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Original Article

Evaluation on the immunotherapy efficacies of synthetic peptide vaccines in asthmatic mice with group I and II allergens from Dermatophagoides pteronyssinus

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Abstract: To assess the immunotherapy efficacies of recombinant vaccines containing T-cell epitopes derived from group I and allergens from Dermatophagoides pteronyssinus (Der p1, Der p2). Forty female BALB/c mice were randomized into groups of negative control (PBS group), positive controls (Asthma group), immunotherapy with rDer p1 and rDer p2 protein suspension (rDer p1/rDer p2 group) and specific immunotherapy with fusion peptide T_{1-8} (T_{1-8} group). Asthmatic mouse models were initially established with the crude extract from house dust mites (HDM), and PBS models were solely treated with PBS buffer. The two treatment groups were managed with corresponding protein via subcutaneous injection at the back 30 minutes before inhalation sensitization from day 25 to 27. Twenty-four hour following the final inhalation challenge, sera, bronchoalveolar lavage fluid (BALF) and the supernatant of splenocyte cultures (SSCC) were collected in each group of mice. ELISA was used to assay the levels of IFN-γ, IL-4, IL-10 and IL-17 in the BALF and SSCC, as well as serum levels of specific IgE, IgG_1 and IgG_{2a}. The lung tissue sections were stained with haematoxylin and eosin (H&E) for pathological examination. ELISA detection revealed reduced levels of IL-4 and IL-17 in the BALF and SSCC, yet increased levels of IFN-γ and IL-10, and decreased specific serum IgE and IgG_1, yet increased serum IgG_{2a} in T_{1-8} group and rDer p1/rDer p2 group than asthma group ($P<0.05$). T_{1-8} group had lower IL-4 and IL-17 level and higher IFN-γ and IL-10 level in the BALF and SSCC as well as reduced specific serum IgE and IgG_1, yet elevated IgG_{2a} level compared to rDer p1/rDer p2 group ($P<0.05$). Examination on the lung sections indicated significantly abated pulmonary inflammation, less inflammatory cell infiltration and better remodeled airway epithelia in T_{1-8} group and rDer p1/rDer p2 group than asthma group. However, the airway epithelium structure T_{1-8} group and rDer p1/rDer p2 group remained similar to that of PBS group. In Conclusion, The recombinant protein T_{1-8}, has effectively alleviated the allergic inflammation of airways and lungs in the experimental mice, suggesting that this synthetic peptide may be used as candidate vaccines for asthma on allergen-specific immunotherapy basis.

Keywords: Dermatophagoides pteronyssinus, Der p1, Der p2, T cell epitope, specific immunotherapy

Introduction

Allergen-specific immunotherapy (SIT), also known as desensitization therapy, is currently recognized as the only causal approaches to treatment of allergy, for it can impede progression of the condition and has long-lasting therapeutic outcomes [1, 2]. The underlying treatment target can be achieved by immunize the patients with gradual dosing of the allergen extract via subcutaneous injection or sublingual use to cause a patient's antigen-specific tolerance based on his/her component-resolved diagnosis [3], and its clinical efficacy has been confirmed in the treatment of allergic asthma and allergic rhinitis [4, 5]. SIT can not only reduce the allergen sensitization, but also the relapse of asthma [6, 7].

Previous studies described positive reaction of the serum with Der p1 and Der p2 in 95% of the patients allergic to dust mites [8, 9]. This implies that Der p1 and Der p2 should be the important component as the vaccine for allergic diseases associated with house dust mites. Some clinical studies further demonstrated that SIT by using recombinant allergen derivatives with hypoallergenicity had greatly reduced the side

effects in treatment [10]. Current reports on the hypoallergenic Der p1/Der p2 combination vaccines in *in vitro* tests indicated that the recombinant Der p1/Der p2 protein notably produced lower allergenic activity than crude Der p1 or Der p2 [11]. Toshio Sone *et al* constructed and prokaryotically expressed the Cry j1 and Cry j2 protein containing T-cell epitopes from Japanese Cedar pollen, and found that the T-cell epitope fusion peptide had excellent immunogenicity and lower antigenicity through T-cell proliferation experiments and tests of the allergen-specific binding with IgE antibody [12]. However, few reports are available on the T-cell fusion peptide vaccine derived from the house dust mite allergen Der p1 and Der p2.

The current study was undertaken to recombine the proteins of T_{1-8} containing T-cell epitopes based on the major house dust mite allergen Der p1 and Der p2. Initially, the Der p1 (containing 222 AAs) encoding the 4 T-cell epitopes(amino acid sequences being 21-49, 78-100, 110-131 and 197-212 [13]) and Der p2 (containing 129 AAs) encoding another 4 T-cell epitopes (amino acid sequences being 11-35, 36-50, 87-104 and 105-129 [14]) were designed in accordance with the sequence of T_1-T_2-T_3-T_4-T_5-T_6-T_7-T_8. Then the amino acid sequence between epitopes was dimensionally preserved by insertion with GPGPG. Recombined Der p1/Der p2 protein containing T-cell epitopes was subjected to prokaryotic expression, and tentatively used for specific immunotherapy in asthmatic mouse models for assessing the therapeutic effects.

Materials and methods

Experimental animals

Forty female BALB/c mice, SPF grade, aged 6-8 weeks and weighed (20±2) g, were purchased from the Centre for Comparative Medicine, Yangzhou University (License: SCXK SU 2007-0001).

Experimental materials

Recombinant allergens rDer p1 and rDer p2 from the house dust mites' group I and group II as well as the frozen dry powder of the house dust mites were preserved in our laboratory.

Main reagents

RMPI.1640 culture solution, Australia fetal bovine serum and trypan blue were purchased from Sangon Biotech (Shanghai, China) Mouse IgE ELISA Kit, Mouse IgG_1 ELISA Kit, Mouse IgG_{2a} ELISA Kit, Mouse IFN-gamma ELISA Kit, ELISA Kit, Mouse IL-4 IL-10 ELISA Kit and Mouse IL-17 ELISA Kit were products of sed from R&D company (Switzerland) R&D Systems, Ampicillin and streptomycin were purchased from Sigma company. Analytic reagents were domestic products.

Experimental methods

Preparation of crude extract from Dermatophagoides pteronyssinus: Dermatopha-goid-es pteronyssinus was isolated, identified and fed in our laboratory. After repeated washing, the mites were stored at -20°C. Two grams of the frozen dry powder of Dermatophagoides pteronyssinus was taken, and subjected to repeated inactivation with acetone and de-fatting 3 to 4 times till purification. Then the samples were dried at 37°C by natural ventilation. 40 ml Coca's solution (containing NaCl 5 g, sodium bicarbonate 2.75 g and carbolic acid 4 g that was made constant volume to 1 L by adding double-distilled water) was added at ratio of 1:20 (W/V). Then the samples were subjected to ultrasonication (200 V) for 5 min, and transferred into a magnetic stirrer for extracting for 48 h at 4°C by 50 r/min. The extract solution was centrifuged at 2 500× g for 30 min, and the supernatant was obtained. A 0.22 μm syringe filter was used to sterilize the samples. Bradford's test was performed to determine the protein concentration. After that, the samples were stored at -80°C for following use.

Animal grouping

Forty female BALB/c mice were equally randomized into four groups, namely PBS group (negative control), Asthma group (positive control), immunotherapy with rDer p1 and rDer p2 protein (Der p1/Der p2 group, treated with the protein suspension by ratio 1:1) and immunotherapy with T_{1-8} fusion peptide (T_{1-8} group).

Establishment of asthmatic mouse models

Each mouse in PBS group was intraperitoneally injected with 100 μl PBS suspension (containing 2% Al $(OH)_3$, pH 7.4) at day 0, 7 and 14, respectively. The remaining three groups were individually sensitized with the extract solution of Dermatophagoides pteronyssinus (dissolved

in 100 μl containing 2% Al $(OH)_3$ PBS suspension liquid, pH 7.4) via intraperitoneal injection in dose of 10 μg at day 0, 7 and 14, respectively. From day 21, the mouse in each group was successively placed in a box to undergo aerosol inhalation. PBS group were challenged only with PBS suspension (containing 2% Al $(OH)_3$), and the three treatment groups were sensitized with the mite crude extract in dose of 10 μg/mL dissolved in PBS suspension (containing 2% Al $(OH)_3$) for 30 min, once a day, for consecutive 7 days, in which the asthma attack was observed and recorded.

Specific immunotherapy for asthmatic mice

On day 25, 26 and 27 rDer p1/rDer p2 group were given immunotherapy with 5 μg rDer p1/rDer p2 dissolved in 100 μl PBS suspension via multiple subcutaneous injection at the back, 30 min prior to the inhalation challenge, whereas $T_{1\text{-}8}$ group were immunized with 10 μg $T_{1\text{-}8}$ fusion peptide dissolved in 100 μl PBS suspension with the same protocol. PBS group exclusively received 100 μl PBS suspension for negative control. Injection was performed once a day for consecutive 3 days. Twenty-four after the final inhalation challenge, mice in each group were treated as follows.

Specimen sampling and index measurement

Collection and detection of the bronchoalveolar lavage fluid (BALF): Twenty-four hour after the final challenge, five mice were randomly selected and anesthetized by 10% chloral hydrate via intraperitoneal injection. Then the airways were exposed for easy endotracheal cannula. BALF was collected through application of the pre-chilled sterile PBS, a total of 1 ml of it that was applied in fractionated dose of 0.3 ml, 0.3 ml and 0.4 ml to irrigate the trachea. Then the fluid recovered was centrifuged at 4000× g at 4°C for 5 min. The supernatant was harvested and stored at -20°C for following use. ELISA was performed as the user instructions to detect the IL-4, IL-10, IL-17 and IFN-γ cytokine levels in the BALF. The absorbance was measured at 450 nm (A450 value) in a microplate reader.

Determination of the serum specific antibody IgE, IgG_1 and IgG_{2a} content

The blood samples were obtained via the orbital cavity from the remaining five mice in each group, and were allowed to stand still at 4°C till serum precipitation. Then the serum was centrifuged at 4000× g at 4°C for 5 min, and collected and stored at -20°C for following use. ELISA was used to detect levels of specific antibody IgE, IgG_1 and IgG_{2a} in the serum.

Culturing of the splenocytes

After completing the blood sampling, the mice in each group were soaked for 3 to 5 min in 75% ethanol. The spleens were isolated in sterile condition, and then cut into pieces and filtered by using a 300-steel mesh. Simple splenocyte suspension was prepared, and the cells were lysed with EDTA-NH_4Cl, an erythrocyte lysate, and homogenated. Then the suspension was allowed to stand still for 10 min and centrifuged by 4 000 r/min for 10 min to discard the supernatant. Re-suspension was performed by applying the D-hank's solution to the precipitated cells that were washed twice. Then the splenocytes were re-suspended by adding perfect RPMI-1640 medium (containing 10% fetal bovine serum, 100 μg/ml streptomycin and 100 U/ml penicillin). The cell density in the medium was adjusted to 5×10^6 /ml, and 1 ml splenocyte suspension was taken and injected into a sterile 24-well plate. The final concentration in each culture well was adjusted to t 25 μg/mL by adding crude extract from the dust mites. The culture plate was maintained in the thermostatic CO_2 incubator (5% CO_2) at 37°C for 72 h. The supernatant was obtained and detected with ELISA for the levels of IL-4, IL-10, IL-17 and IFN-γ cytokines. The absorbance of the wells in the plates was measured using microplate reader at 450 nm.

Staining of the lung tissue sections

Lung tissues were isolated from each mouse in all groups, and fixed in 10% paraformaldehyde overnight at 4°C. The tissues were then embedded in paraffin, and sectioned in conventional technique. After HE staining, the sections were examined under the light microscope for the infiltration of the lymphocytes and eosinophils (EOS) as well as incidence of the edema and bronchial epithelial damage.

Results

Measurement of the concentration of the mite crude extract

The protein concentration was 271 μg/mL for the crude mite extract that was measured with modified Bradford's method.

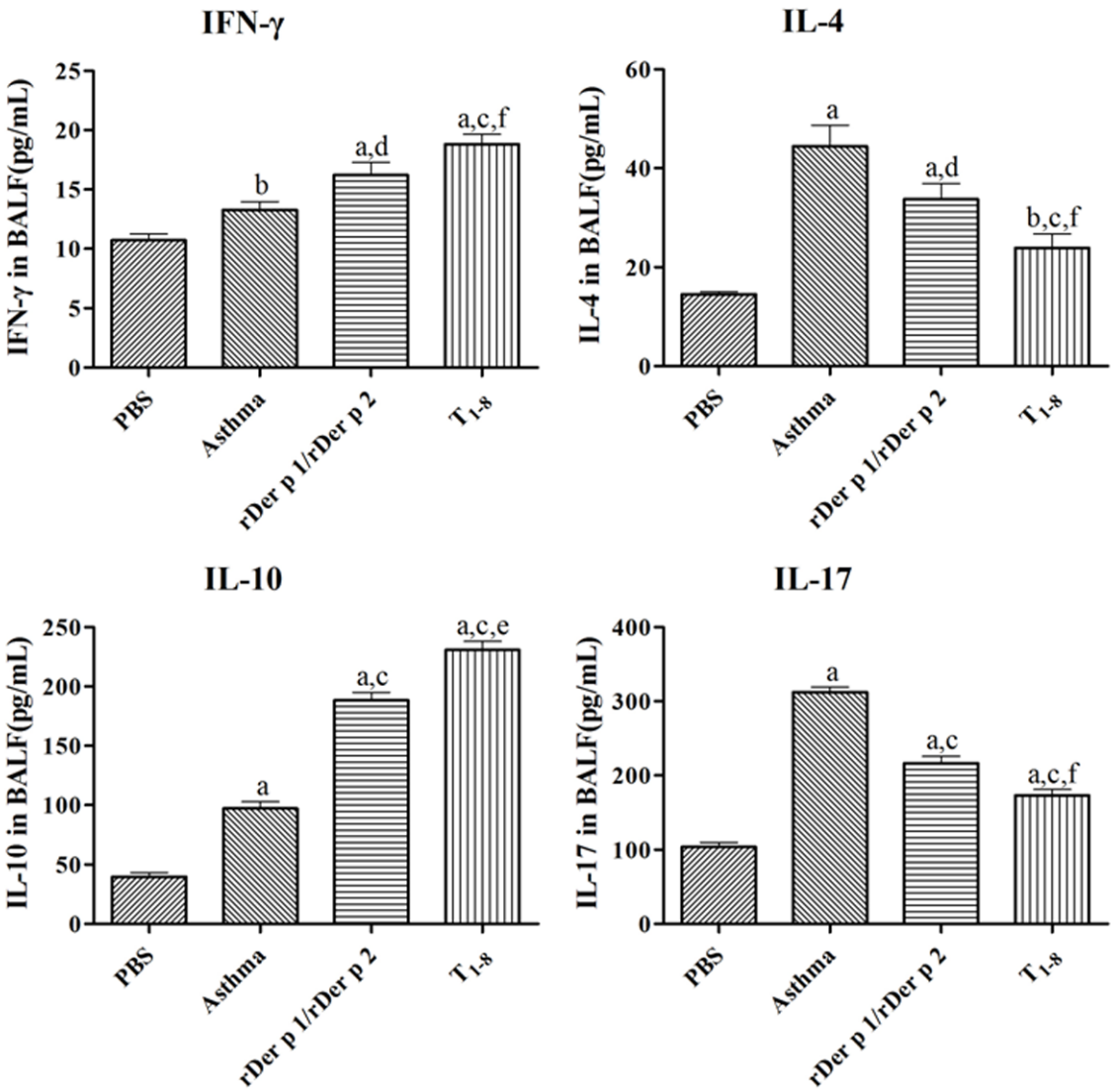

Figure 1. The level of IL-4, IL-10, IL-17 and IFN-γ in BALF from each group of mice. Note: *Vs* PBS group, [a]*P*<0.01, [b]P<0.05; *Vs.* Asthma group, [c]*P*<0.01, [d]*P*<0.05; *Vs.* rDer p1/rDer p2 group, [e]*P*<0.01, [f]*P*<0.05.

Changes of the symptoms and signs in each group of mice

The signs, including restlessness, quiet wheezing and shortness of breath to a certain degree, occurred on day 21 in the mice of asthma group and two groups of immunotherapy during aerosol inhalation. In the following excitation, mice in the three groups developed different degrees of wheezing, deepened and fast breath, nod-like breathing and immediate asthmatic reaction, and attack appeared to advance than usual. However, the symptoms looked somewhat relieved in the two groups of mice after specific immunotherapy. Although the asthmatic signs disappeared following withdrawal of the inhalation challenge, wheezing persisted for several minutes before complete recovery. The similar signs were free in The PBS negative controls by the end of inhalation sensitization. Typical signs and symptoms of asthma attack were faded gradually in the treatment group after specific immunotherapy.

Measured levels of IL-4, IL-10, IL-17 and IFN-γ in the BALF

ELISA detection (see **Figure 1** and **Table 1**) indicated that the IFN-γ level in BALF from each group of mice was somewhat increased (*P*<0.01 or *P*<0.05). However, asthma group had higher IL-4 level than PBS group, and decreased or

Table 1. Changes of IL-4, IL-10, IL-17 and IFN-γ in the BALF ($\bar{x}$ ± s, pg/mL, *n*=5)

Group	IL-4	IL-10	IL-17	IFN-γ
PBS group	14.54±1.05	39.68±7.78	103.98±13.40	10.71±1.23
Asthma group	44.43±9.64[a]	97.12±12.71[a]	312.14±15.56[a]	13.28±1.52[b]
rDer p1/rDer p2 group	33.70±7.12[a,d]	188.44±14.79[a,c]	216.42±20.79[a,c]	16.22±2.38[a,d]
T_{1-8} group	23.89±6.38[b,c,f]	230.73±16.20[a,c,e]	173.26±17.69[a,c,f]	18.82±1.88[a,c,f]

Note: *Vs* PBS group, [a]*P*<0.01, [b]*P*<0.05; *Vs* Asthma group, [c]*P*<0.01, [d]*P*<0.05; *Vs* rDer p1/rDer p2 group, [e]*P*<0.01, [f]*P*<0.05.

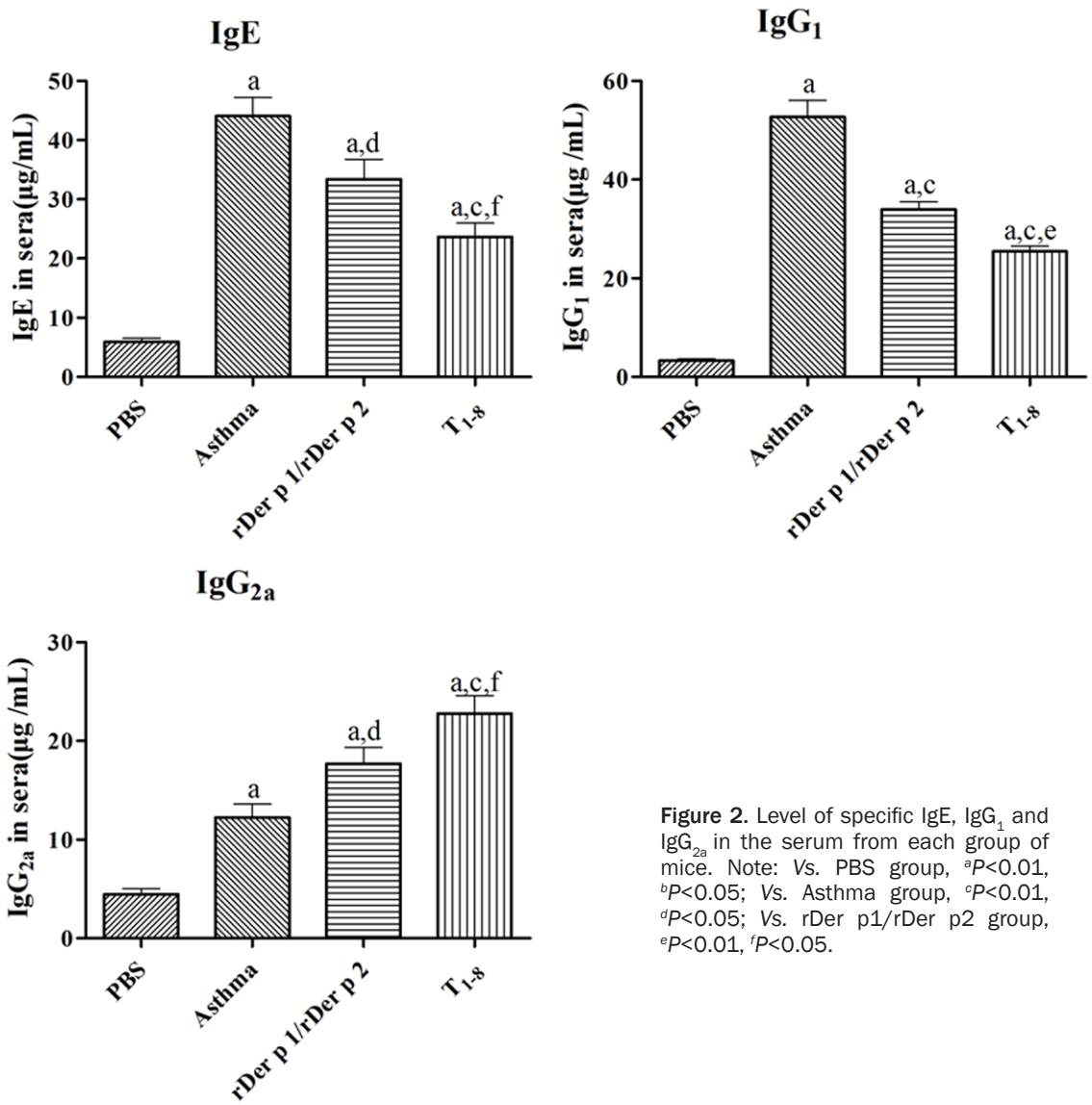

Figure 2. Level of specific IgE, IgG_1 and IgG_{2a} in the serum from each group of mice. Note: *Vs.* PBS group, [a]*P*<0.01, [b]*P*<0.05; *Vs.* Asthma group, [c]*P*<0.01, [d]*P*<0.05; *Vs.* rDer p1/rDer p2 group, [e]*P*<0.01, [f]*P*<0.05.

lower IL-4 level was seen in rDer p1/rDer p2 group and T_{1-8} group (*P*<0.01 or *P*<0.05). The level change of IL-10 remained similar to IFN-γ in each group, and the difference was significant (*P*<0.01). Asthma group had significantly higher level of IL-17 than that in PBS group as well as that in rDer p1/rDer p2 group and T_{1-8} group, and the IL-17 level was lower in T_{1-8} group than rDer p1/rDer p2 group (*P*<0.01 or *P*<0.05).

Measured content of serum specific IgE, IgG_1 and IgG_{2a}

ELISA determination of the serum cytokines (**Figure 2** and **Table 2**) demonstrated results as

Table 2. Changes of IgE, IgG_1 and IgG_{2a} in the blood serum in the four group of mice ($\bar{x} \pm s$, μg /mL, n=5)

Group	Amo-unt	IgE	IgG_1	IgG_{2a}
PBS group	5	5.88±1.43	3.29±0.90	4.45±1.34
Asthma group	5	44.06±7.01[a]	52.69±7.60[a]	12.23±3.04[a]
rDer p1/rDer p2 group	5	33.39±7.53[a,d]	33.92±3.46[a,c]	17.69±3.72[a,d]
T_{1-8} group	5	23.66±5.19[a,c,f]	25.41±2.45[a,c,e]	22.77±4.08[a,c,f]

Note: *Vs.* PBS group, [a]P<0.01, [b]P<0.05; *Vs.* Asthma group, [c]P<0.01, [d]P<0.05; *Vs.* rDer p1/rDer p2 group, [e]P<0.01, [f]<0.05.

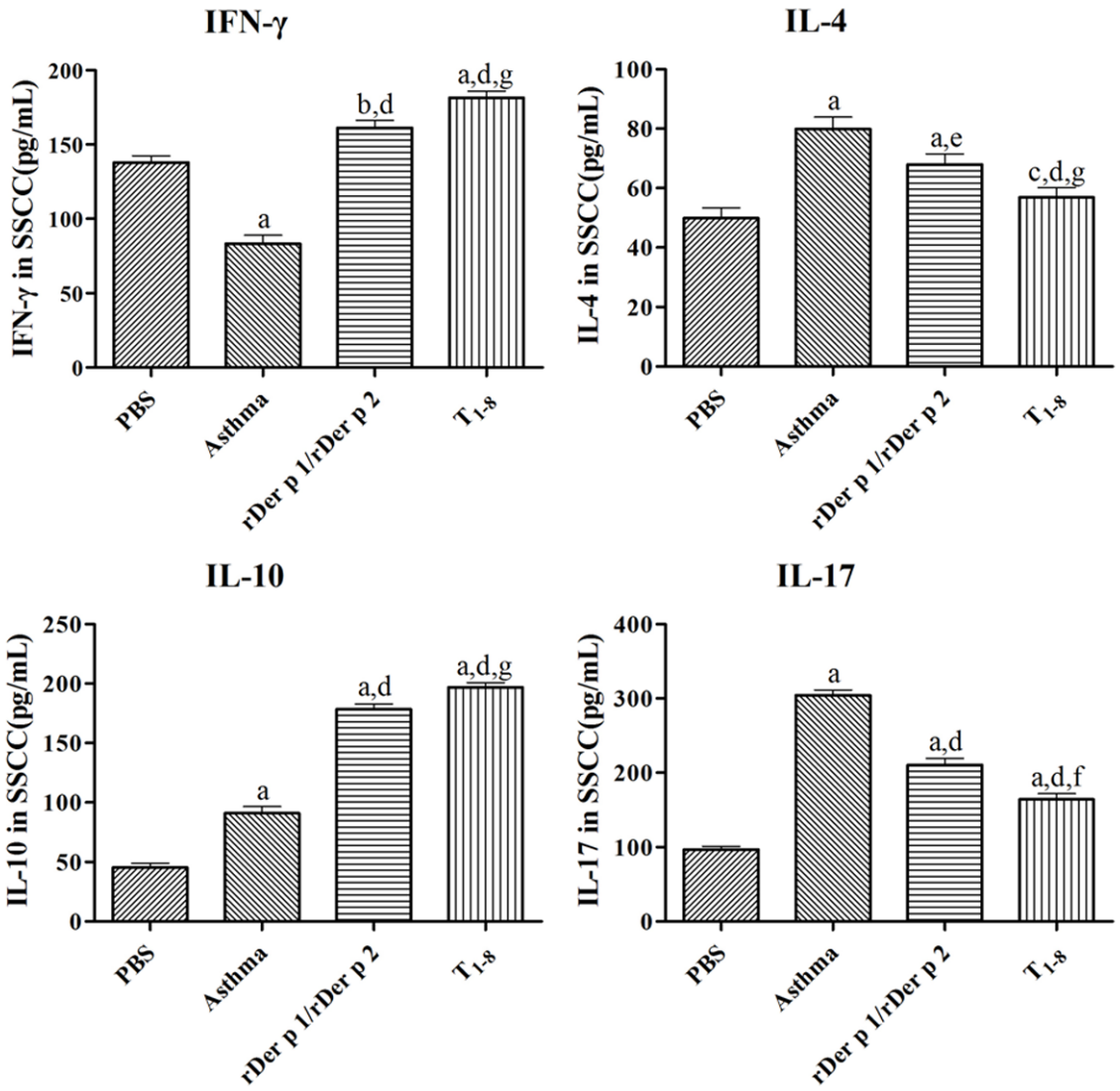

Figure 3. The level of IL-4, IL-10, IL-17 and IFN-γ in the supernatant of splenocyte culture. *Note*: *Vs.* PBS group, [a]P<0.01, [b]P<0.05, [c]P>0.05; *Vs.* Asthma group, [d]P<0.01, [e]P<0.05; *Vs.* rDer p1/rDer p2 group, [f]P<0.01, [g]P<0.05.

follows: 1) IgE was increased in asthma group compared to PBS group (P<0.01); 2) IgE was decreased to a certain degree in groups of rDer p1/rDer p2 and T_{1-8} as compared with asthma group (P<0.01, P<0.05); 3) After immunotherapy of with T_{1-8}, this group had lower IgE than rDer p1/rDer p2 treatment (P<0.05); 4) Serum IgG_1 antibody level was significantly increased

Table 3. Change of IL-4, IL-10, IL-17 and IFN-γ in the cultured splenocyte supernatants ($\bar{x}$ ± s, pg/mL, n=5)

Group	IL-4	IL-10	IL-17	IFN-γ
PBS group	49.85±7.91	45.24±8.25	96.92±9.31	137.91±10.00
Asthma group	79.94±8.73[a]	90.92±12.46[a]	304.14±16.66[a]	83.20±12.79[a]
rDer p1/rDer p2 group	67.92±8.13[a,e]	178.44±9.73[a,d]	210.42±20.54[a,d]	159.51±12.03[b,d]
T_{1-8} group	56.85±7.58[c,d,g]	196.73±9.39[a,d,g]	164.06±17.20[a,d,f]	179.27±10.55[a,d,g]

Note: *Vs.* PBS group, [a]*P*<0.01, [b]*P*<0.05, [c]*P*>0.05; *Vs.* Asthma group, [d]*P*<0.01, [e]*P*<0.05; *Vs.* rDer p1/rDer p2 group, [f]*P*<0.01, [g]*P*<0.05.

in asthma group by comparison with PBS group ([a]*P*<0.01); 5) IgG_1 level was declined in rDer p1/rDer p2 group and T_{1-8} group, in which the latter had lower IgG_1 content than the former ([c]*P*<0.01, [e]*P*<0.01); 6) IgG_{2a} went up in asthma group over the PBS group (*P*<0.01), and was significantly increased in groups of T_{1-8} and rDer p1/rDer p2 compared to asthma group (*P*<0.01, *P*<0.05); 7) T_{1-8} group had slightly higher IgG_{2a} level than rDer p1/rDer p2 group ([f]*P*<0.05).

Antigen-specific IL-4, IL-10, IL-17 and IFN-γ levels detected in the spleen cell culture supernatant by ELISA

ELISA detection and comparison of the cytokine levels in the spleen cell culture supernatant among groups (**Figure 3** and **Table 3**) revealed that: 1) IFN-γ was expressed in significantly lower level in asthma group than PBS group (*P*<0.01); 2) IFN-γ was boosted in groups of rDer p1/rDer p2 and T_{1-8} (*P*<0.01), and T_{1-8} group had higher IFN-γ content than that of rDer p1/rDer p2 group (*P*<0.05). However, the change of IL-4 level in the four groups appeared inversely to that of IFN-γ, as was shown that asthma group had significant level of IL-4 over the PBS group (*P*<0.01), whereas IL-4 levels were decreased in groups of rDer p1/rDer p2 and T_{1-8} by comparison with asthma group (*P*<0.01, *P*<0.05), and the level lower in T_{1-8} group than rDer p1/rDer p2 group ([g]*P*<0.05). Of IL-10 and IL-17 level in the four groups, both IL-10 and IL-17 were significantly increased in asthma group compared to PBS group (*P*<0.01, [a]*P*<0.01); By comparison with the asthma group, IL-10 was markedly elevated, yet IL-17 was fallen in groups of rDer p1/rDer p2 and T_{1-8} (*P*<0.01, [d]*P*<0.01). However, T_{1-8} group had higher IL-10 level, yet lower IL-17 level than rDer p1/rDer p2 group (*P*<0.05, [f]*P*<0.01).

Pathological changes of the lung tissues

Light microscopic examination of sections after HE staining demonstrated that the lung tissues in asthma group (**Figure 4A** and **4B**; indicating PBS group) presented with notably broadened interalveolar septum, thickened bronchial walls, partially fractured bronchial epithelial cells, proliferated goblet cell, lymphocytes surrounding the entire bronchia, inflammatory cell infiltration of primary eosinophils, dilated small vessels, edema at the vascular walls and exuded erythocytes within the lung tissues. The pathological condition was generally improved in the rDer p1/rDer p2 group and T_{1-8} group after immunotherapy, since inflammatory cell infiltration into the lung tissues was significantly alleviated in rDer p1/rDer p2 group (**Figure 4C**), with relatively well-restored airway epithelial structure as compared with asthma group. Furthermore, treatment with the recombinant protein had effectively inhibited the inflammatory cell infiltration into the airways in T_{1-8} group (**Figure 4D**) and led to excellent recovery of the airway epithelial structure that appeared similar to that of negative controls (**Figure 4A**).

Discussion

Allergic asthma from house dust mite represents one of the important allergic disorders, and it prevalence tends to climb year by year worldwide. The pathogenesis of this entity, a chronic allergic airway inflammation mediated by IgE, is strongly involved in a variety of inflammatory cells (e.g. mast cells, eosinophils, T lymphocytes, etc.) and cytokines [15].

Although pathogenesis of asthma is very complex, yet most researchers consider that this disorder is closely associated with imbalanced secretion of Th1/Th2 cytokines, because in

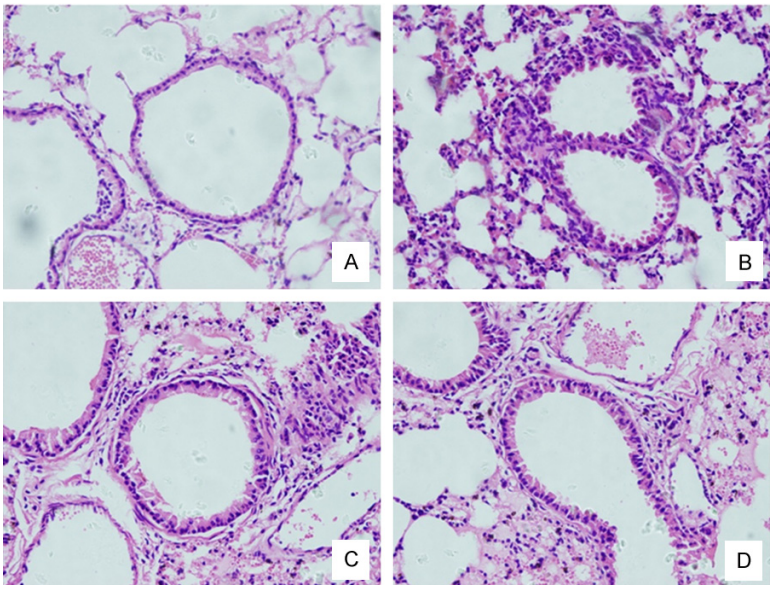

Figure 4. Lung tissue sections by light microscopy (H&E staining, ×400). Note: A. PBS group. B. Asthma group. C. rDer p1/rDer p2 group. D. $T_{1\text{-}8}$ group.

allergic asthma attack, IL-4, IL-5 and IL-13 secreted by Th2 cells are prominent, whereas IFN-γ and IL-2 generated by Th1 cell are downward, which results in activation of the eosinophils and its infiltration into the airway, eventually causing airway hyper-responsiveness symptoms [16]. However, as knowledge on the immune nature of asthma evolves, the theory on Th1/Th2 ground cannot fully clarify the pathogenesis of asthma, now that Th1 cells are considered as pro-inflammatory cells, primarily producing more pro-inflammatory effect than anti-inflammatory action, therefore, Th1 cells fail to attenuate Th2 cell-induced airway hyperreactivity and inflammation [17]. In recent years, Th17/Treg imbalance in the pathogenesis of allergic asthma has become the focus of study interest. HU *et al* [18] found significantly higher percentage of Th17/Treg in asthmatic mouse models, and that Th17/Treg was negatively related to IL-10, yet positively to IL-17 as well as negatively to the count of eosinophils, lymphocyte and neutrophils. Their findings indicated that Th17/Treg proportion was positively correlated with airway inflammation, and functional imbalance of Th17/Treg potentially led to the onset and development of asthma.

IFN-γ and IL-4, respectively secreted by Th1 and Th2 cells, are essential cytokines that can generate immune effects, and these two cytokines may inversely promote Th1 and Th2 cell activation and differentiation as well as inhibit differentiation of homologous cells. Thus, IFN-γ and IL-4 are cross-sectional cytokines indicative of the Th1/Th2 balance in immune response as well as the functional status of T cell subsets. Th2 cells secreting IL-4 can induce B lymphocytes to secrete specific IgE [19], promote proliferation and differentiation of mast cells, and are involved in the development of asthma [20, 21]. Contrarily, Th1-type cytokine IFN-γ, an antagonistic factor synthesized by IL-4, is powered to neutralize IgE production by inhibiting the transcription content of IL-4 mRNA and IL-4-induced B cells to express low-affinity IgE receptor [22]. Th17 cell, a newly described lineage differing from Th1/Th2, is another subtype of $CD4^+$T cells, and characteristically its development depends on IL-23 generating IL-17 [23]. IL-17, strongly associated with asthma attack, is one of vital classes of inflammatory cytokines, and IFN-γ can inhibit its production [24]. Accumulating data demonstrated that that the serum IL-17 mRNA and protein levels were markedly increased in the lung tissues, sputum and BALF from patients with asthma [25-29], positively correlated with the degree of airway hyperreactivity [30]. IL-10 is an immunosuppressive factor, and roles control effect on the development of allergic asthma [31]. This cytokine is primarily generated by Th2 cells, macrophages and Treg cells or others, and able to induce Th0 to Th2 differentiation, which suggests that it may play an important role in immune regulation through suppressing pro-inflammatory factors. Previous studies concluded that antigen-specific Th2 cells can be anergized by IL-10, and that Th2 cell tolerance may suppress eosinophilic inflammation in allergic asthma [32]. Although intricate mechanisms may exist in Th17/Treg and Th1/Th2, yet Th17/Treg imbalance and the pathogenesis and development of asthma may be theoretical basis for thorough investigation on the IgE-mediated allergy and additional evi-

dence to recognize the mechanisms of asthma in conventional conception.

Epitope-based vaccine, a promising therapeutic strategy for allergic disorders, has been much concern in recent years. This vaccine is designed by artificially synthesizing the epitopes or *in vitro* expressing the epitopes in pathogenic vectors [33]. Epitope, also known as antigenic determinant, is a functional chemical group that can decide the antigen-specificity, and can specifically bind with the B cell antigen receptor (BCR) or T cell antigen receptor (TCR) to stimulate the body immune response and generate immunity to the pathogenic microorganisms. Importantly, epitope-based vaccine has advantages over the traditional preparation in that: 1) it can be recognized by and bound with diverse MHC molecules, resulting in improved efficiencies in antigen-presenting; 2) this vaccine can be stable and directly induce specific immune response upon use without toxic effects; 3) it can exclude autoimmune reaction or immunosuppression because of simple molecular structure and low molecular weight of this peptide; and 4) it is relatively easier to produce, store and use. These merits indicate that epitope-based vaccine should be wider implications.

Current vaccines for SIT include recombinant wild-type vaccine, recombinant hypoallergenic vaccine, DNA vaccine and peptide vaccine. Recombinant hypoallergenic vaccine is designed by destroying B cell epitopes on the allergen in order to reduce its allergenicity, while the T cell epitopes are preserved to maintain the antigen recognition and immunogenicity of T cells [34]. Previous work tentatively applied the recombinant allergen to SIT in animal models by using Bet v1 (antigen derived from birch pollen) with T cell epitopes being retained and B cell epitopes being modified, and found that the rBet v 1 was capable of reducing specific IgE production and disrupting the binding of allergens bind to IgE, and had potentially triggered the body to induce inability of antigen-specific T cells [35]. Unfortunately, application of SIT to individuals prone to allergies may be associated with sporadic anaphylaxis that, if ever, can be fatal. Therefore, rational vaccine for SIT purpose should be reduced allergenicity (B epitopes being minimized) and boosted immunogenicity (T epitopes being increased).

Presently, vaccines for SIT of allergic asthma are crude extract vaccine and DNA vaccine available in clinic, however, the two preparations are prone to toxic effects on the individuals. Therefore, successful and safe SIT strategies, allergen variants should be created of which recognition sites for T cells remain intact, whereas binding sites for IgE antibody are removed [2]. With the development of molecular immunology and gene recombination technology, epitope vaccine can overcome the disadvantages of traditional allergen-extract, particularly, this type of vaccine can better generate the targeted cellular immune responses, yet reduce the side effects and improve safe use.

In our previous laboratory work, we successfully constructed the PET-28a (+) - $T_{1\text{-}8}$ plasmid that underwent prokaryotic expression and purification, and verified that its molecular mass was matchable to that as expected by SDS-PAGE and Western blotting. Subsequently, we tentatively applied this fusion peptide to SIT in mouse models and observed the therapeutic outcomes.

However, reliable outcomes from SIT should rely on rational development of mouse models with asthma, for which we established such animal models by modifying the technique as described by Secor *et al* [36], and applied the fusion peptide $T_{1\text{-}8}$ to SIT in mice. The immune-regulation effects on Th1/Th2/Th17/Treg after therapy were verified through determining the serum levels of IFN-γ, IL-4, IL-10 and IL-17 as well as levels of serum specific IgE, IgG_1 and IgG_{2a} antibodies.

The results showed that SIT of the asthmatic mice with $T_{1\text{-}8}$ had greatly reduced the specific serum IgE and IgG_1 antibody levels and boosted the IgG_{2a} antibody. Apart from that, the cytokine levels of IL-4 (Th2-type) and IL-17 (Th17-type) were decreased in the BALF and cultured splenocyte supernatant from the experimental animals, whereas Th1-type cytokine IFN-γ was up-regulated and the inhibitory cytokine IL-10 secreted by T-regulatory cells were increased. Following pathological examination on the pulmonary sections from the mice received SIT indicated that the entire bronchia and the alveolar structure remained relatively intact as compared with the asthma group. No evident inflammatory infiltration into the smaller bron-

chia and surrounding vessels were observed, and the pathological change of the lung tissue was similar to that of PBS group. These findings prove that our fusion peptide T_{1-8} has effectively modified the progression of asthmatic mice after SIT.

In conclusion, the experiments testify that our fusion peptide T_{1-8} can not only inhibit the production of IgE antibody, but also regulate the balance of Th1/Th2 and Th17/Treg in compliance with the cytokine changes observed. Ideally, this fusion peptide demonstrates excellent specific immunotherapy effects based on recombinant allergens. However, we fail to clarify the mechanisms of the fusion peptide vaccine in SIT, which needs further efforts in the following works.

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Disclosure of conflict of interest

None.

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