expiratory volume

in 1 second) and safety concerns (2). Recently, one strategy aimed at downregulation of the allergic response is based on the development of DNA vaccine. DNA-based therapeutics has shown great promise in rodent models of allergic disease (3). DNA immunization had turned out as a promising novel type of immunotherapy against allergy (4).

We had demonstrated that vaccination with DNA encoding Der p 2 allergen could generate immunologic protection in Der p 2 allergen-induced allergic airway inflammation mouse model. DNA vaccines have prevented subsequent sensitization of IgE reactions. This vaccine could markedly decrease Th2-mediated responses, enhance Th1-mediated responses, and suppress the allergic response (5). But the therapeutic effect has not been studied using plasmid DNA encoding allergen. In our present study, we investigated whether DNA vaccine encoding Der p 2 could generate therapeutic efficiency in allergen-induced allergic airway inflammation mouse model and explored the mechanism of DNA vaccine immunization in asthma specific-allergen immunotherapy.

Materials and Methods

Construction of pcDNA-Der p 2 immunization vector

The expression plasmid pcDNA-Der p 2 was constructed as previously described (3). The plasmids were propagated in

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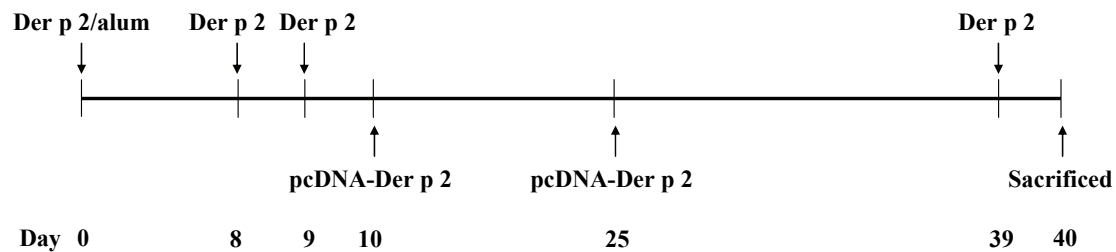


Figure 1. Immunization scheme for treatment of allergen-induced allergic airway inflammation by DNA vaccine.

Escherichia coli TOP10. Large scale purification of the plasmid pcDNA-Der p 2 was conducted with a QBIogene MAXIPREP GFII™ Endo-Free Kit (QBIogene, USA) according to the manufacturer's instructions.

Immunization protocols

BALB/c mice (8-wk-old, male, 20–25 g) were maintained under standard conditions with free access to water and rodent laboratory food. Mice were handled according to experimental procedures involving mice. Thirty-two mice were divided randomly into normal control group, pcDNA control group, DNA vaccine group and asthma group. Mice were anesthetized and immunized by the injection of 100 µl of inoculum into the tibialis anterior muscle using a 1 ml tuberculin syringe. pcDNA control group were immunized with pcDNA3.1 plasmid (100 µg/mouse). DNA vaccine group were treated with pcDNA-Der p 2 (100 µg/mouse). Asthma group were treated with phosphate-buffered saline (PBS). To determine whether DNA vaccination could generate therapeutic efficiency in allergen-induced allergic airway inflammation mouse model, sensitization, vaccination and challenge were performed as described previously (6). Mice were first sensitized *i.p.* with 10 µg rDer p 2 and 10 µg mite dust extract adsorbed onto 4 mg aluminum potassium sulfate (alum), and intranasal instillation of rDer p 2 (25 µg in 50 µl PBS) on days 8, 9 to establish allergen-induced allergic airway inflammation mouse model. The mice were then vaccinated with pcDNA-Der p 2 (100 µg/mouse), PBS or pcDNA3.1 plasmid (100 µg/mouse) on days 10 and 25. On day 39 the mice were boosted again with intranasal instillation of rDer p 2 (25 µg in 50 µl PBS) (Figure 1). Normal control group only received PBS. Twenty four hours after the last challenge, blood was taken, mice were sacrificed, lungs were removed and fixed, and splenocytes were isolated for *in vitro* culture.

Determination of Der p 2 specific antibody levels in serum

Blood from mice in the four groups was collected 24-hour after the last challenge. Der p 2-specific IgE were determined by ELISA as follows: 96 microtiter plates were coated overnight at 4°C with 100 µl of Der p 2 (10 µg/ml in 0.1 mol/L carbonate buffer, pH 9.6). The antigen-coated plates were washed five times with PBST (0.5% Tween-20 in PBS).

Mouse sera were added to the antigen-coated wells, the plates were incubated with peroxidase-conjugated anti-mouse IgE antibody (Southern Biotech, USA) overnight at 4°C, and then washed five times with PBST before adding citric acid-phosphate buffer (pH 5.0) containing 0.5 mg/ml of O-phenylenediamine (Sigma, USA). Color was developed at 37°C, and the reaction stopped with 2.5 mol/L sulfuric acid, and measured at 450 nm.

Bronchoalveolar lavage

To investigate the cellular infiltrate in the airways, the trachea were exposed and cannulated and lungs were gently instilled twice with 500 µl of cold PBS. The volume, total cell number and composition of BAL samples were determined. Samples were centrifuged (500× g for 5 minutes at 4°C) and resuspended, cytopspins were made onto slides. Slides were air-dried, and stained by Liu's stain using standard procedures (Taiwan). Different cell counts were performed in duplicate on coded slides for 200 cells from each sample. BAL fluid was stored at -70°C and levels of the cytokines IL-4 and IL-13 were determined using ELISA kit (R&D systems, USA; sensitivity > 2 pg/ml).

Histologic evaluation

Twenty-four hours after the last allergen challenge, lungs were excised. The tissues were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections (5 µm) of specimens were put onto 3-amino propyltriethoxy silane (APES)-APES-coated slides. The tissues were assessed for general morphology and cellular infiltration using haematoxylin and eosin (H&E) staining. Mucus-producing goblet cells were identified using periodic acid-Schiff (PAS)/alcian blue. The degree of cellular infiltration was scored by the method of Kwon. This scoring method discriminates between the presence of mononuclear cells around blood vessels (score 0-3) and around bronchioli (score 0-3), and the number of patchy cellular infiltration (score 0-3) (7).

Detection of STAT6 and NF-κB in lung by immunohistochemistry staining

Mice were sacrificed 24 h after last allergen challenge, and then lungs were removed and fixed overnight in 10% formalin and embedded in paraffin. Sections (5 µm) of

Table 1. Degree of inflammatory cellular infiltration in lung tissue after immunization (mean $\pm$ SD)

Immunization	Bronchioli	Blood vessels	Patchy
DNA vaccine	1.34 $\pm$ 0.17*	1.12 $\pm$ 0.18*	0.67 $\pm$ 0.15*
pcDNA	2.09 $\pm$ 0.34	2.36 $\pm$ 0.25	2.34 $\pm$ 0.21
Asthma	2.12 $\pm$ 0.31	2.71 $\pm$ 0.33	2.18 $\pm$ 0.19

The degree of cellular infiltration was expressed as a histological score that is described in Materials and Methods. * $p < 0.01$ compared with the pcDNA control and asthma groups.

specimens were heated for 1 h at 60°C, rehydrated, and incubated for 30 min with 0.3% H₂O₂/0.2 mol/L NaN₃, followed by blocking with 1% bovine serum albumin (BSA) in TTBS (0.1% Tween, 100 mmol/L Tris-HCl, 0.9% NaCl). Sections were then stained for 1 h with rabbit anti-STAT6 (1:500, labvision, USA) or rabbit anti-NF- κ B p65 (1:200). Followed two washes in TTBS, horseradish peroxidase (HRP)-labeled anti-rabbit IgG2a antibody was applied for 1 h at room temperature. Then the sections were washed again and incubated for 4 min in medium containing 50 mmol/L Tris-HCl buffer (pH 7.6), 0.02% (wt/vol) DAB and 0.01% hydrogen peroxide. Chromogenic reaction was terminated with water followed by hematoxylin counter staining. Specific staining with the STAT6 and NF- κ B p65 antibodies was verified by incubating with an isotype control or the secondary antibody only.

Statistical analysis

Data were expressed as mean $\pm$ standard error. Statistical analysis was performed using ANOVA, and the significant level was defined as $p < 0.05$. The data were analyzed by SPSS statistical software.

Results

Inhibition of allergen-induced airway inflammation by DNA vaccine

Twenty-four hours after last allergen challenge with intranasal instillation of Der p 2, histological analysis demonstrated that sensitization of mice with allergen (asthma group) resulted in the development of significant airway inflammation. Vaccination of mice with DNA vaccine inhibited the development of airway inflammation. Injection of pcDNA3.1 plasmid had no effect on allergen-induced airway inflammation. The degree of cellular infiltration around the central bronchi, alveoli and blood vessels was scored (Table 1). The degree of cellular infiltration around the central bronchi, alveoli and blood vessels in DNA vaccination mice was decreased, as compared with pcDNA control and asthma group ($p < 0.01$). Representative histology was shown for each group (Figure 2). The asthma group or plasmid control group developed extensive infiltration of inflammatory cells such as eosinophils around the central bronchi, alveoli and blood vessels (Figure 2A), goblet cell

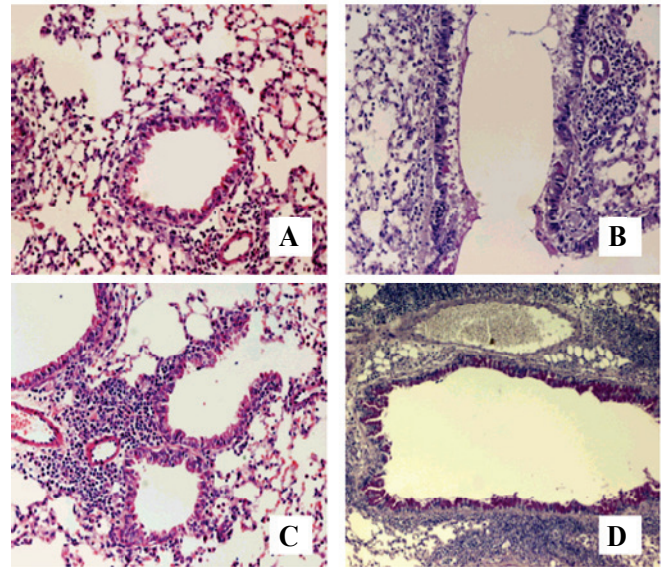


Figure 2. Histological examination of peripheral airway tissue. (A) DNA vaccine group (H&E, 200 $\times$); (B) DNA vaccine group for mucus glycoconjugates (PAS, 200 $\times$); (C) asthma group (H&E, 200 $\times$); (D) asthma group (PAS, 200 $\times$).

hyperplasia, production of mucin (a major constituent of mucus) (Figure 2B). The extent of inflammation and cellular infiltration in the airways were reduced (Figure 2C), and less marked goblet cell hyperplasia and mucin production in epithelial cells were observed in DNA vaccine group (Figure 2D).

Inhibition of IgE synthesis by DNA vaccine

We also analyzed the levels of anti-Der p 2 IgE in serum collected from these mice by ELISA. After last allergen challenge, the levels of Der p 2-specific IgE were high in Der p 2-immunized mice. Vaccination with DNA vaccine reduced the levels of Der p 2-specific IgE compared with asthma group ($p < 0.05$). In contrast, pcDNA 3.1 plasmid had little effect on IgE production. The data indicated that mice treated by DNA vaccine suppressed allergen-induced IgE production (Figure 3).

Infiltration of eosinophils and cytokine production in BAL fluid

We assessed allergen-induced infiltration of eosinophils in BAL fluid. By morphometric analysis, the number of eosinophils in BAL fluid was high in Der p 2-immunized mice, but vaccination with DNA vaccine reduced the number of eosinophils in BAL fluid compared with asthma group ($p < 0.01$, Figure 4). In contrast, pcDNA 3.1 plasmid had little effect on infiltration of eosinophils. The levels of IL-4 and IL-13 in BAL fluid were high in Der p 2-immunized mice. Vaccination with DNA vaccine reduced the levels of IL-4 and IL-13 in BAL fluid compared with asthma group ($p < 0.01$, Figure 5). In contrast, pcDNA 3.1 plasmid had little effect on the production of IL-4 and IL-13.

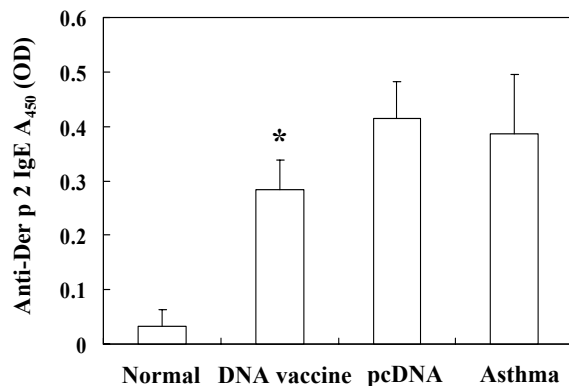


Figure 3. IgE level in the serum were determined by ELISA. Serum 1:10 dilution for IgE. * $p < 0.05$ compared with pcDNA control and asthma groups.

Expression of STAT6 and NF- κ B in lung tissue

Expression of STAT6 and NF- κ B is directly related to the induction of an allergic peribronchial inflammation of Th2-mediated cytokine responses. Therefore, histological analysis by anti-STAT6 and anti-NF- κ B p65 staining was performed to investigate the effect of DNA vaccine on express of STAT6 and NF- κ B in lung tissue. The expression of STAT6 and NF- κ B in lung was high in Der p 2-immunized mice. Vaccination with DNA vaccine reduced the expression of STAT6 and NF- κ B in lung. As shown in Figure 6, the expression of STAT6 and NF- κ B in Der p 2-immunized mice scattered in all biopsies studied and localized predominantly to inflammatory tissue of the lung. In contrast, pcDNA 3.1 plasmid had little effect on expression of STAT6 and NF- κ B.

Discussion

Over the last decades the prevalence of allergic disorders,

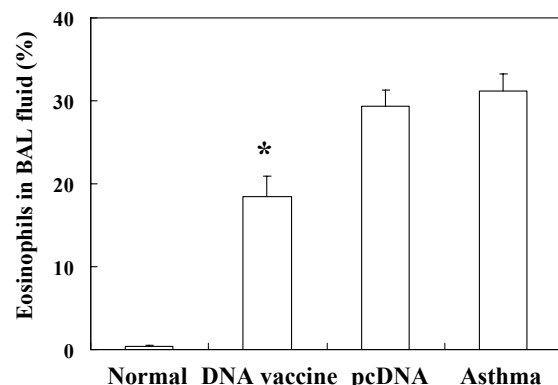


Figure 4. Infiltration of eosinophils into the airways is suppressed by DNA vaccine. * $p < 0.01$ compared with the pcDNA control and asthma groups.

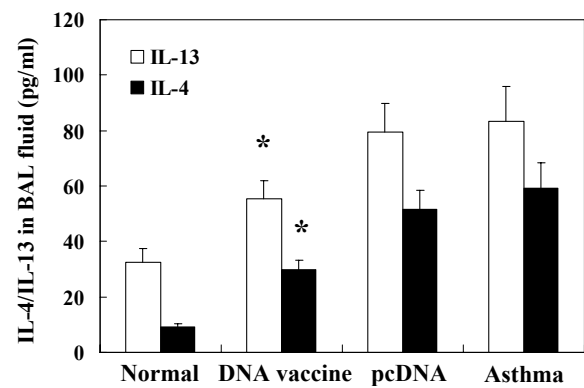


Figure 5. The levels of IL-4 and IL-13 were determined by ELISA in the BAL fluid. * $p < 0.01$ compared with pcDNA control and asthma groups.

such as asthma, has increased worldwide, especially in westernized countries, where up to 20% of the population are affected (8). In China, the prevalence of asthma is also increasing. Indoor allergen exposures were positively associated with asthma diagnosis and persistent respiratory symptoms among Chinese adolescents (9). It was also confirmed that sensitization to house dust mite was significantly associated with current wheezing and allergic hyperresponsiveness in Chinese schoolchildren (10).

Previously, our study has demonstrated the usefulness of allergen DNA immunization in the prevention of allergen-induced allergic airway inflammation. DNA vaccine coding Der p 2 allergen induced a Th1 biased immune response and prevented the development of airway inflammation induced by allergen (5). In present study, the mice developed allergic airway inflammation in response to an inhaled allergen, extensive infiltration of inflammatory cells such as eosinophils were found around the central bronchi, alveoli and blood vessels. Vaccination of mice with DNA vaccine inhibited the development of airway inflammation. Our data demonstrated that DNA vaccine coding Der p 2 allergen was also effective in treating allergen-induced allergic airway inflammation.

The mechanisms of DNA vaccines that trigger different branches of the immune system remained unclear. It has been confirmed that the effect of DNA immunization is dominant. Not only does pre-immunization with plasmid DNA prevent the subsequent induction by protein in alum of either an IgE antibody response or activation of Th2 cells producing IL-4 and IL-5, but it can also reduce a pre-existing antigen-specific IgE response (11). In our study, the results showed that DNA vaccination inhibited the production of Der p 2-specific IgE antibody. Suppression of IgE synthesis was also associated with the decrease in levels of the Th2-derived cytokines IL-4 and IL-13 in BAL fluid. These results indicated that DNA vaccination in the treatment of allergen-induced allergic airway inflammation also inhibited Th2 immune response, and suppressed IgE production.

Over-expression of IL-4 or IL-13 in lungs of mice results

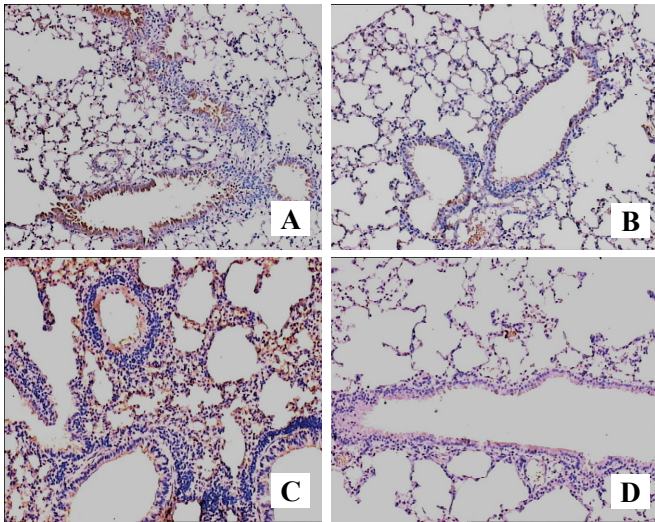


Figure 6. Immunohistological analysis of STAT6 and NF- κ B in lung tissue. Representative sections of lung tissue were obtained from different treatment groups 24 h after allergen challenge. Expression of STAT6 and NF- κ B were detected on general morphology and cellular distribution following staining with an anti-STAT6 and anti-NF- κ B p65. (A) The expression of STAT6 in asthmatic group (200 $\times$); (B) the expression of STAT6 in DNA vaccine group (200 $\times$); (C) the expression of NF- κ B in asthmatic group (200 $\times$); (D) the expression of NF- κ B in DNA vaccine group (200 $\times$).

in typical changes of asthma, such as inflammation, goblet cell hyperplasia and hypertrophy. STAT6 mediates IL-4 responses. When IL-4 binds to IL-4 receptor, the complex activates STAT6. Within the IL-4/IL-13 pathway, genetic variants in the STAT6 gene significantly contribute to the regulation of serum IgE levels (12). It has been confirmed that STAT6 protein and STAT6 mRNA were found strongly expressed in rat asthma model and the epithelial cells were the chief expressing cells (13). In our study, the levels of IL-4 and IL-13 in BAL fluid were high in Der p 2-immunized mice. The expression of STAT6 in lung was high in Der p 2-immunized mice. Vaccination with DNA vaccine reduced the levels of IL-4 and IL-13 in BAL fluid, and the expression of STAT6 in lung. Increases in phospho-STAT6 were largely observed in epithelial cells and fibroblasts in asthmatic mouse model (14). Unfortunately, phospho-STAT6 had not been tested in our study, so we were not able to provide direct evidence of STAT6 transcriptional activity inhibited by DNA vaccine.

NF- κ B plays an important role in the regulation of various inflammatory diseases including asthma. Its increased activation has been demonstrated in the lungs after allergen challenge and in airway epithelial cells and macrophages of asthmatic patients (15). Knockout mice studies have revealed that NF- κ B plays a critical role in Th2 cell differentiation and is therefore required for induction of allergic airway inflammation and airway remodeling (16, 17). In our study, the expression of NF- κ B in lung was also high

in Der p 2-immunized mice, and vaccination with DNA vaccine reduced the expression of NF- κ B in lung.

In conclusion, DNA vaccine encoding allergen might suppress allergen-specific IgE formation, and inhibit the production of IL-4/IL-13 and the expression of STAT6 and NF- κ B in lung. This efficiency might suppress airway inflammation induced by allergen. Thus, DNA vaccine encoding mite dust major allergen Der p 2 might generate therapeutic efficiency in allergen-induced allergic airway inflammation mouse model.

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