

Clinical Response to Subcutaneous *Dermatophagoides pteronyssinus* Immunotherapy in Children with Allergic Rhinitis and Asthma Is Independent of Sensitization to *Blomia tropicalis* Allergens

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Keywords

Allergic asthma · Allergic rhinitis · *Blomia tropicalis* · *Dermatophagoides pteronyssinus* · Subcutaneous specific immunotherapy · IgE · IgG4

Abstract

Background: *Dermatophagoides pteronyssinus* (DP) and *Blomia tropicalis* (BT) are the dominant house dust mites inducing allergic diseases in tropical climates. It is not known whether the efficacy of DP subcutaneous immunotherapy (SCIT) is similar in patients sensitized to DP alone or to both DP and BT. **Method:** Ninety-five children (5–17 years old) affected by asthma with rhinitis and sensitized to both DP and BT received 3 years of DP-SCIT. Clinical symptom and medication scores, serum-specific IgE and IgG₄ were evaluated during DP-SCIT. Patients were grouped based on DP and BT co-sensitization or cross-reactivity, according to positive or negative IgE to BT major allergen (BTMA). **Results:** After 3 years of DP-SCIT, all patients had significant reductions in

symptoms and medication use. In all, 65% of the patients were free of asthma symptoms and medication use; in addition, 3% was free of rhinitis symptoms. FEV₁ in all patients were greater than 95% of predicted. DP-SCIT induced significant increases in DP- and BT-specific IgG₄. In 50% of patients, DP-specific IgG₄ increased more than 67-fold. BT-specific IgG₄ increased more than 2.5 fold. A moderate correlation ($r = 0.48\text{--}0.61$, $p < 0.01$) was found between specific IgE against DP and BT in the BTMA– group ($n = 34$) before and after DP-SCIT, whereas no correlation was found in the BTMA+ group ($n = 61$). The 2 BTMA groups responded similarly with regard to clinical improvement and increase in specific IgG₄ to both DP and BT. No safety finding of concern were reported in either group. **Conclusion:** DP-SCIT may be of clinical benefit to patients with IgE sensitizations to both DP and BT. DP-SCIT induces IgG₄ that cross-react with BT allergens.

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Introduction

House dust mite is the most important inhaled allergen source causing allergic rhinitis (AR) and asthma. In China, the indoor allergen exposure study [1] showed that the most common house dust mite species presented in households were *Dermatophagoides pteronyssinus* (DP) and *Dermatophagoides farinae* (DF). However, in the tropical island of Hainan, the southernmost part of China, the predominant species of house dust mites are DP and *Blomia tropicalis* (BT), both of which have been described in tropical cities [2, 3]. DP and BT have also been shown to be the most common allergen sources causing allergic respiratory diseases in tropical and subtropical regions with prevalence rates in patients affected by asthma and/or rhinitis of 70–90% in India [4], Singapore [5, 6], Taiwan [7] and Hainan in China [8]. These data should be considered in the light of several studies that have demonstrated a low to moderate IgE cross-reactivity between DP and BT [9–12].

Subcutaneous allergen immunotherapy (SCIT) is a disease-modifying treatment for allergy [13, 14]. DP-SCIT treatment is clinically effective in Chinese house dust mite allergic patients [15]. SCIT induces substantial increases in allergen-specific IgG₄, which is a blocking antibody that can compete with IgE for allergen binding. The increase of specific IgG₄ is occurring more rapidly and in higher concentrations in children than in adults [16]. This study aimed to investigate whether DP-SCIT provided clinical benefit to children affected by allergic asthma (AA) and rhinitis, when they are exposed to and sensitized to both DP and BT, and whether DP-SCIT-induced allergen-specific IgG₄ antibodies cross-react with BT antigens. The primary endpoint was the combined symptom and medication scores (CSMS). According to the recommendations of the World Allergy Organization the minimal clinically relevant efficacy should be at least 20% higher than placebo [17]. The placebo effect in SCIT studies can be up to 40% [18]. Thus, if the placebo effect was in line with earlier studies, an improvement of >60% in CSMS would likely have met clinical significance.

A previous study performed on 121 mite allergic children in Hainan [8] showed that only 0.8% of patients were IgE negative to DP, while 12.3% of the patients were BT IgE negative. In the same study, the IgE cross-inhibition test between DP and BT showed that BT extract can only inhibit less than 50% of DP IgE, and conversely DP extract only inhibit 40–80% BT IgE. These results indicate that either patients can be co-sensitized

or IgE may cross-react between DP and BT. Studies on the major allergen level show limited IgE cross-reactivity between the DP major allergen Der p 1 and Blo t 1 [19, 20]; and vice-versa between BT major allergen (BTMA) Blo t 5 and Der p 5 [21]. We hypothesized that patients sensitized to BTMA would most likely be co-sensitized to DP, while patients not sensitized to BTMA, would have a high probability of having IgE cross-reactive between DP and BT. A *major allergen* is defined as an allergen to which more than 50% of patients with allergy to the species are sensitized [22]. Studies have shown that more than 80% of BT sensitized patients react to Blo t 5 and Blo t 21 [23]. Blo t 5 and Blo t 21 share a moderate degree of homology, but the IgE cross-reactivity between the 2 allergens is very limited [24–26]. Thus, Blo t 5 or Blo t 21 are defined as BTMA [27, 28]. In this study, the BTMA+ group, with specific IgE to either Blo t 5 or Blo t 21, was denoted as “co-sensitized”; the BTMA– group, where patients had no detectable IgE to neither Blo t 5 nor Blo t 21, was denoted as “cross-reactive” to DP.

Material and Methods

Subjects

The study recruited 95 children (5–17 years old) diagnosed with house dust mite AA with rhinitis as determined by medical history and specific IgE test in the pediatric clinic of Hainan General Hospital, Haikou, China. The severity of asthma is mild to moderate according to the Chinese Guideline for diagnosis and optimal management of asthma in China [29]. All subjects had specific IgE (larger than 0.7 kUA/L) to DP and among these, 87 also had specific IgE (larger than 0.35 kUA/L) to BT. The subjects received 3 years of SCIT treatment and rescue medication according to need.

The study was approved by the local Ethics Committee and informed consent was obtained from the guardian of each participant. Serum samples were collected from each patient at 4 research visits: before treatment and after 1, 2, and 3 years of SCIT.

SCIT Protocol

The patients were treated with subcutaneous injections of standardized aluminum-formulated house dust mite DP Alutard SQ® (ALK-Abello A/S, Hørsholm, Denmark) allergy immunotherapy. The treatment protocol followed the recommended dose escalation schedule of 15 weeks before reaching a maintenance dose of 100.000 SQ-U of Alutard SQ® given every 6–8 weeks for 3 years.

Clinical Assessments

Patients were asked to rate their AA and AR symptoms during daytime and night-time over the past week at each research visit during the study period. For asthma, daytime symptoms were scored on a scale from 0 to 5 with respect to duration, severity of the symptoms, and the influence on work and daily activities. For

night-time asthma symptoms, a score of 0–4 was used, and patients scored frequency and time of awakening because of asthma symptoms. For rhinitis (daytime and night-time), a score between 0 and 3 was assigned according to the severity of the following 4 symptoms: the number of sneezing episodes, the severity of runny nose, and blocking and itching of nose according to the EAACI guideline [30]. The symptom scores (SS) were calculated as the mean of the daytime and night time SS in the past week. The maximum AASS is 4.5 and the maximum ARSS is 3.

Rescue medication use was scored according to World Allergy Organization recommendations [17]: one point was assigned to each dose of anti-histamine, anti-leukotriene or inhaled β_2 -agonist; 2 points was assigned for the use of inhaled or nasal corticosteroids; and 3 points was assigned for the use of an oral corticosteroid. The daily medication score (MS) was the sum of each medication used in a day, and the mean of 7 days was used as the score for the week. The maximum MS is 8.

The CSMS was calculated as the sum of (AASS+ARSS+MS). The maximum of CSMS is 15.5 at each research visit. The percentage of improvement of the CSMS = $100 \times (\text{CSMS}_{T_i} - \text{CSMS}_{T_0}) / \text{CSMS}_{T_0}$, where T_i is the treatment time-point at research visit, T_0 is the baseline before DP-SCIT.

The lung function test was performed using SpiroDyn'R (Muret Cedex, France) at each research visit. Forced expiratory volume in the first second of expiration (FEV₁%) in percent of the predicted value was recorded.

Adverse Events

Safety was assessed by recording both immediate and late phase adverse events (AEs). Immediate AEs were recorded during the 30-min observation period after SCIT injection and rated by the investigator as mild, moderate, severe, or life-threatening [17]. The late phase AEs were recorded outside the clinic by subject/parent/guardian.

Environmental Allergen Testing

Dust samples from beddings were collected from the households of 52 of the recruited patients, using same dust collection and extraction methods as previously reported [1]. The content of mite major allergen Der p 1 and Der f 1 allergens and BT antigen were measured by ELISA as previously described [1]. The results are reported as $\mu\text{g/g}$ dust for both Der p 1 and Der f 1 content. The lower limit of ELISA detection (LOD) is 0.0060 $\mu\text{g/g}$ for Der p 1; 0.0054 $\mu\text{g/g}$ for Der f 1. The BT antigen results are reported as μg BT extract/g dust, the LOD is 0.15 μg BT extract/g.

Immunological Tests

DP- and BT-specific IgE were measured using the ADVIA Centaur® immunoassay system (Siemens AG, Erlangen, Germany), a reverse sandwich immunoassay using direct chemiluminescent technology [31]. The results are reported as kUA/L. Specific IgE results were calibrated against ImmunoCAP-specific IgE. A result of more than 0.35 kUA/L was defined as positive.

Blo t 5- and Blo t 21-specific IgE were also tested using the ADVIA Centaur® system. However, the method was not calibrated against ImmunoCAP, and results were reported as AU/L. Results of >1 AU/L were considered positive.

DP-specific IgG₄ in serum samples was measured by a 4-layer sandwich ELISA as reported previously [16]. The previous report presented results in AU/mL. However, for this study, the assay was

further calibrated against ImmunoCAP specific IgG₄, and is here reported in mgA/L. The BT-specific IgG₄ concentration was measured by ImmunoCAP 100 system (Phadia AB, Uppsala, Sweden) and reported as mgA/L.

Statistical Analysis

Samples with IgE results below the lower limit of quantification were reported as 0.175 kUA/L. For IgG₄, values below a limit of quantification of 0.07 mgA/L were reported as 0.035 mgA/L.

Repeated measures of IgE and IgG₄ over treatment time were fitted to linear mixed models as the change from baseline of $\log_{10}$ transformed values, $\text{Log}_{10} \text{IgE or IgG}_4 (\text{time} = 1 \text{ or } 2 \text{ or } 3 \text{ years}) - \text{Log}_{10} \text{IgE or IgG}_4 (\text{time} = \text{baseline}) = \text{Log}_{10} \text{IgE or IgG}_4 (T_i/T_0)$. The same model fitting was used for the repeated measures of clinical responses (As SS, Rh SS, MS, and FEV₁%) over treatment time. The fitting mixed model contained both fixed and random effects. The fixed effect was constructed by two-ways analysis of variance with interaction. The fixed factors were Reaction group (BTMA+ or BTMA-) and Time; the interaction was Reaction group $\times$ Time. The random factor was Patient ID with reaction group nested inside, where the individual difference of patients was accounted by random effects in the mixed model. The models were set up and analyzed with JMP version 13.2.0 using the JMP add-in "repeated measures – full factorial design (Mixed model)." Least square means were plotted as a function of time in Figures 1 and 2. The difference between least squares means was obtained by Tukey's honest significant difference test adjusting for multiple comparisons.

Spearman rank correlation analysis was used to assess the correlation between IgE and IgG₄ levels between reaction groups.

JMP®, version 13.2 (SAS Institute Inc., Cary, NC, USA) and GraphPad Prism 7.0 for Windows (GraphPad Software, La Jolla, CA, USA) were used for the statistical analysis in this study. All corrected *p* values less than 0.05 were considered significant.

Results

Patients

Demographic data as well as clinical and immunological parameters before and during DP-SCIT are shown in Table 1. All 95 recruited patients completed 1 year of DP-SCIT, and 74 (78%) patients completed 3 years of treatment. Eleven dropouts were caused by dissatisfaction with clinical improvement and unwillingness to accept residual rhinitis symptoms even though asthma symptoms significantly improved; 4 patients dropped out because a change of residence prevented further participation in the study; 2 dropouts were due to side-effects, 1 dropout because of heart issue, and the reason of the rest 3 dropouts was unknown.

Environmental Allergens

Before treatment, allergen exposure was investigated in 52 bedding dust samples from the patients' house-

Fig. 1. Clinical responses before and during DP-SCIT in the 2 BTMA groups. Both scatter plots and model fitting plots of the change from baseline are shown for AASS (a1, a2), ARSS (b1, b2), MS (c1, c2), CSMS (d1, d2). The scatter plot and model fitting plot of FEV₁% predicted was shown in (e1, e2). BTMA- group is denoted as circles, while BTMA+ group was denoted as squares; the horizontal line in the scatter plot indicated the median level at each time-point, and the stippled vertical line in scatter plot is to separate the 2 groups. The horizontal dot line in model fitting plot (a2–d2) is 60% improvement of each parameter (the improvement threshold in this study). The Tukey honest significant difference tests shows that DP-SCIT 1, 2, and 3 years' data are significantly different from data of baseline and from each other ($p < 0.0001$) in all 5 clinical responses. However, no difference was observed between the 2 BTMA groups.

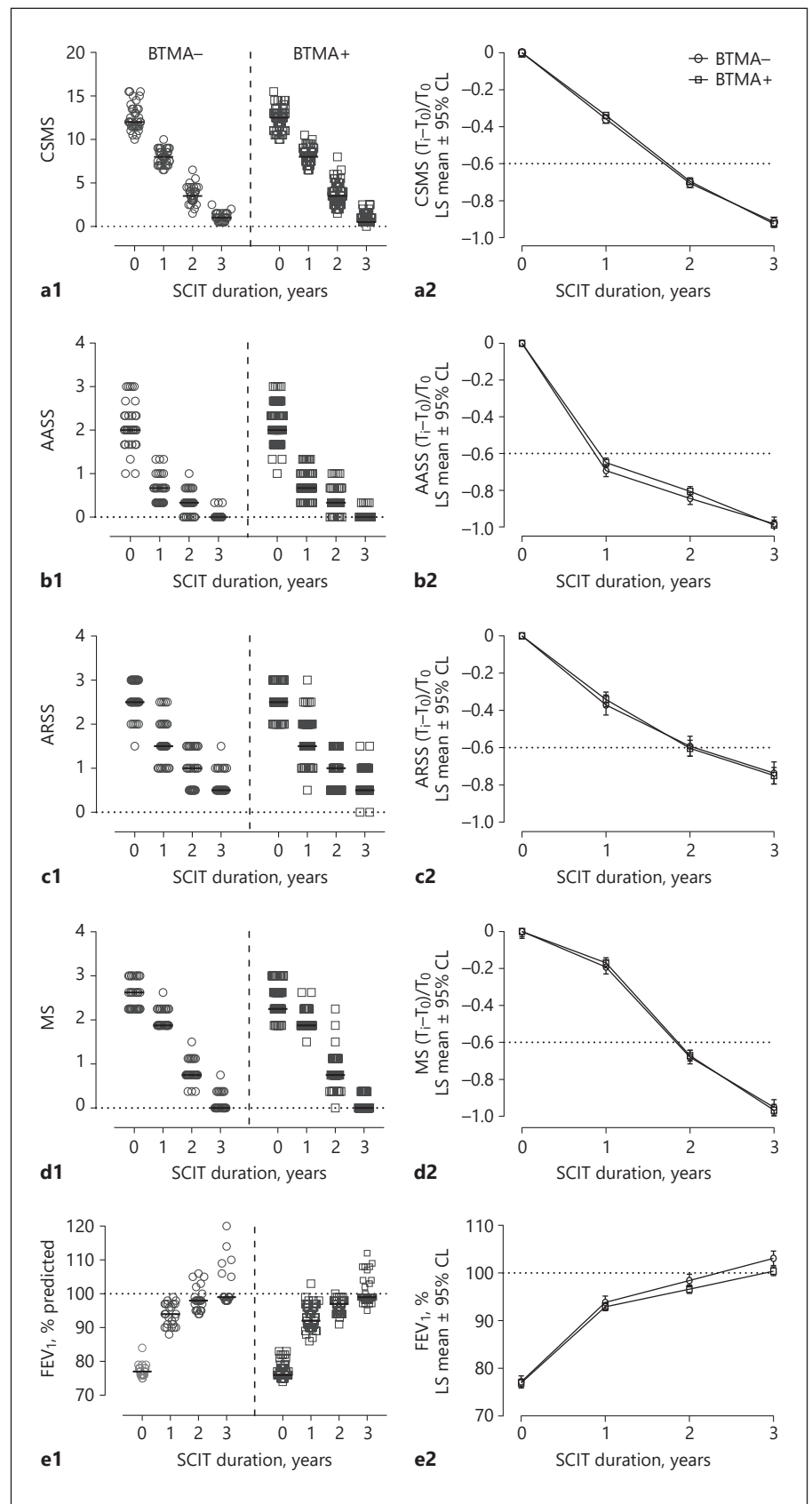


Fig. 2. Immunological change from baseline during DP-SCIT in the 2 BTMA groups. DP-specific IgE (a); BT-specific IgE (b); DP-specific IgG4 (c); BT-specific IgG4 (d). BTMA- group is denoted as circles, while BTMA+ group is denoted as black squares. The significance of the differences between time-points (T_i) compared to baseline (T_0) in BTMA- and BTMA+ groups are shown as $p < 0.05$: *, +; $p < 0.001$: ***, +++; $p < 0.0001$: ****, ++++ respectively. The difference between the 2 BTMA groups at the same time-point was observed only for the BT IgE change, 1 year: $p = 0.02$; 2 years and 3 years: $p < 0.0001$.

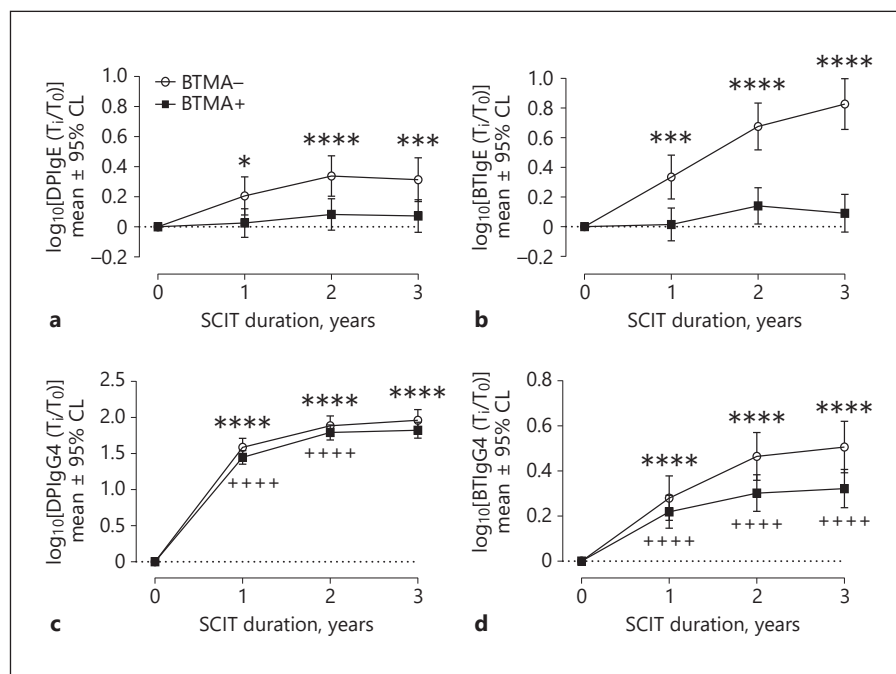


Table 1. Demographic, clinical, and immunological characteristics before and during DP-SCIT

	0	1	2	3
AIT duration, years				
Subjects, <i>n</i> (BTMA+/BTMA-)	95 (61/34)	95 (61/34)	85 (52/33)	74 (47/27)
Age, years, mean (range)	9 (5–17)	9 (5–17)	9 (5–17)	9 (5–17)
Gender, <i>n</i> (male/female)	71/24	71/24	64/21	59/15
AASS, mean (range)	3.2 (1.5–4.5)	1.1 (0.5–2)	0.6 (0–1.5)	0.1 (0–0.5)
ARSS, mean (range)	2.6 (1.5–3)	3.2 (0.5–3)	1.0 (0.5–1.5)	0.7 (0–1.5)
MS, mean (range)	6.7 (5–8)	5.4 (4–7)	2.1 (0–6)	0.3 (0–2)
CSMS, mean (range)	12.4 (10–15.5)	8.1 (6.5–10.5)	3.8 (1.5–8)	1.0 (0–2.5)
%improvement in CSMS, mean (range)	–	35 (14–54)	70 (46–87)	92 (79–100)
FEV ₁ % predicted, median (range)	76 (74–84)	93 (86–103)	97 (91–106)	100 (95–120)
DP IgE, kUA/L, median (range)	144 (0.7–1208)	157 (1.8–1129)	202 (1.8–1543)	179 (0.8–1483)
BT IgE, kUA/L, median (range)	10.5 (0.2–1343)	14.3 (0.2–1046)	22.1 (0.2–1095)	23.1 (0.2–341)
DP IgG4, mgA/L, median (range)	0.3 (0.04–3.6)	8.7 (0.5–55.4)	19.0 (1.1–93.9)	20.1 (2.7–167.3)
BT IgG4, mgA/L, median (range)	0.16 (0.04–2.76)	0.28 (0.04–3.22)	0.46 (0.04–3.43)	0.43 (0.04–3.58)

AASS, allergic asthma symptom scores; ARSS, allergic rhinitis symptom score; CSMS, combined symptom and medication score; MS, medication scores.

holds. The median level of allergen was Der p 1: 3.8 µg/g dust, Der f 1: 0.1 µg/g dust, and BT: 34.2 µg extract/g dust. Der p 1 and BT could be detected in all dust samples, whereas Der f 1 was detected in 62% of dust samples; 73% of samples had levels of Der f 1 of <1 µg/g dust. These data confirmed that patients are predominantly exposed to both DP and BT, whereas DF is a minor species in Hai-kou city.

Clinical Benefit

The primary outcome of CSMS change during DP-SCIT is shown in Figure 1-a1. The mean percentage of improvement after 1 year of allergen immunotherapy (AIT) is 35%; none of the patients reached 60% improvement, which is considered significant improvement. After 2 years of AIT, the mean improvement is 70%; about 75% of the 85 patients reach significant im-

provement. After 3 years of AIT, the mean improvement is 92%; all 74 patients achieved significant improvement (see Table 1).

When looking at the individual outcomes, asthma (Fig. 1-b1) and rhinitis symptoms (Fig. 1-c1) as well as the use of rescue medication (Fig. 1-d1) were significantly reduced from baseline. After 1 year of treatment, the mean improvement of AASS, ARSS, and MS is 67, 37, and 19%, respectively. After 2 years of treatment, the mean improvement of AASS, ARSS, and MS is 82, 61, and 67% respectively. After 3 years of treatment, the mean improvement of AASS, ARSS, and MS is 98, 74, and 96% respectively. Furthermore, 54 out of 74 (73%) patients were free of medication use for both asthma and rhinitis; 48 (65%) patients no longer experienced asthma symptoms; and 2 (3%) patients no longer had rhinitis symptoms. The majority of patients still had mild rhinitis symptoms after 3 years of treatment. The median ARSS after 3 years of treatment was 1.3 (Table 1); 42 (57%) patients improved more than 80% in ARSS. The lung function FEV₁% was significantly improved from 76% of predicted before treatment to 100% after 3 years of treatment (Fig. 1-e1).

Immunological Responses

Before DP-SCIT, all 95 patients had positive DP-specific IgE, with a median level of 144 kU/L. Among them, 87 patients had positive BT-reactive IgE, with a median level of 10.5 kU/L (Table 1). During DP-SCIT, DP-specific IgE increased after 1 year and then remained unchanged until the end of the study. BT-reactive IgE increased through 2 years of treatment, and remained unchanged until the end of the study.

Both DP- and BT-reactive IgG₄ significantly increased during DP-SCIT. After 3 years of DP-SCIT, the median increase of DP-specific IgG₄ was 67 fold, and for BT-reactive IgG₄, the increase was 2.5-fold.

No Difference in Clinical Improvement between the Two BTMA Groups

The recruited patients were grouped according to BTMA (Blo t 5 and Blo t 21) sensitization. There were 34 patients in the BTMA- group and 61 patients in the BTMA+ group (Table 1). The clinical changes from baseline during DP-SCIT between the 2 BTMA groups are shown in Figure 1. There were no differences in the change from baseline of CSMS (Fig. 1-a2), AASS (Fig. 1-b2), ARSS (Fig. 1-c2), MS (Fig. 1-d2) and FEV₁% predicted (Fig. 1-e2) between the 2 BTMA groups at any time point during DP-SCIT. The percentage of patients who

were free of medication use (BTMA-: 70%; BTMA+: 74%) and asthma symptoms (BTMA-: 63%; BTMA+: 66%) were similar in both groups.

Significant Difference in IgE Response between the Two BTMA Groups

During DP-SCIT, DP- and BT-specific IgE significantly increased from baseline only in the BTMA- group (Fig. 2a, b). At the end of 3 years of treatment, the median increase was 1.5-fold in DP-specific IgE, and 10-fold in BT-specific IgE in the BTMA- group, while no increase at all was observed in the BTMA+ group. BT-specific IgE in the BTMA- group was about 30-fold lower than in the BTMA+ group before DP-SCIT, and about 2.5-fold lower at the end of the study.

No Difference in IgG₄ Response between the Two BTMA Groups

DP-specific IgG₄ increased substantially after 1 year of DP-SCIT, continued to increase after 2 years and was sustained at a high level at 3 years in both BTMA groups (Fig. 2c). The median increase from baseline after 3 years of DP-SCIT was 84- and 59-fold in the BTMA- and BTMA+ groups respectively. The 2 BTMA groups showed no difference in DP IgG₄ levels or increase in ratio at any of the investigated time points.

BT-reactive IgG₄, on the other hand, increased significantly during DP-SCIT in both BTMA groups (Fig. 2d). The median-fold increase after 3 years was 2.9 and 2.4 in BTMA- and BTMA+ groups, respectively, which was about 20–30-fold less than the DP IgG₄ increase.

IgE and IgG₄ Correlation between DP and BT Allergen

Correlations between DP and BT IgE and IgG₄ are shown in Table 2. BT IgE show moderate correlation with DP IgE in the BTMA- group ($r = 0.48$, $p = 0.004$) before treatment. After DP-SCIT, a similar IgE correlation was maintained at all time points (1 year: $r = 0.58$, $p = 0.0003$; 2 year: $r = 0.55$, $p = 0.003$; 3 year: $r = 0.61$, $p = 0.002$). There was no IgE correlation observed before and after DP-SCIT in the BTMA+ group. The 2 groups had a similar low to moderate correlation between DP and BT IgG₄ before and throughout treatment. The specific IgG₄ correlation seemed to be independent of BT allergen-sensitization.

Adverse Events

Detailed data on AEs in the 2 BTMA groups are shown in Table 3. The total number of injections for the patients in BTMA+ group is 2,663, while for BTMA- group is

Table 2. Spearman correlation between DP and BT IgE and IgG4 during DP-SCIT in the 2 BTMA groups

AIT, years	BT IgE versus DP IgE				BT IgG4 versus DP IgG4			
	BTMA–		BTMA+		BTMA–		BTMA+	
	<i>r</i>	<i>p value</i>	<i>r</i>	<i>p value</i>	<i>r</i>	<i>p value</i>	<i>r</i>	<i>p value</i>
0	0.48	0.0038	0.03	0.82	0.42	0.01	0.39	0.002
1	0.58	0.0003	0.10	0.43	0.37	0.03	0.38	0.003
2	0.55	0.0025	0.29	0.05	0.38	0.05	0.41	0.005
3	0.61	0.0024	0.22	0.17	0.30	0.18	0.33	0.036

r is the Spearman ranking correlation coefficient.

Table 3. Adverse events in BTMA+ and BTMA– groups

Adverse events	BTMA+ group (total injections, <i>n</i> = 2,663)		BTMA– group (total injections, <i>n</i> = 1,497)	
	<i>n</i>	%	<i>n</i>	%
Throat itching	4	0.2	4	0.3
Cough	20	0.8	18	1.2
Chest tightness	20	0.8	12	0.8
Short of breath	5	0.2	3	0.2
Nose itching and sneezing	0	0.0	1	0.1
Asthma	3	0.1	0	0.0
Eye redness	1	0.0	0	0.0
Swelling and itching at injection site	83	3.1	63	4.2
Papule at injection site	6	0.2	5	0.3
Induration at injection site	1	0.0	0	0.0
Severe system adverse reaction	0	0.0	1	0.1

1,497. There were no differences between the 2 BTMA groups with respect to AEs. All AEs were considered treatment related.

Patients received a total of 4,160 injections. Overall, 158 (3.8%) local skin AEs and 92 (2.2%) respiratory AEs were reported. All AEs were reported during the 30-min observation time after each injection, and no late phase AEs were reported. Two hundred and forty eight AEs (5.96%) were classified as mild reactions, 1 respiratory AE (0.02%) was classified as moderate, and 1 reaction (0.02%) was classified as severe, but not life threatening. The symptom of the severe AE happened 20 minutes after the injection of 0.6 mL of Vial 4. The symptom reported was a systemic reaction with chest tightness, shortness of breath, cough, and asthma, and required an injection of 0.3 mL Adrenaline and 3 mg Dexamethasone.

Discussion

The DP-SCIT treatment is in general a safe treatment. No safety concerns were reported in this study. The results showed that treating with DP-SCIT for 3 years was of significant clinical benefit to patients sensitized to both DP and BT. The treatment duration is important in order to achieve sustainable efficacy. Treatment for only 1 year was not sufficient to achieve the improvement threshold, which was arbitrarily decided to be 60% clinical improvement compared to baseline in light of the lack of a placebo group in this study. After 2 years of treatment, the majority of patients achieved 60% improvement in AASS, ARSS, and MS. After 3 years of treatment, all patients reached nearly 80% reduction in CSMS. Similar to Eifan et al. [32], the improvement in asthma symptoms seemed to be better than the improvement in rhinitis symptoms. Indeed, at the end of the 3-year treatment period, 65% of

patients were free of both asthma symptoms and requirement for asthma medication. Only 3% of the patients, however, were completely free of the rhinitis symptoms.

In this study, patients were naturally exposed to both DP and BT mite allergens. We have grouped the subjects according to the presence or absence of specific IgE against BTMA. The immunological results show that BT-reactive IgE was significantly correlated with DP IgE in the BTMA- group, but not in the BTMA+ group. This indicates that BTMA- patients had IgE cross-reacting between DP and BT allergens. In contrast, the patients in BTMA+ group were more likely to be independently sensitized to both DP and BT. This observation supports that the grouping by BTMA sensitization is rational.

Unlike the clinical responses, the 2 groups showed differences in their immunological responses, which are generally not confounded by placebo effect in AIT studies [33, 34]. The induced cross-reactive IgE is dependent on the BT sensitization. If the patients did not have BT-reactive IgE before treatment, then it was not induced by DP-SCIT. Therefore, the treatment with DP-SCIT did not induce new IgE sensitization. Our study supported low to moderate IgE cross-reactivity between DP and BT, not only before AIT, as previously reported [8, 14], but also while AIT was ongoing.

DP-SCIT gave rise to a 60–80-fold increase in DP-specific IgG₄, and a threefold increase of BT specific IgG₄. Although the BT-specific IgG₄ increase was small, it was statistically significant. This may explain part of the therapeutic effect, similar to the cross-reactive IgG₄ to tree species, such as alder and hazel, induced with birch pollen immunotherapy [35].

The induced BT-reactive IgG₄ showed no difference between the 2 BTMA groups, indicating that the induced IgG₄ is independent of BT sensitization. The correlation of IgE and IgG₄ between BT and DP did not change before and after DP-SCIT in the BTMA group. However, the level of BT- and DP-specific IgE and IgG₄ changed considerably after treatment, especially for BTMA-group. It seems that DP-SCIT induced cross-reactive IgE and IgG₄, but it did not affect the correlation of IgE and IgG₄ between DP and BT during treatment. A probable explanation is that a proportion of induced BT reactive IgE and IgG₄ by DP-SCIT does not change before and after treatment.

In this study, no clinically relevant differences between BTMA+ and BTMA- groups were observed in the improvement of symptoms, medication use and lung function at any investigated time-point during DP-SCIT. Other studies also support that single allergen DP-SCIT

give similar improvement in clinical response on both mono-sensitized and poly-sensitized patients [36, 37].

A recently published study showed no effect on birch pollen-induced allergic symptoms after 4 months of treatment with Timothy grass pollen SLIT-tablet immunotherapy [38]; however, another study of single specie sublingual drop immunotherapy showed significant clinical effect on symptom score and medication use in the birch pollen season of grass pollen immunotherapy and vice versa [39]. Several studies have shown efficacy of single-specie immunotherapy in multisensitized patients, for example, large, randomized, double-blinded, placebo-controlled studies of grass pollen SCIT [40], and house dust mite SLIT-tablet immunotherapy [41]. The practice of treating with only one allergen specie that can be demonstrated to trigger symptoms in multisensitized patients as in Europe, or treat simultaneous with multiple species as in the United States has become a debate [42].

It seems reasonable to speculate, however, that as the allergic reaction has dual phases, an immediate reaction triggered by IgE, and a late phase reaction triggered by eosinophilic inflammation, there is specific as well as general effects. Allergy immunotherapy has a pronounced effect on airway inflammation [43], which is not limited to the allergen used for treatment. This phenomenon has been described as “minimal persistent inflammation” [44, 45], and could account for a more general effect on the airway mucosa in allergic respiratory disease.

In conclusion, DP-SCIT can induce IgG₄ cross-reactive with BT allergens, and single-allergen DP-SCIT seems to be equally effective in patients with IgE sensitizations to only DP or to both DP and BT.

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