

Potential therapy of Fc-antigen combination-encoding DNA vaccination in mouse allergic airway inflammation

Y. Wang,^{*,†} G. Qian,[†] G. Wang,[†]
X. Cheng,[†] C. Bai^{*} and X. Wang^{*}

^{*}Department of Pulmonary Medicine, Zhongshan Hospital, Fudan University, Shanghai, and

[†]Institute of Respiratory Diseases, Xinqiao's Hospital, Third Military Medical University, Chongqing, China

Accepted for publication 24 June 2008

Correspondence: X. Wang and G. Qian, Department of Pulmonary Medicine, Zhongshan Hospital, Fudan University, Shanghai, 200032, China.

E-mail: xiangdong.wang@telia.com

This project was supported by grants from the National Natural Scientific Foundation of China (NSFC 30200120) and by China Postdoctoral Science Foundation (NO 20070420596) and by Shanghai Leading Academic Discipline Project (Project Number: B115).

Summary

Vaccination with allergen-encoding DNA has been proposed as having potential for allergen-specific immunotherapy. In this study, we examine the therapeutic effect of allergen-encoding DNA vaccination directly to dendritic cells (DCs) on allergen-induced allergic airway inflammation in a mouse model and explore potential mechanism. Ovalbumin (OVA)-sensitized and challenged mice were immunized with DNA vaccine and received bronchoalveolar lavage (BAL) 1 day after the last challenge, to measure BAL levels of interleukin (IL)-4, IL-5, interferon (IFN)-gamma and differential cell count. Pulmonary DCs and Spleen DCs were purified and sorted according to the expression of CD_{11c}⁺CD₈₀⁺ and CD_{11c}⁺CD₈₆⁺ co-stimulatory molecules. Our data demonstrated that DNA vaccine therapy with OVA-Fc-pcDNA₃₋₁ significantly prevented OVA-increased levels of IL-4, IL-5 and the percentage of eosinophils and OVA-decreased level of IFN-gamma. OVA-Fc-pcDNA₃₋₁-treated mice had less severity of airway inflammation, and lower expression of CD_{11c}⁺CD₈₀⁺ and CD_{11c}⁺CD₈₆⁺ on pulmonary DCs, as compared with animals with OVA-pcDNA₃₋₁, pcDNA₃₋₁ and OVA respectively. DNA vaccine encoding both Fc and OVA was shown to be more effective than DNA vaccine encoding OVA alone. Our data indicate that Fc-antigen combination-encoding DNA vaccination has better preventive effects on antigen-induced airway inflammation by regulating DCs, and may be a new alternative therapy for asthma.

Keywords: airway, asthma, dendritic cells, Fc, inflammation, vaccination

Introduction

Allergic asthma is an airway inflammation disease characterized by airway eosinophilia, goblet-cell hyperplasia, variable airway obstruction and bronchial hyper-responsiveness [1,2]. The airway inflammation is one of the most contributing factors to pathogenesis of allergic asthma, often correlated with the clinical symptoms, airway obstruction and airway hyperresponsiveness. Allergen-specific immunotherapy (SIT) has been investigated for about a century as one of the few antigen-specific treatments for diseases. SIT is performed by a repeated administration of sensitizing allergen, e.g. subcutaneous injection or sublingual application. SIT is a disease-modifying process and has the duration of action that exceeds the treatment period [3]. It has been shown that SIT could reduce the development of asthma and bronchial responses in patients exposed to inhaled

allergens. However, the mechanisms by which SIT plays anti-inflammatory roles remain unclear. In the current study, we investigated whether allergen-encoding DNA vaccination directly to dendritic cells (DCs) could generate therapeutic efficiency in a mouse model of allergen-induced airway inflammation and explore the potential mechanism of allergen-SIT with DNA vaccination in asthma.

Methods and materials

Animals

Male BALB/c mice, 6–10 weeks old at the onset of experiments, were purchased from the Animal Institute at the Third Military Medical University (Chongqing, China). Animal care and experimental procedures were in accordance with the animal ethics regulations of the Home Office, UK.

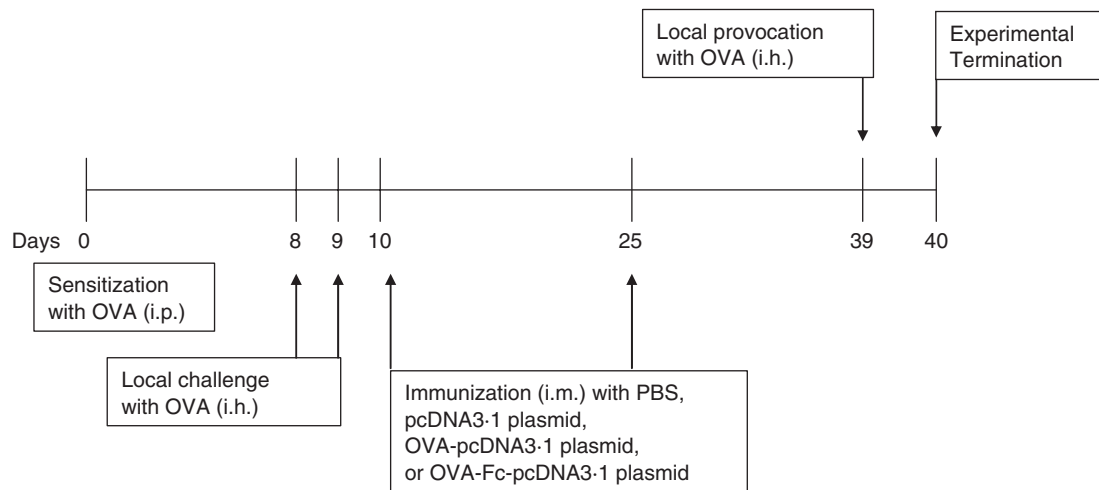


Fig. 1. Immunization scheme for treatment of allergen-induced allergic airway inflammation by DNA vaccination.

Construction of ovalbumin-Fc-pcDNA_{3.1} immunization vector

The PCR products of ovalbumin (OVA) and Fc were selected as target DNA fragments and cloned into pcDNA_{3.1} (+) to construct the recombinant plasmids OVA-pcDNA_{3.1} and OVA-Fc-pcDNA_{3.1} respectively. The plasmids were propagated in *Escherichia coli* and large-scale purification of all plasmids was conducted with the EndoFree Plasmid Giga Kit (Qiagen, Mississauga, Canada) according to the manufacturer's instructions.

Immunization protocols

BALB/c mice were maintained under standard conditions with free access to water and rodent laboratory food. Mice were handled according to experimental procedures. Forty mice were divided randomly into the five groups ($n = 8$ mice): (i) animals treated with saline and sensitized and challenged with saline as processing control group (controls); (ii) animals treated with saline and sensitized and challenged with OVA (saline-OVA); (iii) animals treated with pcDNA_{3.1} plasmid (100 µg/mouse) and sensitized and challenged with OVA (pcDNA_{3.1}); (iv) animals treated with OVA-pcDNA_{3.1} (100 µg/mouse) and sensitized and challenged with OVA (OVA-pcDNA_{3.1}); and (v) animals treated with OVA-Fc-pcDNA_{3.1} and sensitized and challenged with OVA (OVA-Fc-pcDNA_{3.1}). Mice were anaesthetized and immunized by the intramuscular injection of 100 µl inoculum using a syringe. The sensitization, vaccination and challenge were performed as described previously [4]. In brief, mice were sensitized intraperitoneally with 10 µg OVA (grade V, Sigma Chemical Co., St Louis, MO, USA) and 4 mg aluminium potassium sulphate, followed by an inhalation of 1% OVA (grade II) diluted in PBS for 30 min on days 8 and 9. The mice were then vaccinated with PBS, plasmid, pcDNA_{3.1},

OVA-pcDNA_{3.1}, or OVA-Fc-pcDNA_{3.1} plasmid on days 10 and 25. On day 39 the mice were challenged with inhalation of 1% OVA (grade II) diluted in PBS for 30 min (Fig. 1). Twenty-four hours after the last challenge, blood was taken. After mice were sacrificed, bronchoalveolar lavage (BAL) fluid and lungs were harvested for further analysis and histology, and the pulmonary DCs were isolated for *in vitro* culture.

Serum level of OVA-specific IgE

Ovalbumin-specific IgE was determined by ELISA in 96 microtitre plates coated with 100 µl of OVA (10 µg/ml in 0.1 mol carbonate buffer, pH 9.6) overnight at 4°C. The antigen-coated plates were washed with 0.5% Tween-20 in PBS five times. Mouse sera were added and the plates were incubated with peroxidase-conjugated anti-mouse IgE antibody (Biotechnology Associates, Birmingham, AL, USA) overnight at 4°C, and then washed five times before adding citric acid-phosphate buffer (pH 5.0) containing 0.5 mg/ml of O-phenylenediamine (Sigma Chemical Co.). Colour was developed at 37°C and measured at 450 nm after the reaction was stopped with 2.5 mol/l sulphuric acid.

Bronchoalveolar lavage

The trachea were exposed and cannulated and lungs were gently instilled with 500 µl of cold PBS twice. The volume and total cell number of BAL samples were recorded. Samples were centrifuged (500× g for 5 min at 4°C), resuspended, and cytopspined onto slides. Differential 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 interferon (IL)-4, IL-5 and interleukin (IFN)-γ were determined using specific ELISA according to the user's manual (ELISA kits, eBioscience, San Diego, CA, USA).

Histological evaluation

Twenty-four hours after the last allergen challenge, lungs were harvested and fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections (5 µm) of specimens were put onto 3-amino propyltriethoxy saline-coated slides. The morphology and cellular infiltration were assessed using haematoxylin and eosin staining. Inflammatory changes were graded by a scale of 0–5 for perivascular and bronchiolar eosinophilia, epithelial damage and oedema [5].

Generation of DCs from lung and culture

Pulmonary DCs were enriched according to the methods described previously [6]. Briefly, lungs were disrupted and the cells were centrifuged at 1300 rpm for 5 min, resuspended in RPMI 1640 medium supplemented with 10% heat-activated fetal calf serum, 2 mmol/l L-glutamine, 1 mmol/l pyruvate, 50 µmol/l mercaptoethanol, 100 U/ml penicillin and 100 µg/ml streptomycin, and then incubated for 2 h at 37 °C in a 5% CO₂ atmosphere. Culture plates were then washed thrice with RPMI 1640 medium and non-adherent cells were discarded. The residual adherent cells were maintained in the culture medium and incubated overnight. After incubation, DCs adhered during the initial hours of culture were collected and immediately used in the assays.

Generation of DCs from spleen and culture

Spleen DCs were enriched according to the methods described previously [6]. Briefly, spleen were disrupted and the cells were centrifuged at 1300 rpm for 5 min, resuspended in RPMI 1640 medium supplemented with 10% heat-activated fetal calf serum, 2 mmol/l L-glutamine, 1 mmol/l pyruvate, 50 µmol/l mercaptoethanol, 100 U/ml penicillin and 100 µg/ml streptomycin, and then incubated for 2 h at 37 °C in a 5% CO₂ atmosphere. Culture plates were then washed thrice with RPMI 1640 medium and non-adherent cells were discarded. The residual adherent cells were maintained in the culture medium and incubated overnight. After incubation, DCs adhered during the initial hours of culture were collected and immediately used in the assays.

Flowcytometry analysis

Dendritic cells were incubated with FITC-labelled CD_{11c} (eBioscience Inc.), PE-labelled anti-CD₈₀ (B7-1) mAb (eBioscience Inc.), PE-labelled anti-CD₈₆ (B7-2) mAb (Southern-biotech) on ice for 30 min and washed with PBS. Ten thousand cells were collected from each sample, and the data were analysed with flowcytometer and CELLQUEST software (Coulter, Becton Dickinson, Mountain View, CA, USA). DCs were incubated with PE-labelled rat anti-mouse IgG on ice for 30 min and washed again, after which they were used as an isotype control. The expression of the costimulatory molecules CD_{11c}⁺CD₈₀⁺ and CD_{11c}⁺CD₈₆⁺ on the surface of DCs was detected by a flowcytometry.

Statistical analysis

Data were expressed with means ± SD. Difference between groups was analysed using software SPSS for windows (version 8.0) by unpaired two-tailed parametric Student's *t*-test, after ANOVA test. *P*-values below 0.05 were considered statistically significant.

Results

Inhibition of allergen-induced airway inflammation by vaccination with different DNA vectors

Histological analysis demonstrated the significant airway inflammation in sensitized- and challenged-OVA animals treated with saline or pcDNA_{3.1} plasmid, rather than with OVA-pcDNA_{3.1} or OVA-Fc-pcDNA_{3.1} vaccination. The severity of cellular infiltration around the central bronchi, alveoli and blood vessels was shown in Table 1. OVA induced a significant increase of eosinophil infiltration in animals treated with saline or pcDNA_{3.1} (*P* < 0.05 or 0.01 *versus* non-OVA challenge group). The number of infiltrated eosinophils around the central bronchi, alveoli and blood vessels was significantly lower in OVA-sensitized and challenged animals treated with OVA-Fc-pcDNA_{3.1}, as compared with groups treated with saline, pcDNA_{3.1} or OVA-pcDNA_{3.1} (*P* < 0.01). The thickness of small airway walls and the number of infiltrated eosinophils around the airway

Table 1. Severity of inflammatory cellular infiltration in lung tissue after immunization.

Groups	Perivascular and peribronchiolar eosinophilia	Oedema	Epithelial damage
Mice sensitized, challenged and pretreated with saline or plasmid	0+ [†]	0+ [†]	0+ [†]
OVA-sensitized and challenged mice pretreated with saline	4.12 ± 0.25*	4.36 ± 0.34*	4.03 ± 0.21*
OVA-sensitized and challenged mice pretreated with pcDNA _{3.1}	3.31 ± 0.24*	3.34 ± 0.17*	3.12 ± 0.15*
OVA-sensitized and challenged mice pretreated with OVA-pcDNA _{3.1}	2.10 ± 0.19*	2.74 ± 0.30*	2.18 ± 0.33*
OVA-sensitized and challenged mice pretreated with OVA-Fc-pcDNA _{3.1}	1.32 ± 0.15+ [†]	1.24 ± 0.17+ [†]	0.68 ± 0.15+ [†]

*Stands for *P* value less than 0.05 as compared with A and E groups. + stands for *P* value less than 0.05 as compared with B and C groups. [†]Stands for *P* value less than 0.01 as compared with D group.

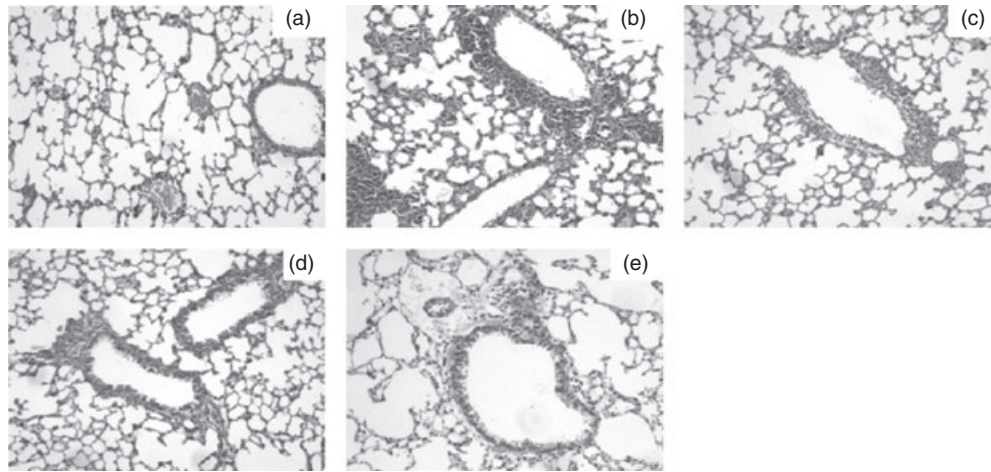


Fig. 2. Histological findings of peripheral airway tissues (H&E, $\times 200$ origin) from mice sensitized, challenged and treated with saline (A), OVA-sensitized and challenged mice treated with saline (B), OVA-sensitized and challenged mice pretreated with pcDNA_{3.1} (C), OVA-sensitized and challenged mice treated with OVA- pcDNA_{3.1} (D), and OVA-sensitized and challenged mice treated with OVA-Fc-pcDNA_{3.1} (E).

increased, eosinophils appeared within the lumen of the airway, and goblet cell hyperplasia and hypertrophy occurred in OVA-sensitized and challenged animals pretreated with saline (Fig. 2b) or pcDNA_{3.1} (Fig. 2c), as compared with those sensitized and challenged with saline (Fig. 2a). Treatment with both OVA-pcDNA_{3.1} (Fig. 2d) and OVA-Fc-pcDNA_{3.1} (Fig. 2e) significantly reduced OVA-induced infiltration of inflammatory cells in the airway, of which OVA-Fc-pcDNA_{3.1} had better therapeutic effects.

Inhibition of IgE synthesis by vaccination with different DNA vectors

Serum levels of OVA-specific IgE were significantly higher in OVA-sensitized and challenged mice treated with saline, as compared with those sensitized, challenged and treated with pcDNA_{3.1} ($P < 0.01$, Fig. 3). Animals treated with OVA-Fc-pcDNA_{3.1} had significantly lower levels of OVA-specific IgE, as compared with OVA-sensitized and challenged mice treated with pcDNA_{3.1} or OVA-pcDNA_{3.1} ($P < 0.01$). Animals treated with pcDNA_{3.1} plasmid had similar levels of OVA-specific IgE to those treated with saline. Allergen-DNA-targeted DCs could suppress allergen-induced airway inflammation and down-regulated the production of OVA-specific IgE.

Effects of combination vaccination on eosinophils in BAL fluid

The number of eosinophils in BAL fluid was significantly higher in OVA-sensitized and challenged mice pretreated with saline, similar to those pretreated with pcDNA_{3.1} plasmid, as compared with these sensitized, challenged and pretreated with saline ($P < 0.01$, Fig. 4). Treatment with OVA-Fc-pcDNA_{3.1} significantly prevented OVA-induced

eosinophilia in BAL fluid, as compared with treatment with saline, pcDNA_{3.1} or OVA-pcDNA_{3.1} ($P < 0.01$).

Effects of combination vaccination on cytokine production in BAL fluid

Ovalbumin induced a significant elevation of IL-4 (Fig. 5I) and IL-5 (Fig. 5II) levels in BAL fluid of animals treated with saline or pcDNA_{3.1} ($P < 0.01$ versus saline-challenged group), but not those with OVA-Fc-pcDNA_{3.1} ($P < 0.01$, versus groups treated with saline or pcDNA_{3.1}). Vaccination with DNA vaccine reduced the levels of IL-4 and IL-5 in BAL fluid

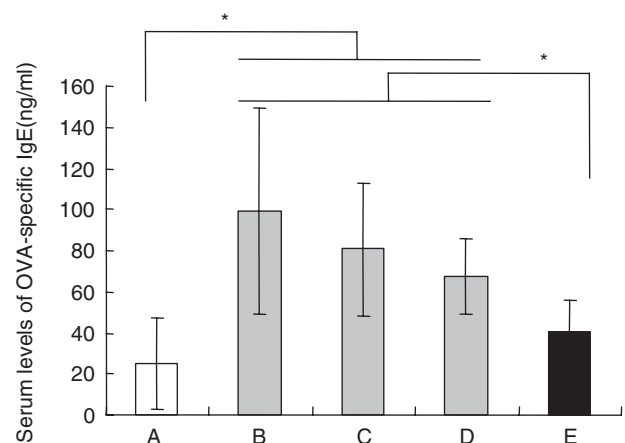


Fig. 3. Serum levels of OVA-specific IgE in mice sensitized, challenged and treated with saline (a), OVA-sensitized and challenged mice treated with saline (b), OVA-sensitized and challenged mice treated with pcDNA_{3.1} (c), OVA-sensitized and challenged mice treated with OVA-pcDNA_{3.1} (d), and OVA-sensitized and challenged mice treated with OVA-Fc-pcDNA_{3.1} (e). *Stands for P value less than 0.01 as compared with the correspondent groups.

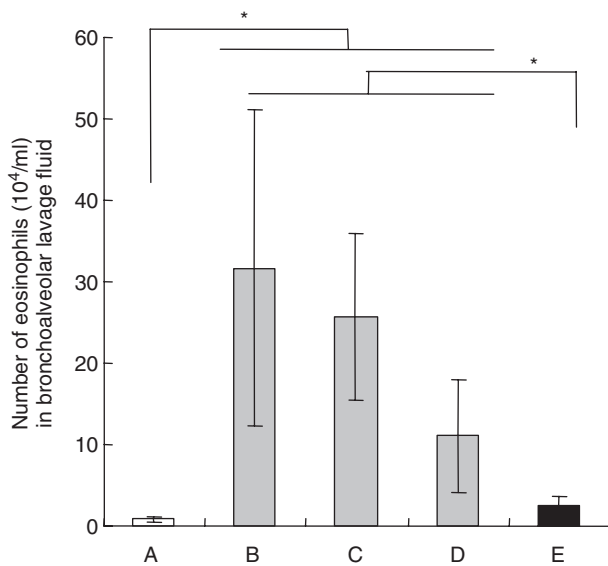


Fig. 4. Number of eosinophils in bronchoalveolar lavage fluid harvested from mice sensitized, challenged and pretreated with saline (A), OVA-sensitized and challenged mice treated with saline (B), OVA-sensitized and challenged mice pretreated with pcDNA_{3.1} (C), OVA-sensitized and challenged mice pretreated with OVA-pcDNA_{3.1} (D), and OVA-sensitized and challenged mice pretreated with OVA-Fc-pcDNA_{3.1} (E). *Stands for *P* value less than 0.01 as compared with the correspondent groups.

compared with the asthma group ($P < 0.01$). Treatment with OVA-Fc-pcDNA_{3.1} significantly prevented OVA-induced reduced level of INF- γ in BAL fluid, as did treatment with saline or pcDNA_{3.1} ($P < 0.01$, Fig. 5III).

Protection against allergic airway inflammation depends upon expression of CD_{11c}⁺CD₈₀⁺ and CD_{11c}⁺CD₈₆⁺ on pulmonary-derived DCs

We characterized expression of surface marker CD_{11c} on DCs isolated from lung and then investigated OVA-induced expression of co-stimulatory molecules CD₈₀ and CD₈₆ on lung DCs in mice (CD_{11c} gated). We found that OVA induced a significant expression of CD_{11c}⁺CD₈₀⁺ (Fig. 6a) and CD_{11c}⁺CD₈₆⁺ (Fig. 6b) on pulmonary DCs harvested from mice treated with saline or pcDNA_{3.1}, as compared with the saline-sensitized and challenged group ($P < 0.01$). Treatment with OVA-Fc-pcDNA_{3.1} significantly prevented OVA-induced over-expression of CD_{11c}⁺CD₈₀⁺ and CD_{11c}⁺CD₈₆⁺ on pulmonary DCs, as compared with that with saline, pcDNA_{3.1} or OVA-pcDNA_{3.1} group ($P < 0.01$, Fig. 6) respectively.

Protection against allergic airway inflammation depends upon expression of CD_{11c}⁺CD₈₀⁺ on spleen-derived DCs

As shown in Fig. 7, we found that OVA induced a significant expression of CD_{11c}⁺CD₈₀⁺ (Fig. 7a) on spleen DCs harvested

from mice treated with saline or pcDNA_{3.1}, as compared with the saline-sensitized and challenged group. Treatment with OVA-Fc-pcDNA_{3.1} significantly prevented OVA-induced over-expression of CD_{11c}⁺CD₈₀⁺ on spleen DCs, as compared with that with saline, pcDNA_{3.1} or OVA-pcDNA_{3.1} group ($P < 0.01$, Fig. 7). No significant difference was observed in the expression of CD_{11c}⁺CD₈₆⁺ on spleen DCs, as compared with the saline, pcDNA_{3.1} or OVA-pcDNA_{3.1} group (Fig. 7b).

Discussion

The current study demonstrated the potential that allergen-DNA-targeted DCs can be an alternative therapy for allergic

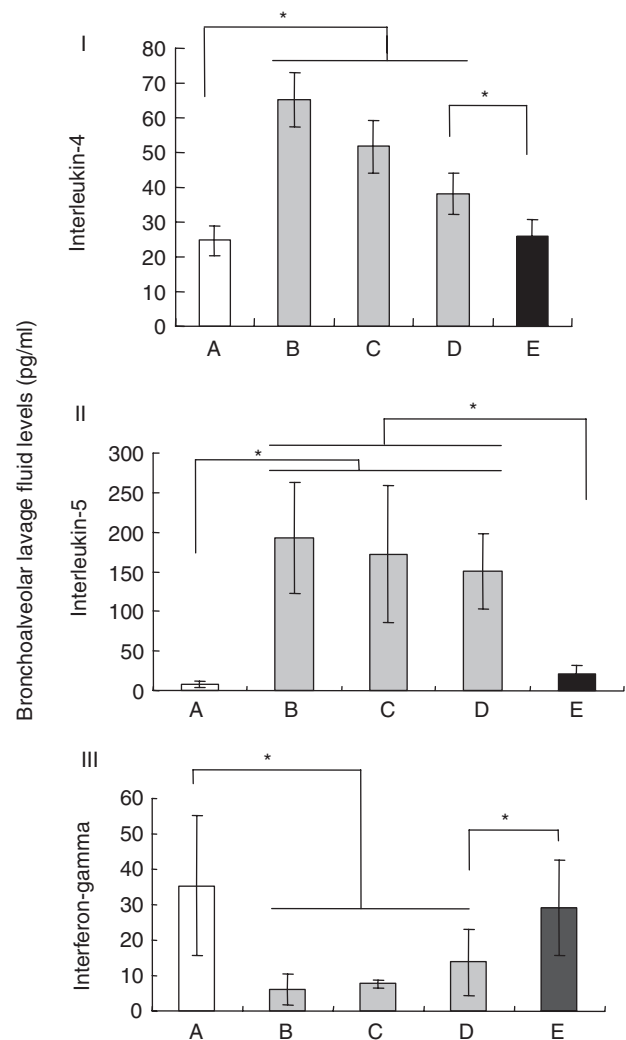


Fig. 5. Bronchoalveolar lavage fluid levels of interleukin-4 (I), interleukin-5 (II) and interferon-gamma (III) in mice sensitized, challenged and pretreated with saline (A), OVA-sensitized and challenged mice pretreated with saline (B), OVA-sensitized and challenged mice pretreated with pcDNA_{3.1} (C), OVA-sensitized and challenged mice pretreated with OVA-pcDNA_{3.1} (D), and OVA-sensitized and challenged mice pretreated with OVA-Fc-pcDNA_{3.1} (E). *Stands for *P* value less than 0.01 as compared with the correspondent groups.

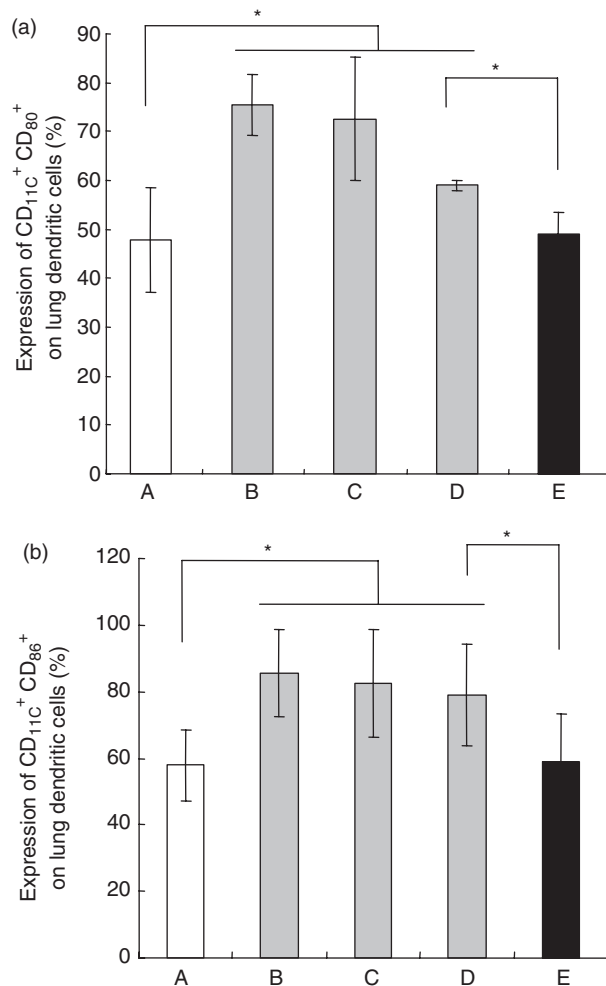


Fig. 6. Expression of CD11c⁺CD80⁺ (a) and CD11c⁺CD86⁺ (b) on lung DCs in mice sensitized, challenged and pretreated with saline (A), OVA-sensitized and challenged mice pretreated with saline (B), OVA-sensitized and challenged mice pretreated with pcDNA_{3.1} (C), OVA-sensitized and challenged mice pretreated with OVA-pcDNA_{3.1} (D), and OVA-sensitized and challenged mice pretreated with OVA-Fc-pcDNA_{3.1} (E). *Stands for *P* value less than 0.01 as compared with the correspondent groups.

asthma, associated with a reduction of CD11c⁺CD80⁺ and CD11c⁺CD86⁺ expression. Fc receptor is expressed on DCs as one of the key players of initiating and maintaining immune responses and as a therapeutic target for the disease. To improve the efficacy of SIT, we constructed a DNA vaccination plasmid containing cDNA for a prototypic allergen, fused to the cDNA of a potent immune modulating Fc (OVA-Fc-pcDNA_{3.1}). This may influence the production of Th1 cytokine synthesis in naïve and memory T cells. Pretreatment with a combination of vaccines (OVA-Fc-pcDNA_{3.1}) could efficiently reduce allergic airway inflammation, significantly prevent OVA-increased levels of IL-4, IL-5 and the percentage of eosinophils and OVA-decreased level of IFN- γ , and have more therapeutic effects than

mono-therapy (OVA-pcDNA_{3.1}). It could become one of the optimal candidates as a strategy for SIT [7,8].

Vaccination with allergen-encoding DNA may induce an allergen-specific Th1-cell response and prevent allergic sensitization, when administered prophylactically in mouse models [9]. However, systemic production of allergen by transfected cells has the potential to cause anaphylaxis. DCs are uniquely situated in the immune cascade to initiate and modulate airway immune responses [10–12]. In addition, immature DCs express a number of specific chemokine receptors, FcR, and Toll-like receptors, through which the DCs are developing [13–15]. DCs can be activated or inhibited through FcR by antibodies or immune complex formed by antibodies depending on the variations of FcR engaged.

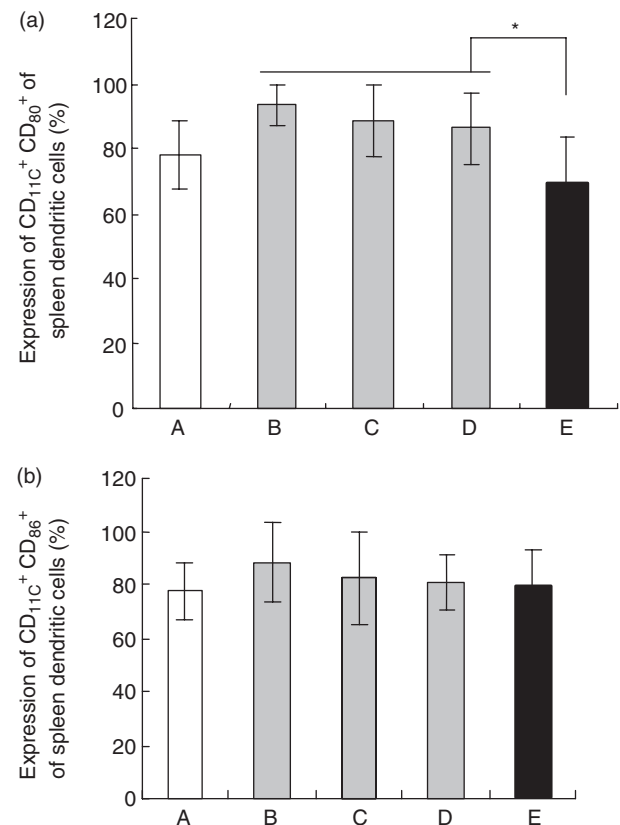


Fig. 7. Expression of CD11c⁺CD80⁺ (a) and CD11c⁺CD86⁺ on spleen DCs (b) in mice. Saline-vaccinated mice sensitized and challenged with saline (A), saline-vaccinated mice sensitized and challenged with OVA (B), pcDNA_{3.1}-vaccinated mice sensitized and challenged with OVA (C), OVA-pcDNA_{3.1}-vaccinated mice sensitized and challenged with OVA (D), and OVA-Fc-pcDNA_{3.1}-vaccinated mice sensitized and challenged with OVA (E). Immunization with OVA-Fc-pcDNA_{3.1} significantly prevented OVA-induced over-expression of CD11c⁺CD80⁺ and CD11c⁺CD86⁺ on spleen DCs, as compared with the saline, pcDNA_{3.1} or OVA-pcDNA_{3.1} groups. (a) Expression of CD11c⁺CD80⁺ on spleen DCs analysed by FACS. **P* < 0.01, *t*-test comparing E versus D. (b) Expression of CD11c⁺CD86⁺ on spleen DCs analysed by FACS. Data are shown as mean $\pm$ SEM (*n* = 8), **P* < 0.05 (OVA-Fc-pcDNA_{3.1} group versus OVA-pcDNA_{3.1} group).

Activating and inhibitory IgG Fc receptors on DCs mediate opposing functions [16,17]. DNA vaccination may directly target DCs involved in allergen-specific T_H1-cell responses, showing preventive effects when delivered at low doses in mice [18,19]. Vaccination with DNA vaccine inhibited the development of airway inflammation [20–22]. Our data demonstrated that DNA vaccine coding OVA allergen directly to DCs was also effective in treating allergen-induced allergic airway inflammation.

Co-stimulatory molecules in pulmonary DCs are known to function as ligands on antigen-presenting cells, enhancing T cell activation, T cell proliferation and cytokine. The present study found an increased expression of CD_{11c}⁺CD₈₀ and CD_{11c}⁺CD₈₆ on pulmonary DCs in the OVA-sensitized and challenged group, similar to the previous findings [23], suggesting that CD₈₀ and CD₈₆ molecules can function as a conduit for extracellular signals to DCs modifying DC functions. These data may be helpful in understanding the requirements for effective cross-presentation and DC maturation in the development of efficacious targeting strategies, though the mechanisms by which allergen induced CD_{11c}⁺CD₈₀ and CD_{11c}⁺CD₈₆ and regulated asthma phenotypes remain unclear. Allergen-DNA modified DCs could inhibit Th2 CD₄⁺ responses and enhance Th1 CD₄⁺ responses *in vitro* [24,25]. DCs activated by cross-linking B7-DC could prevent airway inflammation following SIT [26,27]. In the current study, we demonstrated that DCs can be targeted with allergen-DNA conjugates efficiently with Fc (OVA-Fc-pcDNA_{3.1}). Immune complexes of IgG and antigen can be internalized via FcRs on DC, resulting in DC maturation and priming of antigen-specific CD8⁺ T cells [28,29]. However, DCs express both activating and inhibitory FcRs, and uptake of immune complexes results in naive T-cell activation and protective immunity [30].

The OVA-Fc-pcDNA_{3.1} influenced surface marker expression of targeted DCs, probably down-regulating the general capability of DCs to present antigen [31–33]. OVA-Fc-pcDNA_{3.1}-targeted pulmonary DCs express lower levels of the co-stimulatory molecules CD_{11c}⁺CD₈₀⁺ and CD_{11c}⁺CD₈₆⁺. Our results suggest that DCs both in the pulmonary cells and spleen of allergic mice, via upregulation of CD_{11c}⁺CD₈₀⁺ might play a pivotal role in the maintenance and amplification of the allergic immune response, namely Th2 immune response, whereas no difference was observed in CD_{11c}⁺CD₈₆⁺ expression in the spleen. We hypothesized that spleen-derived DCs had undergone a long time of migration after they captured inhaled antigen in the lung. CD_{11c}⁺CD₈₆⁺ in spleen-derived DCs may play a role in maintaining immune memory. Therefore, pulmonary DCs have a partially mature phenotype and express a range of co-stimulatory molecules that are intermediate between immature and mature DCs, resulting in tolerogenic interaction with T cells in allergen-SIT [34,35]. Costimulatory molecules as potential targets significantly contribute to the therapeutic intervention in allergic airway disease [36–38]. Targeting of antigens to DC

surface receptors elicits effective immune responses [39,40]. Combination vaccination has better therapeutic effects on antigen-associated inflammation [41,42].

In conclusion, treatment with OVA-Fc-pcDNA_{3.1} could prevent OVA-induced over-formation of allergen-specific IgE, local hyper-production of IL-4 and IL-5 and hypo-production of INF- γ , over-expression of costimulatory molecules CD_{11c}⁺CD₈₀⁺ and CD_{11c}⁺CD₈₆⁺ in the lung, and airway inflammation. Thus, DNA vaccine encoding OVA directly to DCs may be a new alternative therapy for patients with allergic asthma.

Acknowledgements

The authors would like to thank Bo Guo for cloning OVA and Fc into pcDNA_{3.1} (+) vector, Kui Wu for excellent advice on flow cytometry, and Yunxi Song for helping with collecting samples. The authors would also like to thank Changzheng Wang for evaluating the question on data. This work was partly supported by grants from the National Natural Scientific Foundation of China (NSFC 30200120) and by China Postdoctoral Science Foundation (NO 20070420596) and by Shanghai Leading Academic Discipline Project (Project Number: B115).

References

- Myers TR. Guidelines for asthma management: a review and comparison of 5 current guidelines. *Respir Care* 2008; **53**:751–69.
- Kuehn BM. New asthma guidelines released. *JAMA* 2007; **298**:1503–4.
- Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. *Nat Rev Immunol* 2006; **6**:761–71.
- Maecker HT, Hansen G, Walter DM, Dekruyff RH, Levy S, Umetsu DT. Vaccination with allergen-IL-18 fusion DNA protects against, and reverses established, airway hyperreactivity in a murine asthma model. *J Immunol* 2001; **166**:959–65.
- Underwood S, Foster M, Raeburn D, Bottoms S, Karlsson JA. Time-course of antigen-induced airway inflammation in the guinea-pig and its relationship to airway hyperresponsiveness. *Eur Respir J* 1995; **8**:2104–13.
- Cheng X, Wang C, Qian G, Zhu B. CD80, but not CD86 were up-regulated on the spleen-derived dendritic cells from OVA-sensitized and challenged BALB/c mice. *Immunol Lett* 2003; **89**:31–8.
- Taube C, Buhl R. Immunomodulation therapy for allergic asthma. What is already possible, what is to come? *Dtsch Med Wochenschr* 2008; **133**:727–32.
- Holgate ST, Polosa R. Treatment strategies for allergy and asthma. *Nat Rev Immunol* 2008; **8**:218–30.
- Klostermann B, Bellinghausen I, Böttcher I *et al.* Modification of the human allergic immune response by allergen-DNA-transfected dendritic cells *in vitro*. *J Allergy Clin Immunol* 2004; **113**:327–33.
- Hammad H, Lambrecht BN. Recent progress in the biology of airway dendritic cells and implications for understanding the regulation of asthmatic inflammation. *J Allergy Clin Immunol* 2006; **118**:331–36.

- 11 Cramer R. Allergy vaccines: dreams and reality. *Expert Rev Vaccines* 2007; **6**:991–9.
- 12 Hammad H, Lambrecht BN. Recent progress in the biology of airway dendritic cells and implications for understanding the regulation of asthmatic inflammation. *J Allergy Clin Immunol* 2006; **118**:331–36.
- 13 Coombes JL, Powrie F. Dendritic cells in intestinal immune regulation. *Nat Rev Immunol* 2008; **8**:435–46.
- 14 Boruchov AM, Heller G, Veri MC, Bonvini E, Ravetch JV, Young JW. Activating and inhibitory IgG Fc receptors on human DCs mediate opposing functions. *J Clin Invest* 2005; **115**:2914–23.
- 15 Bousquet J, Bieber T, Fokkens W *et al.* Important questions in allergy: novel research areas. *Allergy* 2008; **63**:143–7.
- 16 Belostotsky R, Lorberboum-Galski H. Utilizing Fcεpsilon-Bak chimeric protein for studying IgE-FcεpsilonRI interactions. *Clin Immunol* 2004; **110**:89–99.
- 17 Bruhns P, Frémont S, Daéron M. Regulation of allergy by Fc receptors. *Curr Opin Immunol* 2005; **17**:662–69.
- 18 Schmidt-Weber CB, Blaser K. New insights into the mechanisms of allergen-specific immunotherapy. *Curr Opin Allergy Clin Immunol* 2005; **5**:525–30.
- 19 Hammad H, Lambrecht BN. Dendritic cells and epithelial cells: linking innate and adaptive immunity in asthma. *Nat Rev Immunol* 2008; **8**:193–204.
- 20 Sawyer RT, Gergen PJ, Minnicozzi M *et al.* The future of immunotherapy. Report of a National Institute of Allergy and Infectious Diseases (NIAID), Division of Allergy, Immunology and Transplantation (DAIT) workshop. *Allergy* 2006; **61**:1159–61.
- 21 Li R, Yang X, Wang L, Liu E. Respiratory syncytial virus infection reversed anti-asthma effect of neonatal Bacillus Calmette-Guerin vaccination in BALB/c mice. *Pediatr Res* 2006; **59**:210–15.
- 22 Semic-Jusufagic A, Simpson A, Custovic A. Environmental exposures, genetic predisposition and allergic diseases: one size never fits all. *Allergy* 2006; **61**:397–99.
- 23 Köhl J, Baelder R, Lewkowich IP *et al.* A regulatory role for the C5a anaphylatoxin in type 2 immunity in asthma. *J Clin Invest* 2006; **116**:783–96.
- 24 Bellinghausen I, Knop J, Saloga J. Mechanisms of specific immunotherapy. *Hautarzt* 2006; **57**:855–9.
- 25 Ettmayer P, Mayer P, Kalthoff F *et al.* A novel low molecular weight inhibitor of dendritic cells and B cells blocks allergic inflammation. *Am J Respir Crit Care Med* 2006; **173**:599–606.
- 26 Van Rijt LS, Lambrecht BN. Dendritic cells in asthma: a function beyond sensitization. *Clin Exp Allergy* 2005; **35**:1125–34.
- 27 Radhakrishnan S, Iijima K, Kobayashi T, Kita H, Pease LR. Dendritic cells activated by cross-linking B7-DC (PD-L2) block inflammatory airway disease. *J Allergy Clin Immunol* 2005; **116**:668–74.
- 28 Nimmerjahn F, Ravetch JV. Fc gamma receptors as regulators of immune responses. *Nat Rev Immunol* 2008; **8**:34–47.
- 29 de Haar C, Kool M, Hassing I, Bol M, Lambrecht BN, Pieters R. Lung dendritic cells are stimulated by ultrafine particles and play a key role in particle adjuvant activity. *J Allergy Clin Immunol* 2008; **121**:1246–54.
- 30 Masuda A, Yoshida M, Shiomi H *et al.* Fcγ receptor regulation of *Citrobacter rodentium* infection. *Infect Immun* 2008; **76**:1728–37.
- 31 Fiset PO, Tulic MK, Skrablin PS *et al.* Signal transducer and activator of transcription 6 down-regulates toll-like receptor-4 expression of a monocytic cell line. *Clin Exp Allergy* 2006; **36**:158–65.
- 32 Woszczek G, Chen LY, Nagineni S, Shelhamer JH. IL-10 inhibits cysteinyl leukotriene-induced activation of human monocytes and monocyte-derived dendritic cells. *J Immunol* 2008; **180**:7597–603.
- 33 Kercsmar CM. Meeting the challenges of asthma: conference summary. *Respir Care* 2008; **53**:787–95.
- 34 Kallinich T, Beier KC, Gelfand EW, Kroczeck RA, Hamelmann E. Co-stimulatory molecules as potential targets for therapeutic intervention in allergic airway disease. *Clin Exp Allergy* 2005; **35**:1521–34.
- 35 van Rijt LS, Vos N, Willart M *et al.* Essential role of dendritic cell CD80/CD86 costimulation in the induction, but not reactivation, of TH2 effector responses in a mouse model of asthma. *J Allergy Clin Immunol* 2004; **114**:166–73.
- 36 Grayson MH. Lung dendritic cells and the inflammatory response. *Ann Allergy Asthma Immunol* 2006; **96**:643–51; quiz 652–3, 678.
- 37 Bharadwaj AS, Bewtra AK, Agrawal DK. Dendritic cells in allergic airway inflammation. *Can J Physiol Pharmacol* 2007; **85**:686–99.
- 38 Hammad H, Lambrecht BN. Lung dendritic cell migration. *Adv Immunol* 2007; **93**:265–78.
- 39 Wu K, Bi Y, Sun K, Xia J, Wang Y, Wang C. Suppression of allergic inflammation by allergen-DNA-modified dendritic cells depends on the induction of Foxp3+ Regulatory T cells. *Scand J Immunol* 2008; **67**:140–51.
- 40 Zhu B, Zou L, Cheng X *et al.* Administration of MIP-3α gene to the tumor following radiation therapy boosts anti-tumor immunity in a murine model of lung carcinoma. *Immunol Lett* 2006; **103**:101–7.
- 41 Reschner A, Hubert P, Delvenne P, Boniver J, Jacobs N. Innate lymphocyte and dendritic cell cross-talk: a key factor in the regulation of the immune response. *Clin Exp Immunol* 2008; **152**:219–26.
- 42 Bonehill A, Tuyaerts S, Van Nuffel AM *et al.* Enhancing the T-cell stimulatory capacity of human dendritic cells by co-electroporation with CD40L, CD70 and constitutively active TLR4 encoding mRNA. *Mol Ther* 2008; **16**:1170–80.