

Impact of Sublingual Immunotherapy on Specific Antibody Levels in Asthmatic Children Allergic to House Dust Mites

Nerin N. Bahceciler^a Cigdem Arikan^a Alison Taylor^b Mubeccel Akdis^b
Kurt Blaser^b Isil B. Barlan^a Cezmi A. Akdis^b

^aMarmara University Hospital, Division of Pediatric Allergy and Immunology, Istanbul, Turkey;

^bSwiss Institute of Allergy and Asthma Research, Davos, Switzerland

Key Words

Asthma · House dust mites · Specific IgA · Sublingual immunotherapy

Abstract

Objective: To evaluate the clinical outcome and changes in allergen-specific antibodies during sublingual immunotherapy (SLIT) in house dust mite (HDM)-allergic asthma patients and to compare levels of allergen-specific antibodies in HDM-allergic patients before and after treatment with that of healthy controls. **Method:** Thirty-one asthma patients allergic to HDM were studied. Patients in groups I (n = 17) and II (n = 14) received SLIT with a standardized *Dermatophagoides pteronyssinus* plus *Dermatophagoides farinae* 50/50 extract for 6 and 12 months, respectively. A group of healthy children (n = 8) were enrolled as controls. Patients in both groups were evaluated at the start and at the end of treatment according to daily symptom and medication scores, lung function and skin prick tests, PC₂₀, blood eosinophil count, and Der-p-1-specific IgE, IgA, IgG1 and IgG4 levels. **Results:** Drug consumption decreased significantly in both groups. Furthermore, PC₂₀ and forced expiratory flow between 25 and 75% of vital capacity of patients in group II improved significantly. Although specific IgA, IgG1 and IgG4 levels did not change throughout the treatment period, total eosinophil count and specific IgE decreased

significantly in both groups. According to baseline measurements, specific IgA levels of patients in groups I and II were significantly lower than that of controls. This difference disappeared at the end of the treatment period in both groups. **Conclusion:** SLIT seems to be effective in ameliorating clinical symptoms, drug consumption and bronchial hyperreactivity, and results in downregulation of Der-p-1-specific IgE production. Furthermore, at the end of SLIT, specific IgA levels, which were decreased compared to healthy controls initially, did no longer differ between patients and controls.

Copyright © 2005 S. Karger AG, Basel

Introduction

Specific immunotherapy (IT) administered by the subcutaneous route has been shown to be effective in the treatment of respiratory allergic disorders [1–4]. However, subcutaneous IT has been associated with a certain risk of severe or even fatal systemic reactions [5, 6]. Therefore, alternative routes of allergen administration involving mucosal absorption have been proposed, and it has been suggested that the natural mechanisms underlying the induction of oral tolerance at mucosal surfaces may be an effective therapeutic strategy for the suppression of ongoing pathogenic immune responses in allergic diseases [7].

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2005 S. Karger AG, Basel
1018–2438/05/1363–0287\$22.00/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Dr. Nerin N. Bahceciler
Marmara University Hospital, Division of Pediatric Allergy and Immunology
Nilufer Sokak, 5. Gazeteciler Sitesi C-5 Blok D42
TR–34335 Akatlar Istanbul (Turkey)
Tel./Fax +90 216 326 80 30, E-Mail aonder@attglobal.net

Oral tolerance is a long-recognized mechanism of tolerance induction, and has classically been demonstrated by the specific suppression of cellular and/or humoral immune responses to an antigen by prior administration of the antigen by the oral route. During the investigation of mechanisms associated with oral tolerance, a distinct subset of T cells responsible for the regulation of tolerance was recognized [8].

The positive results of studies evaluating the efficiency of specific IT administered by the sublingual route in patients with rhinitis and asthma symptoms due to house dust mite (HDM) or pollen are encouraging [9–16].

In contrast to animal models, the human immunologic response to sublingual IT (SLIT) has not been intensely studied. In a number of studies, some changes have been found in skin sensitivity [10, 15, 17] and specific IgG4 levels [12, 14, 15, 18], whereas other studies have not found any change in specific IgE [12, 15, 17, 18] and IgG4 levels [16, 17, 19].

In this study, we aimed to evaluate (1) the effect of 6 and 12 months of HDM-specific SLIT on clinical and laboratory outcome measures and on serum levels of specific IgA, IgG1, IgG4 and IgE in asthmatic children allergic to HDM, and (2) to compare HDM-specific IgA, IgG1, IgG4 and IgE levels of HDM-allergic asthmatic children with that of healthy controls.

Material and Methods

Study Population

The study comprised 31 children [(mean $\pm$ SD) age 8.27 ± 2.87 years, female to male ratio = 18/13] with a diagnosis of mild-to-moderate persistent asthma [20] and rhinitis sensitized to HDM, who fulfilled the inclusion criteria listed below and who were monitored at the Division of Pediatric Allergy and Immunology at the outpatient clinic of the Marmara University Hospital. None of the patients included had received IT before. The parents of all the patients signed informed consent forms, and the study was approved by the Ethics Committee of the Hospital.

Inclusion criteria were: (1) need for inhaled corticosteroid (ICS) treatment for the control of asthma symptoms, (2) a positive skin prick test (SPT) to *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, and a negative response to all other aeroallergens tested, (3) age >4 years, and (4) ongoing respiratory symptoms in spite of mite avoidance measures and appropriate ICS treatment.

Eight healthy children [(mean $\pm$ SD) age 8.25 ± 1.90 years, female to male ratio = 4/4] with a negative SPT to all allergens tested were included as controls.

Study Design

After enrollment, patients underwent a run-in period of 8 weeks and were asked to complete daily diary cards throughout this period for assessment of frequency and severity of asthma/rhinitis symp-

oms, dose of inhaled and intranasal steroids needed to control symptoms and frequency of need to use β_2 -mimetics for symptom relief.

At the end of the run-in period, a nasal smear, skin prick, lung function and bronchial challenge tests were performed, and a blood sample was drawn to determine the total eosinophil count and serum levels of Der-p-1-specific IgA, IgG1, IgG4 and IgE. Subsequently, patients received SLIT for either 6 (group I, $n = 17$; mean age $\pm$ SD, 8.99 ± 3.27 years, female/male ratio = 12/5) or 12 months (group II, $n = 14$; mean age $\pm$ SD, 7.56 ± 2.47 years, female/male ratio = 6/8). After initiation of IT, patients were assessed every 4 weeks until the end of the study. At each clinical visit, symptom scores, use of rescue β_2 -mimetics, and compliance to inhaled and intranasal steroid treatment were evaluated with the help of symptom-medication scores. The compliance to SLIT was assessed with another diary card. The dose of inhaled and intranasal steroids needed for the control of symptoms was adjusted by one of the authors at each clinical visit. On the last visit, a nasal smear, skin prick, lung function and methacholine bronchial challenge tests were conducted, and a blood sample was taken to determine the total eosinophil count and serum levels of specific IgA, IgG1, IgG4 and IgE. A group of healthy children were included as controls, and a blood sample was obtained to determine serum levels of HDM-specific IgA, IgG1, IgG4 and IgE.

Treatment

Sublingual Immunotherapy. Treatment consisted of progressively increasing doses of a standardized extract of 50% *D. pteronyssinus* and 50% *D. farinae* (Stallergenes, Antony, Paris, France) followed by maintenance therapy. The strength of standardized allergen extracts was established as 'index of reactivity' (IR). The extract was graded in four strengths: 0.1 IR/ml, 1 IR/ml, 10 IR/ml and 100 IR/ml. The in-house reference extract (labeled 100 IR) was defined as the concentration eliciting a wheal with a mean diameter of 7 mm by SPT in 30 subjects sensitive to the corresponding allergen. The *D. pteronyssinus* and *D. farinae* contents of 1 ml of the 100 IR allergen extract used in this study were 8 and 14 μ g, respectively.

Patients received increasing doses from each vial, starting with one drop from the 0.1 IR/ml vial and increasing to 10 drops on day 7. This process was repeated for the 1 (day 8–14) and 10 IR/ml (day 15–21) vials. On days 22–28, 1–20 drops from the fourth vial (100 IR/ml) were given. Once the 20-drop dose was reached, the patient was switched to maintenance therapy consisting of 20 drops of 100 IR/ml every day for 4 weeks and then 2 times a week until the end of the study period. Drops were taken sublingually in the morning before breakfast, kept under the tongue for at least 2 min and then swallowed. According to this schedule, patients in groups I and II received an average cumulative dose of 7,000 IR (equivalent to 0.56 and 0.98 mg of *D. pteronyssinus* and *D. farinae*, respectively) and 14,000 IR (equivalent to 1.12 and 1.96 mg of *D. pteronyssinus* and *D. farinae*, respectively) at the end of the study.

Inhaled Corticosteroids. According to the protocol of our Allergy Outpatient Clinic, for a newly diagnosed patient our initial dose of ICS is 800 μ g/day of budesonide. Patients are then seen every month with symptom-medication diary cards. When an improvement is achieved (usually after 4 weeks), the dose of ICS is reduced to half the initial dose until the minimum dose which controls the symptoms is determined. All patients continued to receive standard inhalation therapy, i.e. of budesonide daily if necessary and of β_2 -agonists as needed during the study.

Patient Monitoring

Symptom-Medication Score. The parents of all the patients were instructed to keep a diary record card throughout the run-in and treatment periods, for the daily evaluation of lung and nasal symptoms and drug intake. Lung symptoms (cough, dyspnea and wheezing) were recorded on a 0–3 (0 = no symptom, 1 = mild, 2 = moderate and 3 = severe symptoms)-point grading scale. For nasal symptoms (sneezing, rhinorrhea or nasal blockage), 1 point was given if any of the symptoms was present and 0 if not. Prophylactic and rescue drug intake (β_2 -agonists, ICS and intranasal steroids) was recorded on the same diary card daily, too. One point was given if a β_2 -agonist rescue drug was taken on that day and 0 if not. The daily dose of inhaled budesonide and intranasal budesonide prescribed by the physician for the run-in period, the graded dosing period and at the last visit was scored as shown in table 1. From these records, daily means of lung symptoms, nasal symptoms and use of β_2 -mimetics were calculated for the run-in period and the last 4 months of maintenance therapy.

Exacerbations, which occurred during the run-in and treatment periods, were also noted. An exacerbation was defined as an abrupt and/or progressive worsening of symptoms of shortness of breath, chest tightness or some combination of these symptoms which does not respond to regular use of β_2 -agonists for a period of 24 h. The mean number of asthma exacerbations experienced per month during the run-in and treatment periods was calculated for each single patient.

Nasal Smear. A nasal secretion specimen was obtained using a cotton-tipped applicator into the anterior nasal cavity. Then, the adherent mucus was smeared on a glass microscope slide and Wright stain was applied. When 10% or more eosinophils were present, nasal smear examination was judged as positive.

Skin Prick Tests. SPT was done employing a panel of 24 allergen extracts (Stallergenes, Antony, Paris, France) in addition to positive and negative controls (histamine dihydrochloride and saline) on the volar surface of the forearm. A wheal size equal or larger than that obtained with histamine was judged as positive.

Lung Function Tests. All patients performed a spirometry test before and after SLIT. Forced expiratory volume in 1 s (FEV_1), FEV_1 /forced vital capacity (FVC), forced expiratory flow between 25 and 75% of vital capacity (FEF_{25-75}), and peak expiratory flow were measured with a spirometer (Puriton Benett, Nellcor, Calif., USA) according to standardized guidelines. The best of three successful maneuvers was recorded. The results are presented as percent of the value predicted for gender, age and height. Normal lung function test standards of Polgar and Promadhat [21] were used to generate the predicted values.

Bronchial Provocation Test. Nonspecific bronchial reactivity to methacholine was measured with the tidal breathing method [22]. The results were expressed as PC_{20} methacholine, which was obtained from the log dose-response curve by linear interpolation of the last two points.

Quantification of Allergen-Specific Antibodies. The IgE, IgA, IgG1 and IgG4 anti-Der-p-1-specific antibody contents in serum were measured by ELISA [23]. ELISA plates (Maxisorb; Nunc) were coated with 10 μ g/ml Der p 1 overnight in PBS at 4°C. Uncoated parts were blocked with phosphate-buffered saline, pH 7.4, containing 3% Top Block™ and 1% Tween 20 (Sigma-Aldrich, Buchs, Switzerland). Standards and serum samples were added in 8-step 1:2 serial dilutions in duplicate and incubated for 2 h at 4°C. Biotinylated anti-IgE mAb 6–7 (Novartis, Basel, Switzerland) and peroxidase-labeled ExtrAvidine (Sigma) were used to develop IgE anti-Der p 1.

Table 1. Scoring for the dose of inhaled and intranasal budesonide

	Dose, μ g/day	Score
Inhaled budesonide	0	0
	0–200	1
	200–400	2
	400–800	3
	800–1,600	4
	<1,600	5
Intranasal budesonide	0	0
	50	1
	100	2
	200	3

Anti-IgG4 mAb PJ4 (Oxoid, Basingstoke, UK) and peroxidase-labeled anti-mouse immunoglobulin antibodies (Tago, Burlingame, Calif., USA) were used in IgG4 anti-Der p 1 ELISA. Mouse anti-human IgG1 mAb (Zymed, San Francisco, Calif., USA), goat anti-human IgA (Chemicon, Hofheim, Germany) and appropriate peroxidase-labeled secondary antibodies were used to detect IgG1 and IgA anti-Der p 1 antibodies. Specific antibody binding to coated plates was controlled with hydrolyzed milk powder and human serum albumin (Sigma). Mouse and human mAbs to different antigens were used as negative controls. Uncoated parts were blocked with PBS, pH 7.4, containing 3% Top Block (Sigma) and 1% Tween 20. A serum pool from four Der-p-1-allergic patients was used as a standard. High amounts of Der-p-1-specific antibody-containing serums were selected separately for each of the antibody isotype or subtype. The cutoff value for allergen-specific antibodies was determined using absorbance (mean +3 SD) obtained in control wells (8 wells for each antibody), to which only dilution buffer (1% BSA in PBS) was added.

Statistical Analysis

Statistical analyses were performed with SPSS (version 5.0). Comparisons between groups were made using unpaired t, Mann-Whitney U, χ^2 and Kruskal-Wallis tests. Changes within groups were tested for significance with paired t, McNemar's χ^2 and Wilcoxon signed ranks tests. A p value of less than 0.05 was considered significant.

Results

HDM-SLIT Improves Clinical Features in Children

All of the 31 patients included completed the study. Seventeen of them received SLIT for 6 months (group I) and 14 for 12 months (group II). At baseline, the treatment groups and healthy controls were comparable regarding age and gender (table 2). Table 3 demonstrates changes in all outcome measures from baseline to final follow-up visit of groups I and II.

Table 2. Comparison of the demographic characteristics of the treatment (groups I and II) and control groups

Characteristics	Group I (n = 17)	Group II (n = 14)	Controls (n = 8)	p value
Age, years	8.9 ± 3.3	7.6 ± 2.5	8.3 ± 1.9	0.82 ¹
Gender, females/males	12/5	6/8	4/4	0.22 ²
Duration of ICS, months	22.7 ± 17.26	17 ± 10.77	–	
Duration of symptoms, months	38.8 ± 26.35	56.14 ± 29.43	–	
Means ± SD.				
¹ Kruskal-Wallis test.				
² χ^2 test.				

Table 3. Changes in outcome measures from baseline to the final follow-up visit in groups I and II

Characteristics	Group I		Group II	
	baseline	6 months	baseline	1 year
<i>Lung function tests</i>				
FVC, %	97.81 ± 10.83	100.4 ± 13.86	102.5 ± 8.4	105.4 ± 15.5
FEV ₁ /FVC, %	93.30 ± 5.46	91.6 ± 5.68	93.8 ± 8	92.45 ± 6.48
FEV ₁ , %	90.83 ± 9.340	94.00 ± 9.42	95.5 ± 8.5	96.5 ± 9.3
FEF _{25–75} , %	85.50 ± 15.36	86.00 ± 18.28	77.3 ± 10.5	92.7 ± 17.9 ¹
PEF, %	90.83 ± 13.70	88.66 ± 20.21	88.5 ± 12.77	87.4 ± 18.5
PC ₂₀ , mg/ml	0.84 (0.01–3.5)	1.4 (0.12–32) ²	0.93 (0.16–16)	2.5 (0.38–16) ²
<i>Laboratory</i>				
<i>D. farinae</i> , wheal size, mm	5.29 ± 1.64	5.29 ± 2.02	4.0 ± 2.25	4.57 ± 1.7
<i>D. pteronyssinus</i> , wheal size, mm	5.41 ± 1.62	6.05 ± 2.13	5.3 ± 1.9	5.2 ± 2.8
Total eosinophil count, mm ³	248.52 ± 224.38	113.23 ± 53.84 ¹	314.6 ± 212.6	85.7 ± 58.6 ¹
Nasal smear, positive/negative	11/1	11/1	12/0	4/1
<i>Symptom/medication</i>				
ICS dose	3 (0–3)	1 (0–3) ²	3 (2–4)	0 (0–2) ²
INS dose	2 (0–3)	1 (0–2) ²	3 (0–3)	0 (0–2) ²
Asthma score	0.78 (0–1.82)	0.21 (0–1.5)	1.12 (0.8–2.8)	0.12 (0–0.64) ²
Rhinitis score	0.39 (0–1)	0.1 (0–0.89)	0.86 (0.13–2.8)	0 (0–2) ²
Asthma attacks	2 (0–4)	0 (0–3) ²	1.5 (0–4)	0 (0–1) ²
β_2 -Mimetic use score	0.28 (0–1)	0 (0–0.28) ²	0.19 (0–0.71)	0 (0–0.14) ²
<i>Der-p-I-specific antibodies</i>				
Specific IgG1, U/ml	11.75 (3.47–119.05)	20.45 (1.55–81.87)	8.87 (0.00–134.40)	11.15 (1.33–163.65)
Specific IgG4, U/ml	10.21 (2.2–103.8)	10.28 (1.8–98.18)	3.06 (0.32–15.09)	2.62 (0.55–223.25)
Specific IgE, U/ml	100 (9–100)	7.8 (0–152) ²	24.6 (1.7–88.9)	3.4 (0–8.9) ²
Specific IgA, U/ml	0.15 (0–10.71)	0.24 (0–32.6)	0.14 (0–3.23)	0.40 (0.007–32.61)

Data are presented as medians (ranges) and means ± SD.

¹ Statistically significant, paired t test.

² Statistically significant, Wilcoxon signed ranks test.

INS = Intranasal steroid.

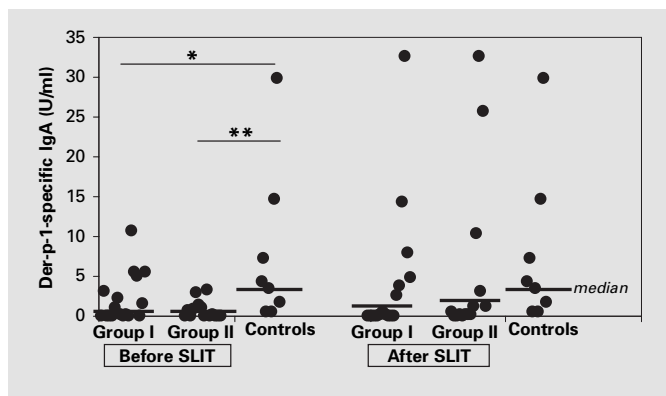


Fig. 1. Der-p-1-specific IgA levels before and after SLIT. Serum Der-p-1-specific IgA was measured in groups I and II before and after 6 and 12 months of HDM-SLIT and in healthy controls. * $p = 0.03$, group I vs. controls, and ** $p = 0.003$ group II vs. controls. Due to increases in Der-p-1-specific IgA, there was no significant difference between patients and controls after SLIT (table 3).

Symptom Scores. Although asthma and rhinitis symptom scores did not show significant improvement in group I, there was a statistically significant decrease in those parameters in group II ($p = 0.001$, respectively, Wilcoxon signed ranks test). On the other hand, the number of asthma exacerbations decreased significantly in both groups when compared to baseline values ($p = 0.0001$ and $p = 0.005$, respectively, Wilcoxon signed ranks test; table 3).

Medication. In both groups, mean daily use of β -mimetic drugs decreased significantly compared to baseline at the end of the treatment period ($p = 0.001$ and $p = 0.003$, respectively; Wilcoxon signed ranks test). In addition, there was a significant decrease in the mean daily dose of inhaled ($p = 0.001$, respectively, Wilcoxon signed ranks test) and intranasal steroids ($p = 0.008$ and $p = 0.001$, respectively, Wilcoxon signed ranks test) in both treatment groups (table 3).

Lung Function and Bronchial Hyperreactivity. Although no improvement was detected in the lung function of patients who received SLIT for 6 months (group I), FEF_{25–75} values of patients treated for 12 months increased significantly ($p = 0.007$, paired t test). On the other hand, PC₂₀ values significantly improved in both treatment groups ($p = 0.04$ and $p = 0.003$, Wilcoxon signed ranks test; table 3).

Blood and Nasal Eosinophilia and Type 1 Skin Reactivity

At the end of the 6- and 12-month treatment periods, the total number of circulating eosinophils had decreased

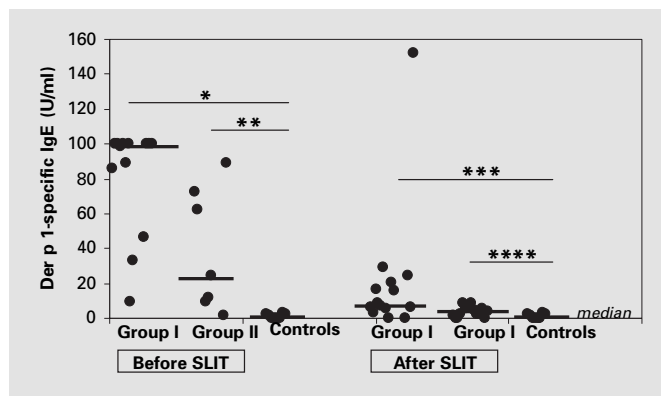


Fig. 2. Specific IgE levels before and after treatment compared to controls. Serum Der-p-1-specific IgE was determined in groups I and II before and after 6 and 12 months of HDM-SLIT and in healthy controls (table 3). Before treatment: * $p = 0.0001$, group I vs. controls, ** $p = 0.004$, group II vs. controls. After treatment: *** $p = 0.002$, group I vs. controls, **** $p = 0.03$, group II vs. controls.

significantly in both groups compared to baseline ($p = 0.02$ and $p = 0.002$, respectively, paired t test). However, skin sensitivity to *D. pteronyssinus* and *D. farinae* did not change significantly in either group (table 3). In contrast, eosinophil-positive nasal smears did not show significant differences at the end of the 6- and 12-month treatment periods in either group (table 3).

Regulation of Specific Antibody Response

To overcome the problems of impurities, non-specific adhesive molecules and non-antigenic contents of singular HDM extracts, we used purified Der p 1 for the detection of antibodies. A group of healthy controls was included for comparison of Der-p-1-specific IgA, IgG1, IgG4 and IgE levels with specific antibody values before and after treatment. According to baseline measurements, specific IgA levels of subjects in groups I and II were significantly lower (fig. 1) and specific IgE and IgG1 levels were significantly higher than in controls (fig. 2, 3). No significant difference was detected in specific IgG4 levels at baseline (fig. 4). After treatment, the only change detected in the comparison of levels was that the significant difference between treatment and control groups regarding specific IgA levels had vanished (fig. 1; $p = 0.74$ for group I and $p = 0.76$ for group II).

Within group comparisons revealed that, although Der-p-1-specific IgA, IgG1 and IgG4 levels did not change, serum-specific IgE levels had decreased significantly in both treatment groups compared to baseline (table 3, fig. 2; $p = 0.005$ and $p = 0.02$, respectively).

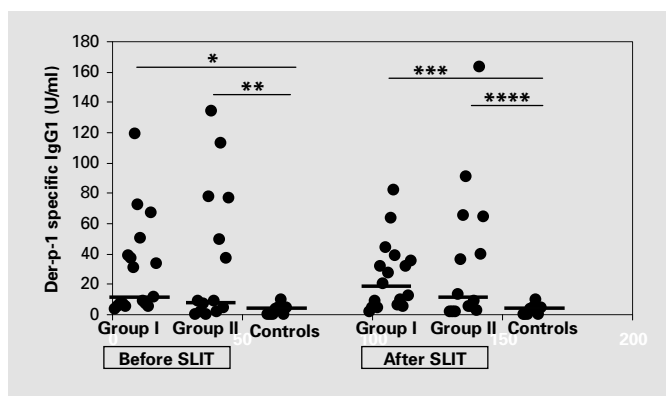


Fig. 3. Specific IgG1 in groups I and II before and after treatment compared to controls. Serum Der-p-1-specific IgG1 was measured in groups I and II before and after 6 and 12 months of HDM-SLIT and in healthy controls. Before treatment: * $p = 0.0001$, group I vs. controls, ** $p = 0.02$, group II vs. controls. After treatment: *** $p = 0.0001$, group I vs. controls, **** $p = 0.01$, group II vs. controls. HDM-specific SLIT did not change IgG1 production in either treatment group (table 3).

Discussion

Today, allergen-specific IT is accepted as the only means that can both alter the outcome of the disease and prevent the formation of new sensitization [24–26]. Sublingual specific IT, which is based on oral desensitization, is another promising application in terms of reliability and efficacy.

In the current study, we observed a significant improvement in drug consumption and the number of asthma exacerbations as early as 6 months. Furthermore, asthma and rhinitis scores improved significantly in the group treated for 12 months.

Bronchial hyperreactivity (BHR) is the main characteristic of the disease [27, 28] and is accepted as one of the parameters that reflect severity. The effect of specific IT on respiratory function tests and BHR is still subject to debate. This study demonstrated a favorable effect of SLIT on BHR in both treatment groups. Furthermore, a significant improvement was achieved in FEF_{25–75} values in the 12-month treatment group. The number of studies investigating the effect of SLIT on BHR and respiratory function tests is limited. In the study by Bousquet et al. [14], respiratory function and asthma symptom scores significantly improved, and PD₂₀ values displayed a 1.75-fold increase after 24 months of SLIT treatment.

In our study, skin reactivity to *D. pteronyssinus* and *D. farinae* did not show any significant change throughout

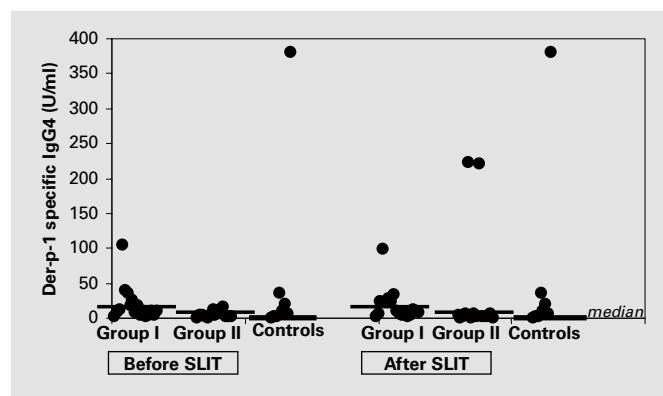


Fig. 4. Specific IgG4 levels before and after treatment compared to controls. Serum Der-p-1-specific IgG4 was measured in groups I and II before and after 6 and 12 months of treatment and in healthy controls. There were no significant differences in IgG4 levels of the patients before and after treatment and controls (table 3).

the study period, whereas HDM-specific IgE levels and total eosinophil counts decreased significantly in both treatment groups. On the other hand, specific IgA, IgG1 and IgG4 levels did not change significantly compared to baseline in either treatment group. In a number of studies, a significant reduction in skin reactivity [10, 15, 17] and a significant increase in specific IgG4 levels [12, 14, 15, 18] were detected following SLIT. On the other hand, other studies have not found any change in specific IgE [12, 15, 17, 18] and IgG4 levels [16, 17, 19]. The decrease in specific IgE levels detected in our study could not be due to the environmental HDM measurements, since all those patients had to meet one of the inclusion criteria at enrollment, which was ‘ongoing respiratory symptoms in spite of mite avoidance measures’.

In one of the first studies on SLIT in children with asthma and rhinitis sensitized to HDM, which was conducted in a double-blind placebo-controlled fashion, our group had reported the efficacy of SLIT. The results of this study showed significant improvement at the end of the 6-month follow-up in asthma scores, use of inhaled β_2 -mimetics, daily dose of intranasal steroids and in the number of asthma exacerbations in the SLIT group, but not in the placebo group [10]. After having shown the clinical efficacy of SLIT, in a subsequent study we aimed to delineate the mechanisms of action in addition to clinical efficacy of this treatment modality. In that study, Der-p-1-stimulated IFN- γ and phytohemagglutinin-stimulated

IL-12 levels were significantly higher in supernatants of peripheral blood mononuclear cells in parallel to the improvement in the parameters of clinical outcome (symptom score, quality of life, number of asthma attacks, inhaled and intranasal steroid and β_2 -agonist use, PC₂₀ and blood eosinophil counts) following 6 months of SLIT [29]. In the current study, we investigated the effects of SLIT on HDM-specific immunoglobulin production, as well as clinical outcome. We acknowledge the fact that the limitation of this study is that it is not a double-blind placebo-controlled study, but an open uncontrolled trial.

Another objective of the current study was to compare specific IgA, IgG1, IgG4 and IgE levels of children allergic to HDM to a group of non-atopic healthy children with comparable age and gender. According to baseline measurements, specific IgG1 and IgE were significantly higher, whereas specific IgA was significantly lower than that of controls in HDM-allergic asthmatic children. At the end of 6- and 12-month treatment periods, Der-p-1-specific IgA of groups I and II were not significantly lower than in healthy controls. These data demonstrate that HDM-allergic asthma is characterized by increased allergen-specific IgE and IgG1 and decreased allergen-specific IgA responses. HDM-SLIT suppresses allergen-specific IgE and may have a stimulatory effect on allergen-specific IgA production.

Although the clinical efficacy and safety of SLIT has been established by a number of controlled trials, the mechanism of action of SLIT is not well known. One of the current hypotheses is induction of tolerance by Langerhans cells. These cells internalize antigens in the sub-

lingual mucosa before migrating to the regional lymph nodes, where they act as antigen-presenting cells. This may result in T-cell response modifications responsible for oral tolerance [30–32]. Animal experiments point to mucosal vaccination with antigens being potential candidates for mucosal tolerance induction [33]. This seems to involve induction of T-regulatory cells [34, 35]. These cells provide help for IgA and IgG4 production and primarily secrete TGF- β and IL-10. The most likely explanation for the mechanism of specific IgA induction could be due to the establishment of mucosal tolerance and production of TGF- β . Therefore, further investigations are warranted to study the role of T-regulatory cells and TGF- β in allergic subjects treated with SLIT.

The results of our study demonstrated that HDM-allergic asthma is characterized by higher specific IgE and IgG1 levels and a lower specific IgA response compared to healthy children. At the end of the 6- and 12-month SLIT periods, the increased specific IgE response was downregulated. In addition, lower IgA levels did no longer differ compared to controls at the end of therapy. These findings were accompanied by accumulating evidence for the clinical efficacy, medication-sparing effect and improvement in BHR following SLIT.

Acknowledgments

This work was supported by the Swiss National Science Foundation (grants 32.65661 and 31.65436). The authors thank Dr. H. Fiebig, Allergopharma AG, Reinbek, Germany, for providing Der p 1.

References

- 1 Bousquet J, Hejjaoui A, Skassa-Brociek W, Guerin B, Maasch HJ, Dhivert H, Michel FB: Double-blind, placebo-controlled immunotherapy with mixed grass pollen allergoids. I. Rush immunotherapy with allergoids and standardized orchard grass-pollen extract. *J Allergy Clin Immunol* 1987;80:591–598.
- 2 Hedlin G, Silber G, Naclerio R, Proud D, Lamas AM, Eggleston P, Adkinson NF Jr: Comparison of the in vivo and in vitro response to ragweed immunotherapy in children and adults with ragweed-induced rhinitis. *Clin Exp Allergy* 1990;20:491–500.
- 3 Warner JO, Price JF, Soothill JF, Hey EN: Controlled trial of hyposensitization to *Dermatophagoides pteronyssinus* in children with asthma. *Lancet* 1978;ii:912–915.
- 4 Machiels JJ, Somville MA, Lebrun PM, Lebecque SJ, Jacquemin MG, Saint-Remy JM: Allergic bronchial asthma due to *Dermatophagoides pteronyssinus* hypersensitivity can be efficiently treated by inoculation of allergen-antibody complexes. *J Clin Invest* 1990;85:1024–1035.
- 5 Committee on Safety of Medicines: Desensitizing vaccines. *BMJ* 1986;293:948.
- 6 Reid MJ, Lockkey RF, Turkeltaub PC, Platts-Mills TA: Survey of fatalities from skin testing and immunotherapy 1985–1989. *J Allergy Clin Immunol* 1993;92:6–15.
- 7 Holt PG: Immunoprophylaxis of atopy. Light at the end of the tunnel? *Immunol Today* 1994; 15:484–489.
- 8 Howard LW: Induction and mechanism of action of transforming growth factor- β -secreting Th3 regulatory cells. *Immunol Rev* 2001;182: 207–214.
- 9 Vourdas D, Syrigou E, Pontamianou P, Carat F, Batard T, Andre C: Double-blind, placebo-controlled evaluation of sublingual immunotherapy with standardized olive pollen extract in pediatric patients with allergic rhinoconjunctivitis and mild asthma due to olive pollen sensitization. *Allergy* 1998;53:662–672.
- 10 Bahceciler NN, Isik U, Barlan IB, Basaran MM: Efficacy of sublingual immunotherapy in children with asthma and rhinitis: A double-blind, placebo-controlled study. *Pediatr Pulmonol* 2001;22:618–626.
- 11 Tari MG, Mancino M, Monti G: Efficacy of sublingual immunotherapy in patients with rhinitis and asthma due to house dust mite. A double-blind study. *Allergol Immunopathol (Madrid)* 1990;18:277–284.

- 12 Troise J, Voltolini S, Canessa A, Pecora S, Negrini AC: Sublingual immunotherapy in *Parietaria* pollen-induced rhinitis: A double-blind study. *J Invest Allergol Clin Immunol* 1995;5:25–30.
- 13 Casanovas M, Guerra F, Morena C, Miguel R, Maranon F, Daza JC: Double-blind, placebo-controlled clinical trial of preseasonal treatment with allergenic extracts of *Olea europaea* pollen administered sublingually. *J Invest Allergol Clin Immunol* 1994;4:305–314.
- 14 Bousquet J, Scheinmann P, Guinneeppain MT, Perrin Fayolle M, Sauvaget J, Tonnel AB, Pauli G, Caillaud D, Dubost R, Leynadier F, Vervoet D, Herman D, Calvin S, Andre C: Sublingual-swallow immunotherapy (SLIT) in patients with asthma due to house dust mites: A double-blind, placebo-controlled study. *Allergy* 1999;54:249–260.
- 15 La Rosa M, Ranno C, Andree C, Carat F, Tosca MA, Canonica GW: Double-blind placebo-controlled evaluation of sublingual-swallow immunotherapy with standardized *Parietaria judaica* extract in children with allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 1999;104:425–432.
- 16 Guez S, Vatrinet C, Fadel R, Andre C: House-dust-mite sublingual-swallow immunotherapy (SLIT) in perennial rhinitis: A double-blind, placebo-controlled study. *Allergy* 2000;55:369–375.
- 17 Pajno GB, Morabito L, Barberio G, Parmiani S: Clinical and immunological effects of long-term sublingual immunotherapy in asthmatic children sensitized to mites: A double-blind, placebo-controlled study. *Allergy* 2000;55:842–849.
- 18 Mungan D, Misirligil Z, Gurbuz L: Comparison of the efficacy of subcutaneous and sublingual immunotherapy in mite-sensitive patients with rhinitis and asthma – A placebo controlled study. *Ann Allergy Asthma Immunol* 1999;82:485–490.
- 19 Quirino T, Iemoli E, Siciliani E, Parmiani S, Milazzo F: Sublingual versus injective immunotherapy in grass pollen allergic patients: A double-blind (double dummy) study. *Clin Exp Allergy* 1996;26:1253–1261.
- 20 National Heart, Lung and Blood Institute: International Consensus Report on Diagnosis and Management of Asthma. Bethesda, US Department of Health and Human Services, 1992, Publication No. 92 3091.
- 21 Polgar P, Promadhat V: Pulmonary Testing in Children. Philadelphia, Saunders, 1971, pp 100–153.
- 22 Sterk PJ, Fabbri LM, Quanjer PhH, Cockcroft DW, O'Byrne PM, Anderson SD, Juniper EF, Malo JL: Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 1993;16:53–83.
- 23 Akdis AC, Akdis M, Blesken T, Wymann D, Alkan SS, Blaser K: Epitope specific T cell tolerance to phospholipase A2 in bee venom immunotherapy and recovery by IL-2 and IL-5 in vitro. *J Clin Invest* 1996;98:1676–1683.
- 24 Mohapatra SS, Juan HS: Novel immunotherapeutic approaches for the treatment of allergic diseases. *Immunol Allergy Clin North Am* 2000;20:625–642.
- 25 Bousquet J, Lockey RF, Malling H-J (eds): World Health Organization Position Paper: Allergen Immunotherapy: Therapeutic vaccines for allergic diseases. *Allergy* 1998;53(suppl 44):1–42.
- 26 Ledford DK: Efficacy of immunotherapy. *Immunol Allergy Clin North Am* 2000;20:503–525.
- 27 Williams PV: Inhalation bronchoprovocation in children. *Immunol Allergy Clin North Am* 1998;18:133–148.
- 28 Cockcroft DW: Nonallergic airway responsiveness. *J Allergy Clin Immunol* 1998;81:111–119.
- 29 Arikian C, Bahceciler NN, Deniz G, Akdis M, Akkoc T, Akdis CA, Barlan IB: Bacillus-Calmette-Guerin-induced interleukin-12 did not additionally improve clinical and immunologic parameters in asthmatic children treated with sublingual immunotherapy. *Clin Exp Allergy* 2004;34:398–405.
- 30 Passalacqua G, Bagnasco M, Mariani G, Falagiani P, Canonica G: Local immunotherapy: Pharmacokinetics and efficacy. *Allergy* 1998;53:477–484.
- 31 Van Wilsem EJJ, Van Hoogstraaten MW, Scheper RBJ, Kraal G: Dendritic cells of the oral mucosa and the induction of oral tolerance. A local affair. *Immunology* 1994;83:128–132.
- 32 Strobel S, Mowat AM: Immune response to dietary antigens: Oral tolerance. *Immunol Today* 1998;12:156–161.
- 33 Wiedermann U, Kraft D: Induction of mucosal tolerance to Bet V 1, the major birch pollen allergen. *Res Trends* 2002;17–24.
- 34 Chen Y, Kuchro UK, Inobe J, Hafler DA, Weiner HL: Regulatory T cell clones induced by oral tolerance: Suppression of autoimmune encephalomyelitis. *Science* 1994;265:1237–1240.
- 35 Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, De Vrieg JE, Roncanolo MG: A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 1997;389:737–742.