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Suppression function against environmental dust exposure after *Dermatophagoides pteronyssinus* immunotherapy is associated with production of specific and cross-reactive immunoglobulin G4

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Abstract

Background: Whether *Dermatophagoides pteronyssinus* (Der-p) allergen immunotherapy (AIT) can induce *Dermatophagoides farina* (Der-f)-specific immunoglobulin (slg) G4 production, and tolerance to environmental allergens has not been fully investigated.

Objective: We aimed to determine serum Der-p-slgG4 and Der-f-slgG4 levels in asthma and/or rhinitis patients undergoing Der-p AIT and their ability to reduce immune responses triggered by indoor dust extracts.

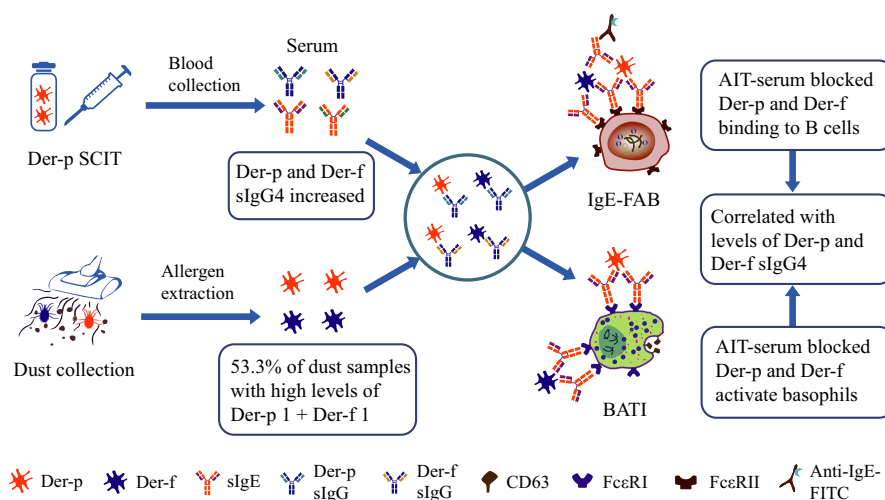
Methods: We performed a real-world prospective trial and enrolled patients with allergic rhinitis and/or asthma in Guangzhou, China. These patients received either Der-p AIT (SCIT group) or routine medications (non-SCIT group) for 156 weeks. Clinical outcomes were assessed by the combined symptom medication score (SMS) and FEV₁% changes. House dust samples were collected to analyse allergen levels. Serum levels of Der-p-slgG4 and Der-f-slgG4, serum inhibitory capacity against Der-p, Der-f and indoor dust extract by slgE-facilitated allergen binding to B cells (IgE-FAB), and serum blocking indoor dust extract-induced basophil activation inhibition assays (BATI) in peripheral blood monocytes were carried out at weeks 0, 4, 12, 16, 52, 104 and 156 after the initiations of the treatments.

Results: Our study enrolled a total of 60 participants, with 30 patients in each group. Patients in the SCIT group had significantly improved SMS when compared with the baseline and the patients in the non-SCIT group. Median levels of Der-p 1 and Der-f 1 in indoor dust extract were 1.86 µg/g and 4.74 µg/g, respectively. Serum Der-p-slgG4 and Der-f-slgG4 levels in SCIT patients showed a significant increase from weeks 12 to 156. Serum in these SCIT patients could significantly block Der-p, Der-f and indoor dust extract formation of allergen-slgE complex and reduced the threshold of IgE-FAB from 16 weeks after the initiation of the treatment. The capacity to inhibit Der-p, Der-f and indoor dust extract BATI was observed in SCIT serum after 12 weeks. Der-p-slgG4 and Der-f-slgG4 had a significant correlation with IgE-FAB and BATI in SCIT patients at all time points.

Conclusion: Single Der-p immunotherapy induced both Der-p-sIgG4 and Der-f-sIgG4 production, which might cross-reactively induce tolerance against environmental allergen exposure in patients with asthma and/or rhinitis.

KEYWORDS

allergen immunotherapy, asthma, environmental allergen, house dust mite, rhinitis



GRAPHICAL ABSTRACT

We demonstrated that 53.3% of home dust samples in Guangzhou had high levels of *Dermatophagoides pteronyssinus* (Der-p) and *Dermatophagoides farina* (Der-f). Single Der-p immunotherapy might cross-reactively induce suppression function against environmental allergen exposure in dual Der-p and Der-f sensitized allergic rhinitis and/or asthma.

1 | INTRODUCTION

House dust mites (HDMs) are common environmental aeroallergens that are present ubiquitously and play a pivotal role in the pathogenesis of respiratory diseases. Exposure to HDM allergens is associated with the development of allergic diseases.¹ Our previous study found that two HDM allergens, *Dermatophagoides pteronyssinus* (Der-p) and *Dermatophagoides farina* (Der-f), were the dominant allergens in China.² Exposures to 2 and 10 µg of group I Der-p and Der-f per gram of dust are considered the exposure thresholds for sensitization and acute asthma attack, respectively.³

Allergic diseases are common chronic health conditions. The treatment strategies include avoiding allergens, patient education, appropriate drug therapy and allergen immunotherapy (AIT). AIT is an effective treatment for HDM allergic disease.^{4,5} The therapeutic mechanisms of AIT involve induction of allergen-specific immunoglobulin G (IgG) 4 antibodies. IgG4 antibodies can compete with specific immunoglobulin E (sIgE) to combine with allergens, which inhibits allergen-immunoglobulin E (IgE) complex formation and binding to mast cells and basophils.⁶ Some studies have also found that IgG antibodies can inhibit allergen-activated effector cells^{7,8} or reduce allergen sensitivity.⁹ The safety and effectiveness of single AIT have been tested in polysensitized patients with asthma

Key Messages

- We investigated the immunologic response to sensitization to multiple allergens during single-allergen immunotherapy.
- Suppression function against environmental dust exposure was demonstrated by sIgE-facilitated allergen binding to B cells and basophil activation inhibition assays.
- Single Der-p immunotherapy induced both Der-p-sIgG4 and Der-f-sIgG4 productions, which might cross-reactively induce suppression functions against environmental allergen exposures.

and rhinitis.¹⁰ However, patients with polysensitization seeking AIT for respiratory allergies are more prevalent than those with monosensitization.¹¹ Our previous study found that more than 90% of patients with asthma and/or rhinitis were sensitized to two or more allergens, and 83.7% were sensitized to Der-p and Der-f simultaneously.² The two group I mite allergens (Der-p 1 and Der-f 1) had very similar physicochemical properties and were highly cross-reactive.¹² Single-Der-p immunotherapy was effective for patients with allergic asthma and rhinitis that were sensitized to

both Der-p and Der-f.⁴ However, very few studies have been performed to investigate the immunologic response to sensitization to multiple allergens during single AIT. Here, we carried out a real-world trial to assess the effects of single Der-p immunotherapy on immunologic responses related to Der-f, including the induction of cross-reactive sIgG4 and its inhibitory capacity against environmental dust.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

We performed a prospective clinical trial and enrolled patients who visited the Allergy and Clinical Immunology Department of the Guangzhou Institute of Respiratory Diseases due to asthma or rhinitis between June 2015 and May 2019 (Figure 1). The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University, China, and registered at <http://www.chictr.org.cn> (ChiCTR-OOC-15006207). Written informed consent was obtained from all adult patients or the parents of children.

The inclusion criteria were as follows: 1) age <60 years old; 2) mild to moderate asthma and/or rhinitis that aligned with the Allergic Rhinitis and its Impact on Asthma (ARIA) guideline for allergic rhinitis and/or the Global Initiative for Asthma (GINA) guideline for asthma^{13,14}; 3) confirmed allergy to Der-p and Der-f by the skin prick test (SPT) and serum sIgE detection (ImmunoCap, Phadia); 4) forced vital capacity in 1 s (FEV₁) greater than 70% of the predicted value. Patients with previously existing cardiopulmonary diseases, such as coronary artery disease, emphysema or bronchiectasis, or signs of acute infection were excluded from the study. Among the AIT-eligible subjects, those willing to receive AIT were assigned to the AIT group, while those unwilling to receive AIT were assigned to the control group and were given medication treatment alone.

2.2 | Study protocol

Patients received either single Der-p subcutaneous immunotherapy (SCIT group) or routine medications (non-SCIT group). Serum samples were collected before the initiation of treatments and at weeks 4, 12, 16, 52, 104 and 156 during the treatments.

2.2.1 | Dust collection and analysis

House dust samples from the participant families in Guangzhou were obtained to analyse group I mite allergen (Der-p 1 and Der-f 1) levels. The detailed steps are described in our previous ISAAC (International Study of Asthma and Allergies in Childhood) study.¹⁵ Briefly, dust samples were collected from the living room and bedroom (bed sheet, pillowcase, pillow and mattress) by the study staff. Endotoxin and allergen levels were analysed as described previously.¹⁵ Endotoxin levels were measured using the kinetic chromogenic Limulus amoebocyte lysate (LAL) assay (Hycult Biotech) and are presented as micrograms per gram dust following unit transformation (i.e. one nanogram of endotoxin is equivalent to 5 EU in the LAL assay¹⁶). Allergen levels were extracted and analysed for Der-p 1 and Der-f 1 using enzyme-linked immunosorbent assay kits.¹⁷ Dust extraction allergen (dust extract) levels are presented as micrograms per gram of dust. Based on the Der-p 1 and Der-f 1 allergen concentrations, we mixed 15 samples of dust extract as an environmental allergen pool (the concentration of Der-p 1 + Der-f 1 = 8.13 µg/ml).

2.2.2 | SCIT procedure and clinical evaluation

Patients received SCIT with standardized aluminium-formulated Der-p Alutard-SQ vaccine (ALK-Abelló A/S). The treatment protocol and clinical evaluations were performed in the same way as described in our previous study.¹⁸ The combined symptom medication score (SMS) was defined as the sum of symptom scores and medication scores.

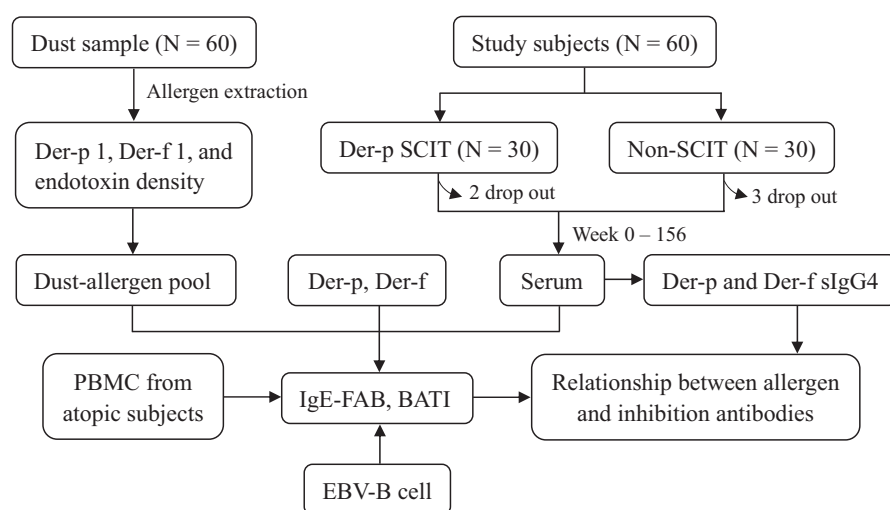


FIGURE 1 Flowchart of the study design. BATI, basophil activation inhibition assay; Der-f, *Dermatophagoides farinae*; Der-p, *Dermatophagoides pteronyssinus*; IgE-FAB, IgE-facilitated allergen binding to B cells assay; PBMC, peripheral blood mononuclear cells; SCIT, subcutaneous allergen immunotherapy; sIgG4, specific immunoglobulin G4

2.2.3 | Detection of serum IgE and IgG4

Levels of total IgE and sIgE against Der-p and Der-f were measured with a fluorescence enzyme immunoassay system (Phadia ImmunoCAP). sIgE results are reported as kU/L, with a reference range 0.35–100 kU/L. The samples with a sIgE level >100 kU/L were diluted and detected again. A four-layer sandwich enzyme-linked immunosorbent assay (ELISA) system was used to measure the serum Der-p and Der-f sIgG4 levels, as previously reported.¹⁹

2.2.4 | Serum allergen-sIgE-facilitated allergen binding assay

Serum inhibitory activity against allergen-sIgE binding to B cells (IgE-FAB) was performed, as described in our previous study.¹⁸ Briefly, stock indicator serum containing a high concentration of Der-p and Der-f sIgE was preincubated with Der-p, Der-f or indoor dust extract to form allergen-IgE complexes. Indicator serum was mixed with test serum or RPMI 1640 as a control. Epstein-Barr virus (EBV)-transformed B cells (a gift from ALK-Abelló A/S) were then added to the allergen-IgE mixture and incubated. Cells were washed, stained with anti-IgE antibody (AbD Serotec) and detected using flow cytometry.

2.2.5 | Basophil activation inhibition assay

Basophil activation inhibition assay (BATI) was performed, as in our previous study²⁰ and reported at weeks 0, 4, 12, 16, 52, 104 and 156 after the initiation of SCIT. Serum (20 µl) from SCIT patients or control participants was incubated with 60 µl of Der-p, Der-f or indoor dust extract at 37°C for 1 h. Then, 80 µl (2×10^6 cells/ml) of peripheral blood mononuclear cells (PBMCs) from three HDM atopic adult volunteers were added to the serum-allergen mixture for 30 min at 37°C. Cells were stained with CD123-PE-Cy5 (BD), HLA-DR-APC (BD), CD203c-PE (Beckman Coulter) and CD63-FITC (Beckman Coulter). Basophils gated on SSC^{low}/CD203c⁺/CD123⁺/HLA-DR⁺ were detected using flow cytometry (BD FACSVerser) and analysed using FCS Express software version 4 (De Novo Software).

2.3 | Statistical analysis

Data analysis was performed in SPSS (version 16.0, SPSS Inc) and GraphPad Prism 8 (GraphPad Software Inc). Allergen levels are presented as median and interquartile range. The Mann-Whitney U test was used to analyse intergroup differences for the allergen concentrations. A two-way analysis of variance with Sidak's multiple comparisons test for two or more groups was used to analyse repeated-measure data. Data are presented as mean $\pm$ standard error of the mean (SEM). Linear regression was used to analyse the relationship among IgE-FAB, BATI and sIgG4. Differences were considered significant at $p < .05$.

3 | RESULTS

3.1 | Patient characteristics

This study enrolled a total of 60 participants. There were 22 children (age ≤ 14 years) and 38 adults (age 15–58 years), with 30 patients in the SCIT group and 30 patients in the non-SCIT control group (Table 1). Two participants in the SCIT group dropped out after 12 and 16 weeks of treatments. Three participants in the control group did not return for the follow-up visits after 16, 52 and 156 weeks of treatments. Some patients did not provide blood at each time point. The missing data from these patients were not included in the final analysis.

The demographic data, SMS and antibody levels of all participants are shown in Table 1. There were no differences between the SCIT and non-SCIT groups in terms of sex, SMS and baseline serum IgE levels (Der-p sIgE: SCIT 89.5 ± 12.7 kU/L vs. non-SCIT 91.4 ± 13.8 kU/L, $p > .05$; Der-f sIgE: SCIT 93.2 ± 13.6 kU/L vs. non-SCIT 94.8 ± 14.5 kU/L, $p > .05$).

3.2 | Changes in clinical outcomes

The SMS was significantly decreased in the SCIT group after 52 weeks of treatment, compared with the baseline. The SCIT group had more significant SMS declines at weeks 52, 104 and 156 compared with the non-SCIT group (Figure S1A). No significant changes in FEV₁% were observed during the treatments in either the SCIT or non-SCIT groups (Figure S1B).

3.3 | Indoor dust analysis

A total of 60 patients and 60 dust samples were collected from the bed and living rooms for the dust exposure investigation. The concentration of mite allergens ranged from <0.01 to >100 µg allergen per gram dust. Figure 2 shows the measured results as µg Der-p 1 or Der-f 1 per gram dust and total amount of Der-p 1 + Der-f 1 in dust samples. Median values of Der-p 1 in dust were 1.86 µg/g dust, which corresponded to a median value of 4.74 µg/g dust for Der-f 1. The level of Der-f 1 was higher than that of Der-p 1 in the dust samples ($p < .05$, Figure 2). High levels of Der-p 1 + Der-f 1 (>10 µg/g) were observed in 53.3% of all dust samples. Sensitive levels of Der-p 1+Der-f 1 (>2 µg/g) were observed in 88.3% of all dust samples. The median value of endotoxin in dust was 0.51 µg/g dust.

3.4 | Allergen concentration curves for IgE-FAB and BATI

To identify suitable allergen concentrations for IgE-FAB and BATI, we performed IgE-FAB and basophil activation tests (BATs) with

Variable	SCIT (n = 30)	Non-SCIT (n = 30)	p value
Sex, M/F	18/12	20/10	>.05
Age distribution (years)	19.2 ± 2.5	21.3 ± 2.6	>.05
Children (≤14 years)	13 (9.3 ± 0.6)	11 (8.6 ± 0.5)	>.05
Adults (>14 years)	17 (26.8 ± 2.4)	19 (28.7 ± 2.1)	>.05
SMS	3.8 ± 0.4	3.9 ± 0.5	>.05
FEV ₁ (%predicted)	90.2 ± 2.3	91.7 ± 1.9	>.05
FVC (%predicted)	97.2 ± 1.6	95.4 ± 2.1	>.05
Total IgE (kU/L)	502.3 ± 66.2	549 ± 53.6	>.05
Specific IgE to Der-p (kU/L)	89.5 ± 12.7	91.4 ± 13.8	>.05
Specific IgE to Der-f (kU/L)	93.2 ± 13.6	94.8 ± 14.5	>.05

Note: Data are presented as mean ± standard error of the mean (n = 30 per group).

Abbreviations: Der-f, *Dermatophagoides farinae*; Der-p, *Dermatophagoides pteronyssinus*; FEV₁, forced expiratory volume at 1 s; FVC, forced vital capacity; IgE, immunoglobulin E; SCIT, subcutaneous allergen immunotherapy; SMS, combined symptom medication score.

TABLE 1 Basic information of all patients in the SCIT and non-SCIT groups

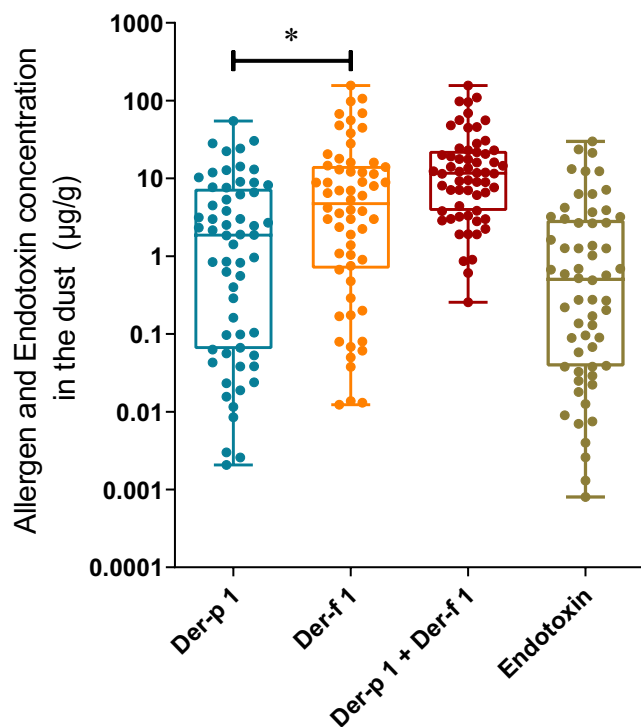


FIGURE 2 Allergen and endotoxin levels in Guangzhou. Data are expressed as median with interquartile range. * $p < .05$ when compared between Der-p 1 and Der-f 1. Der-f, *Dermatophagoides farinae*; Der-p, *Dermatophagoides pteronyssinus*

six concentration levels for Der-p, Der-f, dust extract and lipopolysaccharides (LPS) in 10 volunteers sensitized to HDM. The results showed that IgE-FAB and BAT were allergen dose-dependent in mite-allergic patients (Figure S2A,B). An allergen concentration of 0.15 µg/ml, which evoked submaximal cell stimulation, was chosen to further investigate the time course of IgE-FAB and BATI. Analysis of LPS concentration curves showed no significant change in IgE-FAB and BAT ($p > .05$; Figure S2A,B).

3.5 | Changes in serum antibodies and IgE-FAB

Levels of Der-p and Der-f sIgG4 were significantly increased in the SCIT group starting from week 12 and were more obvious after 16, 52, 104 and 156 weeks of AIT (Figure 3A). Intergroup differences were observed starting from week 16 (Figure 3A). Der-p and Der-f sIgG4 did not change significantly during the treatment in the non-SCIT group (Figure 3A). IgE-FAB showed the capacity to inhibit Der-p, Der-f and indoor dust extract. sIgE complex binding to B cells increased significantly beginning at week 16, with increasingly higher levels at 52, 104 and 156 weeks of AIT in the SCIT group (Figure 3B). The non-SCIT group did not show these changes (Figure 3B).

3.6 | Inhibition of basophil activation

The capacity to inhibit basophil activation stimulated by Der-p, Der-f and dust extract was significantly increased starting from week 12 and kept increasing until week 156 of AIT in the SCIT group (Figure 3C). In the non-SCIT group, there were no altered basophil responses to Der-p, Der-f and dust extract during the treatment process (Figure 3C).

3.7 | Correlations between sIgG4, IgE-FAB and BATI

Levels of Der-p-sIgG4 and Der-f-sIgG4 had significant linear associations with IgE-FAB and BATI in the SCIT group at all time points during the 3 years of treatment (Figures 4 and 5). There were no correlations between Der-p-sIgG4 and Der-f-sIgG4 levels and the BATI and IgE-FAB in the non-SCIT group at the corresponding time points during the 3 years of treatment (Figures S2 and S3).

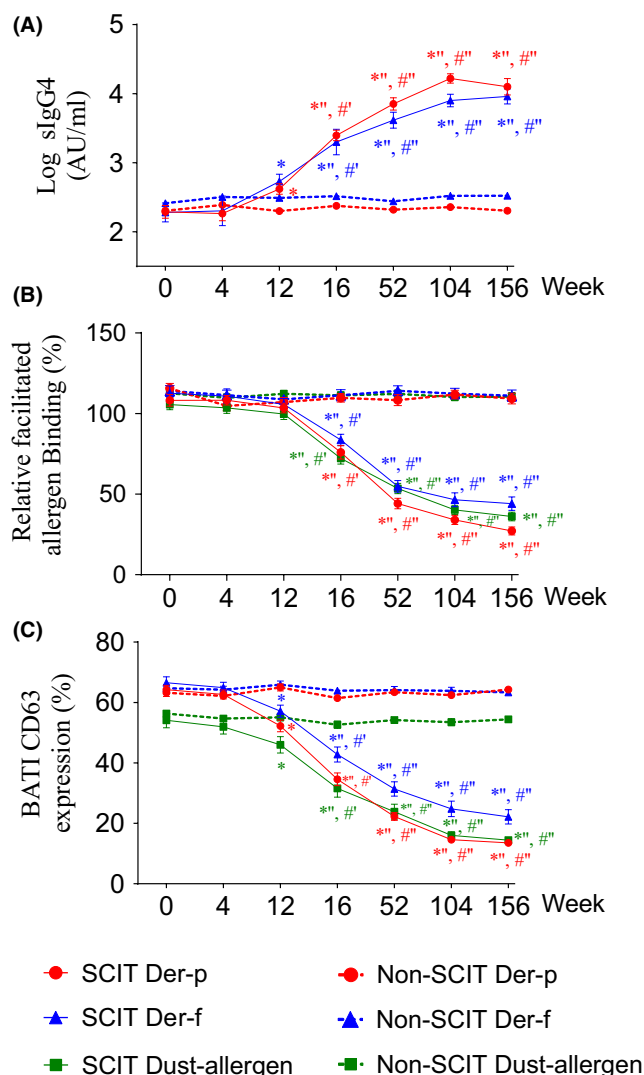


FIGURE 3 Time course of mean Der-p and Der-f sIgG4 (A), Der-p, Der-f and dust allergen IgE-FAB (B), Der-p, Der-f and dust allergen BATI (C) in the SCIT group at visit time points. Data are shown as mean ± standard error of the mean. * $p < .05$, ** $p < .01$, *** $p < .001$ when compared with week 0; # $p < .05$, ## $p < .01$, ### $p < .001$ when compared with the non-SCIT group. BATI, basophil activation inhibition assay; Der-f, *Dermatophagoides farinae*; Der-p, *Dermatophagoides pteronyssinus*; IgE-FAB, IgE-facilitated allergen binding to B cells assay; SCIT, subcutaneous allergen immunotherapy; sIgG4, specific immunoglobulin G4

4 | DISCUSSION

In this study on SCIT-induced blocking antibodies and their association with environmental dust allergen exposure, we found that 53.3% of dust samples showed high levels of Der-p 1 + Der-f 1. We demonstrated that serum Der-p-sIgG4 and Der-f-sIgG4 were significantly increased starting from week 12 after the initiation of SCIT. SCIT patients also had significantly reduced serum Der-p, Der-f and dust extract formation of allergen-sIgE complex binding to B cells, with subsequent inhibited Der-p, Der-f and dust extract to activate basophils. Der-p-sIgG4 and Der-f-sIgG4 had a significant association

with inhibition function in the SCIT group, based on linear regression analysis.

House dust mite allergens are the dominant allergen in China. Exposure to HDM allergens is a major aetiology of perennial allergic respiratory disease.² In this study, we found that 53.3% of dust samples showed high levels of Der-p 1 + Der-f 1 ($\geq 10 \mu\text{g/g}$). Zhang et al.¹⁷ found that 88% of Guangzhou dust samples from bedding had high levels of group I mite allergens. Allergen concentrations above $10 \mu\text{g/g}$ are considered as an exposure risk threshold for acute asthma attack³ and are associated with increased risks of asthma at age 7 years.²¹ Der-f and Der-p are the most common *Dermatophagoides* species found in warm and humid climates.²² These HDMs are the predominant perennial indoor allergen sources in China.² These species are likely to be the cause of the high prevalence of positive mite SPTs in 64% of outpatients.² We also found higher levels of Der-f 1 than Der-p 1 in dust samples from our patients. Similarly, Lian et al.²³ and Zhang et al.¹⁷ showed that concentrations of Der-f 1 were higher than those of Der-p 1 in dust extracts from air conditioner filters or bedding in Guangzhou. In contrast, Lai et al.²⁴ and Chen et al.²⁵ identified and counted more Der-p than Der-f mites in Guangzhou household dust and Guangzhou school dormitory dust. The discrepancy between these studies might be due to the distribution of Der-f and Der-p, which depends on several factors including relative humidity, the biology of these two species and temperature influences on reproduction and survival.²² Nevertheless, these two nearly identical *Dermatophagoides* species are the most common and evenly distributed indoor allergens in Guangzhou city.

The therapeutic strategy for treating allergic asthma is related to both avoidance of the relevant allergens and immunotherapy. Most HDM allergic patients are co-sensitized to Der-p and Der-f due to a high cross-reactivity between these two species, which enabled us to use a single allergen (Der-p) for HDM allergy treatment.^{20,26} Indeed, studies on single Der-p AIT have been shown to be effective in patients with asthma and rhinitis who are sensitized to both Der-p and Der-f.^{10,11} The current investigation is consistent with our previous^{4,19} and other studies,^{27,28} which confirmed that sole Der-p immunotherapy had similar clinical improvements for HDM allergic disease.

Induction of sIgG4 has been found to be a prominent immunological change induced by AIT.⁶ In agreement with our previous findings and those of other studies,^{19,29} we found that AIT could induce a substantial increase of sIgG4. In addition, Der-p single AIT could also induce Der-f sIgG4. These results were consistent with that of Xiao et al.,³⁰ who found that both Der-p and Der-f sIgG4 levels were significantly increased after Der-p AIT. Investigations on the molecular characterization of mite allergens have shown an 81% sequence homology between Der-p 1 and Der-f 1.³¹ Der-p 1 and Der-f 1 were also reported to have similar molecular sizes, stereochemical structures and allergenicities.³² Additionally, a cross-reactive epitope between Der-p 1 and Der-f 1 has been defined,³³ which could play a key role in the cross-reactivity of allergens.³⁴ This structural characteristic indicates a possible cross-reaction between Der-p and Der-f, which have very similar physicochemical properties. The immune system is inclined to respond similarly whether the exposure

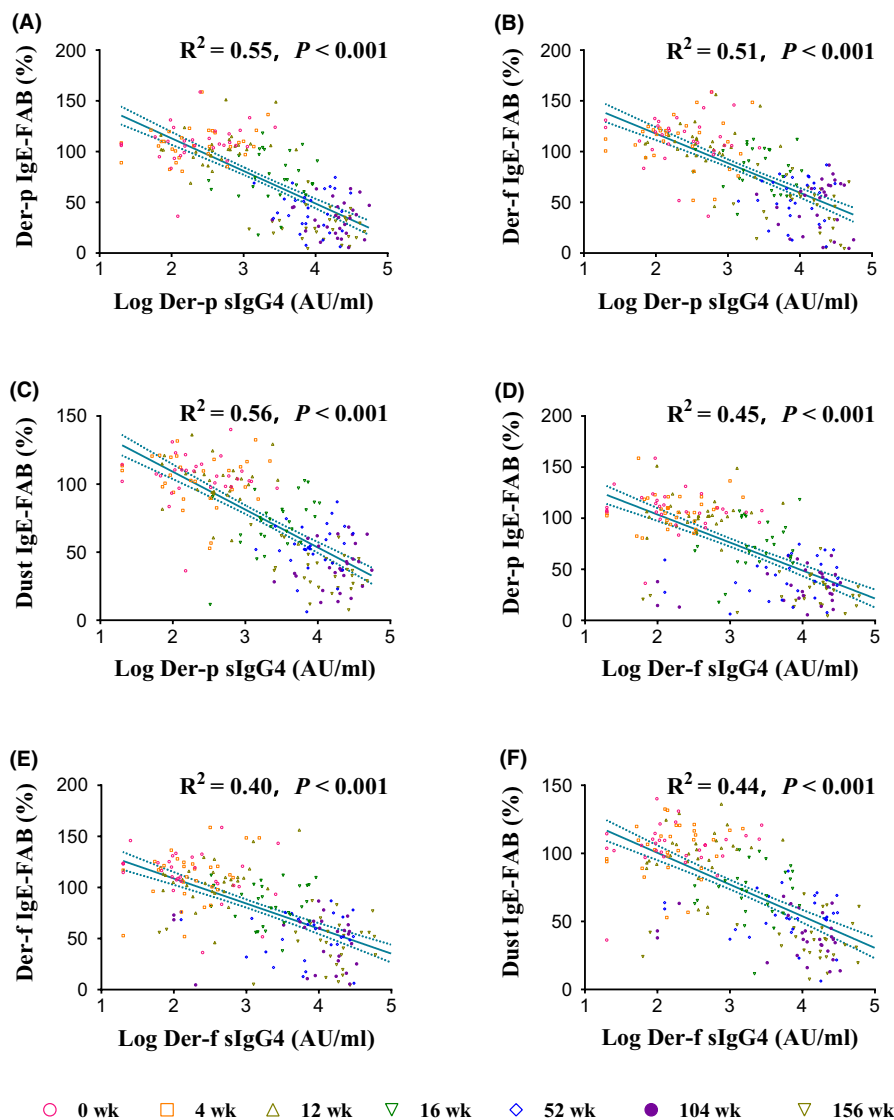


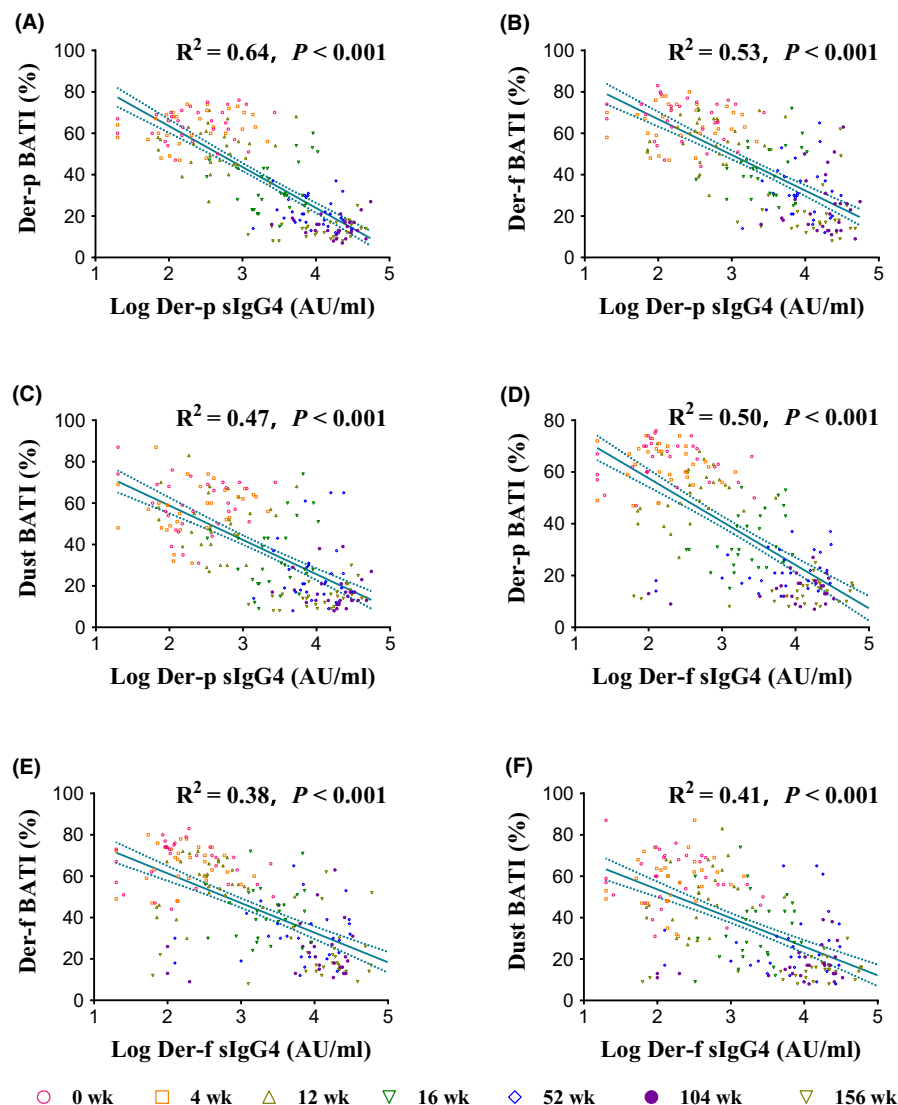
FIGURE 4 Linear regression between Der-p and Der-f sIgG4 and IgE-FAB in the SCIT group at all time points during the 3 years of treatment. Log Der-p sIgG4 versus Der-p IgE-FAB (A); log Der-p sIgG4 versus Der-f IgE-FAB (B); log Der-p sIgG4 versus dust IgE-FAB (C); log Der-f sIgG4 versus Der-p IgE-FAB (D); log Der-f sIgG4 versus Der-f IgE-FAB (E); log Der-f sIgG4 versus dust IgE-FAB (F). *** $p < .001$ (simple linear regression, mean and error, $n = 30$ per group). Der-f, *Dermatophagoides farinae*; Der-p, *Dermatophagoides pteronyssinus*; IgE-FAB, IgE-facilitated allergen binding to B cells assay; SCIT, subcutaneous allergen immunotherapy; sIgG4, specific immunoglobulin G4

is mainly to Der-p or mainly to Der-f, which might explain why 83.7% of participants with asthma and/or rhinitis were simultaneously sensitized to Der-p and Der-f² and why Der-p SCIT-induced sIgG4 antibodies could recognize both Der-p and Der-f.³⁵ Due to the high level of sequence homology, allergens produced by different species can also cause T cell cross-reactivity. T cell-activating regions were identified in corresponding homologous regions to demonstrate the T cell cross-reactivity between Bet v 1 and the related food allergens Mal d 1 (apple) and Api g 1 (celeriace).³⁶ However, there is 85% sequence homology between *Euroglyphus maynei* group 1 allergen and Der-p 1, but the T-cell cross-reactivity between them is only modest.³⁷ T cell cross-reactivity is not only dependent on amino acid sequence similarity but also on structural stability,³⁸ which may explain the diversity. The influence of T cell cross-reactivity, including the allergic response period and the immune response outcome,³⁹ should be taken into consideration when cross-reaction is involved.

Our previous study²⁰ and other studies have found that Der-p and grass pollen AIT-induced sIgG4 exhibited an increased capacity to inhibit allergen-sIgE complex binding to B cells and prevent

the allergen-dependent activation of T cells by interfering with IgE-facilitated antigen presentation.^{29,40,41} In the present study, we further demonstrated that the serum from Der-p SCIT patients could also inhibit Der-f-sIgE and dust allergen-sIgE complex binding to B cells, with the inhibition function increasing persistently during the time course of treatment and reaching a plateau after 2 years of SCIT. This inhibition was associated with cross-reactive sIgG4. The EBV-transformed B cells in the present IgE-FAB assay expressed high levels of low-affinity IgE receptor FcεRII (also called CD23). CD23 is a negative regulator of IgE synthesis^{42,43} and could be proteolytic by Der-p 1 on the surface of cultured human B cells.⁴⁴ Disrupting the CD23 negative feedback signal by Der-p 1 may result in potentially upregulated IgE synthesis.⁴⁵ Therefore, AIT-induced IgG antibodies might interfere with these IgE-dependent mechanisms by inhibiting allergen complex binding to CD23 and reducing the production of IgE antibodies in susceptible atopic individuals. Besides IgG, the existence of other antibody isotypes such as IgA may also influence the inhibition ability.⁶ Our previous study showed that Der-p sIgE and Der-f sIgE levels decreased after 1 year of AIT¹⁸; those results were similar

FIGURE 5 Linear regression between Der-p and Der-f sIgG4 and BATI in the SCIT group at all time points during the 3 years of treatment. Log Der-p sIgG4 versus Der-p BATI (A); log Der-p sIgG4 versus Der-f BATI (B); log Der-p sIgG4 versus dust BATI (C); log Der-f sIgG4 versus Der-p BATI (D); log Der-f sIgG4 versus Der-f BATI (E); log Der-f sIgG4 versus dust BATI (F). BATI, basophil activation inhibition assay; Der-f, *Dermatophagoides farinae*; Der-p, *Dermatophagoides pteronyssinus*; SCIT, subcutaneous allergen immunotherapy; sIgG4, specific immunoglobulin G4



to another study³⁵ that demonstrated that Der-p 2, Der-f and Der-f 2 sIgE levels decreased after 1 year of AIT. However, some studies did not demonstrate a change in sIgE levels during AIT treatment,⁴⁶ or found that AIT-induced serum sIgE increased initially and then declined to the baseline values after 1 year of treatment.¹⁰ In general, the change in sIgE during AIT might be associated with the secretion of IL-10 by Treg cells and regulatory B cells, switching allergen-specific B cells from sIgE production to IgG4 production.⁴⁷ In addition, IL-10 may play a role in reducing proinflammatory cytokines released from mast cells,⁴⁸ downregulating CD86,⁴⁹ and inducing T-cell anergy.⁵⁰

Basophils and mast cells are the key effector cells responsible for clinical symptoms. Previous studies showed that grass pollen immunotherapy induced IgG antibodies associated with reduced basophil sensitivity.^{51,52} Our previous study found that serum obtained after immunotherapy significantly reduced basophil threshold sensitivity under the submaximal Der-p allergen concentration stimulation.²⁰ In the present study, we further demonstrated that serum obtained from patients receiving Der-p SCIT inhibited Der-p, Der-f, and dust extract allergen-activated basophils and had a strong relationship with sIgG4 levels. Basophils express high-affinity

IgE receptor (FcεRI) for IgE. Therefore, we demonstrated the effectiveness of cross-reactive IgG blocking activities on environmental allergens in the FcεRI expression effector cells. Studies have found that inhibition of IgG antibodies reduced basophil allergen threshold sensitivity rather than basophil reactivity, based on IgG depletion experiments.^{20,53} Although AIT-induced IgG antibodies are associated with a reduction in basophil allergen threshold sensitivity, the dose-effect relationship between allergen and inhibition antibodies has still not been completely elucidated. Moreover, AIT might alter the specificity⁵⁴ or affinity⁵⁵ of IgG4 in addition to increasing the antibody levels in serum. Moreover, reduced allergen exposure through use of an air purifier improved clinical efficacy in patients with *Artemisia* pollen-allergic rhinitis.⁵⁶ Thus, the factors related to inhibition of immunological reactions triggered by environmental allergens in allergic individuals may involve the interaction of effector cells, IgE, IgG and environmental allergens.

This is the first study to assess the blocking function of Der-p AIT induced Der-p and Der-f IgG in allergic rhinitis and/or asthma patients using IgE-FAB and BATI assay. There were several limitations, however. First, we sampled the dust from the families of our

patients in Guangzhou City and detected Der-p 1 and Der-f 1 allergens and endotoxin. This sampling method might include other elements such as pet allergens, fungi, bacterial components and pollutants. The interactions of the sampled elements might affect the formation of the allergen-IgE complex and binding to B cells and basophils. With the present real-world dust exposure design, we used the same dust extract to conduct IgE-FAB and BATI experiments in both the SCIT and non-SCIT groups. Moreover, Der-p and Der-f allergens and LPS were used as stimulating factors, indicating that we fully considered the major components of indoor dust. The results reflect an increased tolerance to real-world dust exposure in patients receiving immunotherapy. Second, the experiments were not based on purified allergen-specific IgG antibodies but rather serum from patients receiving SCIT. Other antibodies, such as IgA and IgG1, might also contribute to the inhibition associations. Additional studies, especially those with purified HDM allergens from dust and sIgG4 from serum, are needed to confirm our preliminary findings.

In conclusion, we demonstrated that 53.3% of home dust samples in Guangzhou had high levels of *Dermatophagoides*. Der-p AIT induced both Der-p sIgG4 and Der-f antibodies, with concurrent inhibition of FcεRI and FcεRII functional inhibitory activity triggered by the environmental HDM allergens. Der-p AIT-induced sIgG blocking antibodies might improve immunological tolerance of environmental allergen exposure in patients sensitized to multiple allergens. Der-p AIT may be sufficient to treat dual Der-p- and Der-f-sensitized allergic rhinitis and/or asthma.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

J.L. designed the study, collected the data and performed the statistical analysis. M.L.F. conducted the experiments, performed the statistical analysis and drafted the manuscript. T.L., X.H.Z. and R.D.Q. were involved in the statistical analysis. Q.J.S., M.X. and X.S. were involved in the data collection.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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