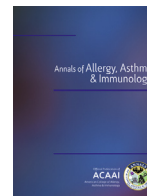




Contents lists available at ScienceDirect



Clinical and cytokine responses to house dust mite sublingual immunotherapy

Paul C. Potter, MD, MBChB, DCH, FCP^{*}; Sheila Baker, MSc^{*}; Bartha Fenemore, Dip Med Tech^{*}; and Barbara Nurse, PhD[†]

^{*} Allergy Diagnostic and Clinical Research Unit, University of Cape Town Lung Institute, Cape Town, South Africa

[†] Division of Immunology, National Health Laboratory Service and Department of Clinical Laboratory Sciences, University of Cape Town, Cape Town, South Africa

ARTICLE INFO

Article history:

Received for publication September 22, 2014.

Received in revised form November 28, 2014.

Accepted for publication December 22, 2014.

ABSTRACT

Background: Cytokine responses accompanying sublingual immunotherapy (SLIT) responder phenotypes have not previously been reported.

Objective: To investigate clinical and cytokine responses of house dust mite (HDM) sensitive patients with allergic rhinitis receiving HDM SLIT or placebo for 2 years.

Methods: Sixty adults were randomized to receive SLIT or placebo. Clinical symptoms were measured using the Total 5 Symptom Score (TSS5) and Juniper Rhinitis Quality of Life Questionnaire. HDM specific IgE, IgG, skin prick tests, and HDM-stimulated release of interleukin (IL) 5 and interferon γ (IFN- γ) in peripheral blood mononuclear cells was studied at 0, 6, 12, and 24 months and IL-13, IL-4, and IL-10 at 0 and 24 months.

Results: A total of 32 of 39 SLIT and 16 of 21 placebo patients completed the study. There was significant clinical improvement in both the SLIT and placebo groups. Median TSS5 decreased from 14.75 to 5.25 in the SLIT group ($P < .001$) and 12.7 to 6.0 in the placebo group ($P = .003$). The median quality-of-life score also decreased in the SLIT group ($P < .001$) and the placebo group ($P < .001$). A subgroup analysis of patients found a 60% or greater improvement (on the TSS5 and the Juniper Rhinitis Quality of Life Questionnaire) in the good responders group and a 30% to 59% improvement or no improvement in the intermediate responders group. This subgroup analysis also found more good responders in the SLIT group (47%) compared with the placebo group (25%; $P = .07$). Significant decreases in the IL-5/IFN- γ ($P < .001$), IL-13/IFN- γ ($P < .001$), and IL-4/IFN- γ ($P = .03$) ratios were found in the combined good clinical improvement group at 24 months.

Conclusion: A good clinical response ($\geq 60\%$ improvement in both TSS5 and quality of life) is associated with significant decreases in IL-5, IL-13, and IL-4 relative to IFN- γ during 2 years of SLIT therapy for HDMs.

© 2015 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Introduction

Meta-analyses have found sublingual immunotherapy (SLIT) to be effective in adults^{1–4} and children^{4,5} with allergic rhinitis, but immunologic markers accompanying a responder phenotype after SLIT have not yet been reported. There are more studies of clinical efficacy in patients receiving SLIT for pollen allergies than for house dust mite (HDM) sensitivity^{3,6}; thus, recent reviews^{2–4} have called for more clinical trials of HDM SLIT vaccines.

SLIT for mites has been reported to be effective for rhinoconjunctivitis.⁷ In a study of SLIT in rhinitis, Passalacqua et al⁸ observed a significant reduction in the total symptom scores in

the first year but not in the second year of treatment, although relief of nasal obstruction was significantly improved in both years.

Little is known about the natural history of disease in patients with HDM-induced persistent allergic rhinitis. It is clear from previous placebo-controlled HDM SLIT studies that some patients improve, even with placebo treatment,^{3,8} but other studies have found a lack of improvement.⁹ Because patients sensitive to HDM vary with respect to severity, organ involvement, and immune responses to different HDM allergens, the World Allergy Organization¹⁰ has outlined published guidelines for conducting clinical trials using immunotherapy and emphasized the need for the determination of other outcomes of efficacy, such as quality-of-life (QOL) analysis, and the need for more intensive studies of immunologic parameters accompanying immunotherapy, which could be identified as markers of response.

Reprints: Paul C. Potter, MD, MBChB, DCH, FCP, Allergy Diagnostic and Clinical Research Unit, University of Cape Town Lung Institute, George Street, Mowbray, 7700, PO Box 34560, Groote Schuur, 7937, South Africa; E-mail: Paul.Potter@uct.ac.za.

Disclosures: Authors have nothing to disclose.

<http://dx.doi.org/10.1016/j.anai.2014.12.015>

1081-1206/© 2015 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Our study investigated the association among clinical symptom scores; QOL; allergen HDM specific IgE, IgG, and interleukin (IFN- γ) secretion of IL-5, IL-13, IL-4, IL-10, and IFN- γ by HDM-stimulated peripheral blood mononuclear cells (PBMCs) in vitro in patients with rhinitis receiving double-blind, placebo-controlled, 2-year SLIT treatment with a *Dermatophagoides pteronyssinus* sublingual vaccine.

Methods

Patient Selection

Patients aged 18 to 60 years, male or female, with HDM-sensitive persistent or perennial rhinitis for at least 2 years, who manifested blocked nose, runny nose, itchy throat, and/or itchy eyes or palate were enrolled. Patients with mild asthma symptoms were not excluded. At the screening visit, patients underwent skin prick tests for 6 allergens (Der p, Bermuda grass, cat, dog, *Alternaria*, and a 5-grass mix [Stallergenes, Antony, France]). Only patients who tested positive for Der p sensitivity to HDMs as confirmed by a positive skin prick test result, manifesting as a wheal of 4 mm or greater to a *D pteronyssinus* extract (Der p 1 mix), were randomized. Patients were excluded if they had a history of seasonal allergic rhinitis due to spring or summer allergens (tree or grass pollens), were known to be polysensitive, were pregnant, required daily corticosteroid treatment for asthma, or had cardiovascular disease, hypertension, or treatment with β -blockers. The study was mono-center, double-blind, placebo controlled, and approved by the Ethics and Research Committee of the University of Cape Town.

Study Design

Sixty patients (age range, 18–60 years) were randomized (2:1) into active (39) and placebo (21) groups. The active group received Der p HDM SLIT using Staloral 300 index of reactivity units (IR)/mL (Stallergenes) and stepping up treatment with daily doses during the first 4 weeks (titrating 1 to 100 IR/mL during this period) until the maintenance dose of 10 sprays (0.1 mL per spray) of 300 IR/mL of SLIT was achieved and continued to receive treatment 3 days a week for the rest of the 2-year study period. Study participants were recruited during a 6-month period. They were allowed to continue taking their usual baseline medications of intranasal corticosteroids, antihistamines, or bronchodilators as needed.

Symptoms were scored daily by the patients using a diary card for 2 weeks before the commencement of active and placebo treatment (randomization visit) and taken as baseline. Patients were followed up, and symptom scores assessed at 2 weeks, 4 weeks, 8 weeks, 12 weeks, 6 months, 9 months, 12 months, 18 months, and 24 months. The diary cards were completed daily during the first 6 weeks after randomization and on 3 days a week during the 2 weeks before the subsequent follow-up visits (8-week to 24-month visits). All patients were telephoned 2 weeks before the follow-up visits to remind them to complete their diary cards. Adherence for the study population was assessed during the first 6 weeks by summing the daily consumption of IRs.

At each visit, a physical examination was performed including careful examination of the nose and throat. The following were recorded: current rhinitis treatment, review of the diary card recordings of the Total 5 Symptom Score (T5SS; mean daily total of 5 individual graded scores for sneezing; runny nose; itchy ear, nose, or throat; ocular symptoms; and nasal congestion; each scored on a range of 0–5, with 0 indicating absent and 5 indicating intolerable; maximum score, 25), the Total Nonnasal Symptom Score (TNSS; including headache, fatigue, concentration, and irritability scores; maximum score, 20), adverse events, and concomitant medications. Global evaluation of rhinitis symptoms by the investigator and the patient used a visual analog scoring system on a scale of 0 to 10, with 0 indicating absent and 10 indicating very severe.

The QOL was assessed with the Juniper Rhinitis Quality of Life Questionnaire.¹¹ The global QOL was the sum of the scores for the individual domains of activity, nasal, non-hay fever, ocular, sleep, emotional, and practical. The QOL was evaluated as part of the source notes at baseline and at 2 years in patients who completed the trial.

Blood samples were taken for lymphocyte and immunoglobulin studies at baseline, 6 months, 12 months, and 24 months. Der p specific IgE and IgG levels, as well as total IgE levels, were measured using the Pharmacia ImmunoCap System (Pharmacia, Uppsala, Sweden). Samples were batched and stored to avoid batch to batch variations in the results. Skin prick tests to Der p 1 extract were performed at the screening, 12-month, and 24-month visits.

HDM Stimulation of PBMCs

PBMCs were isolated and cultured¹² (2×10^5 /200 μ L of RPMI with 10% AB serum per well for 8 days) with and without HDM extract (200 μ g/mL of total protein) at baseline (0), 6, 12, and 24 months. IL-5/IFN- γ release and proliferation were studied at baseline (0), 6, 12, and 24 months. Pilot experiments (1–9 days) revealed that, at this concentration of HDM, proliferation and release of IFN- γ and IL-5 was maximal at 8 days. Cytokines IL-13, IL-4, and IL-10 were also measured at 0 and 24 months. Culture supernatants (pooled from 5–7 wells per individuals) were stored at -80°C .

HDM extract in phosphate-buffered saline was prepared from lyophilized spent Der p culture (Dome/Hollister-Stier, Spokane, WA) and filter sterilized. Protein concentrations were determined using a Pierce BCA Kit (Thermo Fisher Scientific, Waltham, MA).

Proliferation was assessed in parallel cultures pulsed with titrated thymidine (1 μ Ci per well; 5 Ci/mmol; Amersham Pharmacia, Piscataway, NJ) after 7 days and harvested 18 hours later. Results from quadruplicate cultures are expressed as δ disintegrations per minute (HDM-stimulated proliferation minus spontaneous proliferation).

Cytokines were measured using anti-human monoclonal antibody enzyme-linked immunosorbent assay (ELISA) pairs with recombinant human cytokines as standards (BD Pharmingen, San Jose, CA). Coating and capture antibody was paired with the appropriate biotinylated detection antibody. Assays were performed essentially as described in Pharmingen's ELISA protocol except that AMDEX streptavidin–horseradish peroxidase (Amersham Pharmacia) and tetramethylbenzidine substrate (Kirkegaard & Perry Laboratories, Inc, Gaithersburg, Maryland) were used. Color reactions were amplified by stopping with phosphoric acid and were read at 450 nm (reference filter, 540 nm). The sensitivity of the ELISAs was 15 pg/mL for IL-5; 2 pg/mL for IFN- γ , IL-13, and IL-10; and 0.2 pg/mL for IL-4. Cytokine levels below the sensitivity of the assays were assigned values of 7 pg/mL (IL-5), 1 pg/mL (IFN- γ , IL-13, and IL-10), and 0.1 pg/mL (IL-4) for statistical purposes.

Written informed consent was obtained from each patient according to International Conference on Harmonisation/Good Clinical Practice Declaration of Helsinki guidelines.

Statistical Analysis

Because the clinical data and the PBMC proliferation and cytokine release data were not normally distributed, nonparametric tests were used. The Wilcoxon matched pairs test was used to compare 2 dependent groups and the Mann-Whitney test for 2 independent groups. The Kruskal-Wallis test was used to compare 3 groups (multiple comparisons 2-tailed *P* values). The Spearman rank order test was used to assess correlations. The percentage difference test was used to determine the difference between 2 proportions. A STATISTICA program, release 9 (StatSoft, Inc, Tulsa, OK), was used. *P* < .05 were considered statistically significant.

Table 1

Values of the overall changes in clinical and serologic parameters at baseline and after 2 years of house dust mite sublingual immunotherapy in the active and placebo groups

Parameter	Baseline	2 years
Total 5 Symptom Score, mean		
Active	14.75	5.25
Placebo	12.7	6.0
Total Nonnasal Symptom Score, mean (range)		
Active	7.1	1.75 (0.17–4.0)
Placebo	4.36	3.17 (0.05–6.08)
Investigation global rhinitis score, mean		
Active	8.1	2.4
Placebo	8.1	2.95
Patient global rhinitis score, mean		
Active	8.0	3.00
Placebo	7.1	2.50
Juniper Quality of Life Questionnaire score, mean		
Active	156	55
Placebo	127	60
Wheal diameter for <i>Dermatophagoides pteronyssinus</i> skin prick test, mean, mm		
Active	6.2	5.8
Placebo	6.8	6.5
Specific <i>D pteronyssinus</i> IgE, median (range), kU/L		
Active	13.8	21.1 (0.3–772)
Placebo	11.6	6.2 (0.4–137)
Der p 1 specific IgG, median (range), kU/L		
Active	30.8	17.8 (6.4–53.7)
Placebo	26.8	16.8 (4.5–36.7)
Total serum IgE, median (range), kU/L		
Active	152	181 (37.7–8040)
Placebo	222	147 (40.1–827)

^a $P > .05$ for all variables.

Ethics Approval

The study was approved by the University of Cape Town Research Ethics Committee and by the Medicines Control Council of South Africa.

Results

Clinical and Immunologic Characteristics of the Study Population at Baseline

Sixty patients were randomized (2:1) into active (39) and placebo (21) groups. There were 25 women and 14 men in the active group and 12 women and 9 men in the placebo group. The mean ages were 33.7 and 31.4 years in the active and placebo group, respectively.

No significant differences were found between the active and placebo groups for their baseline T5SSs, median baseline TNNSSs, baseline investigator global rhinitis scores, baseline patient global rhinitis score, mean wheal diameters for *D pteronyssinus* skin prick test, Der p 1 specific IgE level, Der p 1 specific IgG level, and total serum IgE levels (Table 1).

There were also no differences in the daily use of intranasal corticosteroid sprays or oral antihistamine use in the 2 weeks before the randomization visit. Asthma symptoms, such as cough and wheezing, were present in 64.1% and 53.8% of the active group, which was not significantly different when compared with the placebo group.

Changes in the Clinical and Immunologic Characteristics During 2 Years

Of the 60 patients randomized, 32 (82%) of 39 patients in the active group and 16 (76%) of 21 in the placebo group completed the

study. Of the 12 patients who withdrew, 7 did so within the first 6 months.

Medication adherence at the start of the study, recorded on the patient's daily diary cards and determined by summing the number of IR units received during the first 6 weeks, was identical in both groups (median [interquartile range]: 7627.5 [7627.5–7627.5] IR/mL for the active group and 7627.5 [7627.5–7777.5] IR for the placebo group).

Only minor localized adverse events were observed, but these were more frequent in the active vs placebo groups for oral pruritus (58% vs 33.3%), throat irritation (35.9% vs 14.3%), oral paraesthesia (15.6% vs 0%), and oral edema (1% vs 0%). The progressive improvement in the total symptom scores during 2 years is shown in Figure 1.

Although significant clinical improvement at 2 years was observed in both the active and placebo groups when taken as a whole for T5SS ($P < .001$ and $P = .003$) (Fig 2), the TNNSSs, investigator global rhinitis scores, and patient global rhinitis scores (Table 1), the changes observed when comparing the active and placebo groups as a whole were not statistically significant. Values indicating the changes in measured parameters at 2 years compared with baseline for the placebo and active groups as a whole are given in Table 1. Although significant overall improvements in the active ($P < .001$) and placebo ($P = .005$) groups using the Juniper Quality of Life Questionnaire were also found at the 2-year assessment, the difference in improvement in active vs placebo groups was also not statistically significant. In addition, there was a significantly correlation between the change in the QOL and the change in T5SS during the 2 years ($R = 0.56$, $P < .001$).

There were no changes in the use of intranasal corticosteroid sprays between the groups: antihistamine use decreased from 14.3% to 0% in the active group and from 1.4% to 0% in the placebo group. In addition there were no significant changes in the skin prick test wheal diameter, *D pteronyssinus* specific IgE, *D pteronyssinus* specific IgG, or total serum IgE at the 2-year end point

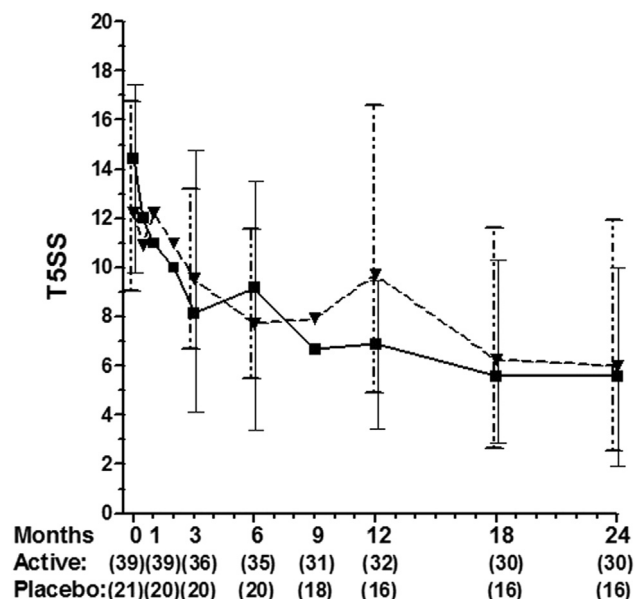


Figure 1. Time course of clinical improvement in the active sublingual immunotherapy (solid line) and placebo (dashed line) patients as measured by the Total 5 Symptom Score (T5SS) (mean daily total of 5 individual scores for sneezing; runny nose; itchy ear, nose, and throat; ocular symptoms; and nasal congestion; each scored on a scale of 0 to 5, with 0 indicating absent and 5 indicating intolerable; maximum score, 25). Symptoms, scored by the patients using a diary card, were recorded for at least 2 weeks before each visit: baseline (0) and the 9 follow-up visits at 2 weeks, 4 weeks, 8 weeks, 12 weeks, 6 months, 9 months, 12 months, 18 months, and 24 months. Median and interquartile range values are shown.

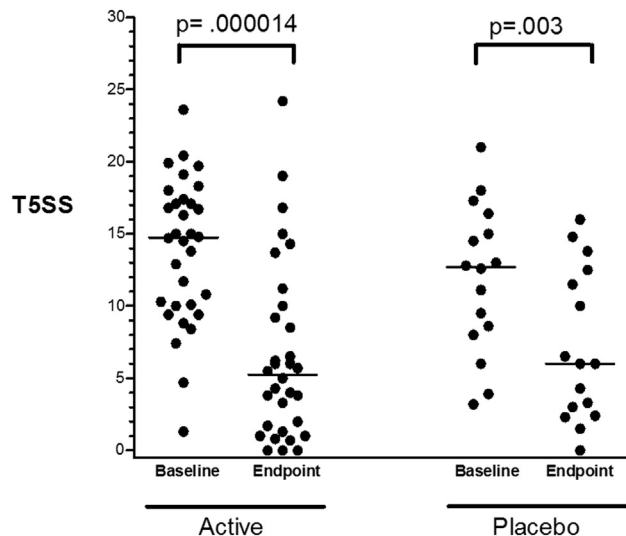


Figure 2. Total 5 Symptom Scores (T5SSs) at baseline and end point for the active sublingual immunotherapy (SLIT) ($n = 32$) and placebo ($n = 16$) groups who completed 24 months of treatment. The decrease in T5SSs reveals significant clinical improvement in both the active SLIT and placebo groups. Horizontal lines represent medians.

between the active and placebo groups, although there was a trend toward an increase in *D pteronyssinus* IgE and total serum IgE in the active group and a decrease in the placebo group (Table 1).

Identification and Analysis of Responder Phenotypes Within Both Study Groups

A clear heterogeneity in the levels of clinical improvement was observed by the investigators in both the active and placebo groups after 2 years; therefore, the patients who completed the study were divided into 3 levels of clinical response. Good responders were those in whom both the T5SS and QOL improved by 60% or more, intermediate responders had 30% to 59% QOL or T5SS improvement, and the no improvement group were those who improved by 29% or less on both scores.

In the active treatment group ($n = 32$) there were 15 good, 11 intermediate, and 6 no improvement responders. In the placebo group ($n = 16$), there were 4 good, 9 intermediate, and 3 no improvement responders (Fig 3). There was a higher proportion of good clinical responders in the active treatment group (47%) than in the placebo group (25%), and although this difference did not achieve statistical significance ($P = .07$), it was concluded to be important to compare the immune responses in this group with the weak responders or nonresponders.

At baseline there were no statistical differences in T5SS, QOL, or Der p specific IgE levels in the good, intermediate, or no improvement subgroups. At 2 years, the HDM specific IgE was not significantly different in the subgroups. Of interest, within the subgroups, only the median HDM IgE in the combined no improvement subgroup was significantly changed (increased) at the 2-year time point relative to baseline (no improvement group: median [interquartile range], 23 [13–64] kU/L at baseline and 66 [15–137] kU/L at 2 years; $n = 9$; $P = .02$).

Studies of IL-5/IFN- γ Cytokine Release From PBMCs During the 2-Year Period

Cytokine release from PBMCs in the active and placebo groups

At baseline, all the PBMC cultures had a positive cytokine response to HDM stimulation, releasing IL-5 or IFN- γ into the

supernatant. At 24 months, one individual did not have a cytokine response to HDM stimulation of PBMCs (IL-5 and IFN- γ were below the sensitivity of the assay and an IL-5/IFN- γ ratio could not be calculated) and was excluded from the final analysis.

There was no statistical difference in the HDM-stimulated IL-5/IFN- γ ratio, IL-5, IFN- γ , or proliferation between the active and placebo patients at baseline or 24 months when the groups were analyzed as a whole. However, after subgroup analyses, significant changes in the IL-5/IFN- γ ratios were observed in the good responder phenotype in the active group ($P = .003$).

Cytokine release in the good, intermediate, and no clinical improvement groups

The good clinical improvement group of active and placebo participants had progressive decreases in the IL-5/IFN- γ ratio during 24 months (Fig 4A). The median IL-5/IFN- γ ratio in the active good group ($n = 15$) was significantly decreased relative to baseline at 24 months ($P < .001$). In the placebo good clinical improvers group ($n = 4$), a similar trend was found but did not reach statistical significance ($P = .07$). In the intermediate and no improvement groups in both the active or placebo participants, no progressive changes or significant differences were found between baseline and 24 months.

In view of the small numbers in the study subgroups, results in the active and placebo subgroups were combined to further define a good responder profile. In the good clinical improvement group (Fig 4B), the median IL-5/IFN- γ ratio progressively decreased over time and at 24 months and was significantly decreased relative to baseline ($P < .001$). There was no decrease over time in the combined groups who had an intermediate response or in the nonresponder group. There was no significant difference in the good, intermediate, and no improvement groups at baseline. At 24 months, the IL-5/IFN- γ ratio was decreased in the good group relative to the intermediate group ($P = .04$; Kruskal-Wallis) (Fig 4B).

At 24 months, HDM-stimulated IL-5 release was decreased and IFN- γ increased in the good responder group relative to the other groups, but the finding was not significant (Kruskal-Wallis test). Direct comparison of the good and intermediate responders groups revealed IL-5 release at 24 months was significantly decreased in the good responders group ($P = .03$; Mann-Whitney test). Median IL-5 release, at 0, 6, 12, and 24 months, was 120, 233, 401, and 89 pg/mL in the good responders group; 234, 430, 696, and 245 pg/mL in the intermediate responders group; and 44, 363, 775, and 159 pg/mL in the no improvement group, respectively; and median IFN- γ levels were 151, 546, 546, and 448 pg/mL in the good responders group; 210, 276, 451, and 340 pg/mL in the intermediate responders group; and 89, 245, 417, and 343 pg/mL in the no improvement group, respectively.

IL-13, IL-4, and IL-10 were only measured at 0 (baseline) and 24 months. In the good responders group, the HDM-stimulated IL-13/IFN- γ ratio and the IL-4/IFN- γ ratio were significantly decreased at 24 months relative to baseline ($P < .001$ and $P = .03$, respectively; Fig 5A and B). At 24 months, the IL-13/IFN- γ ratio and the IL-4/IFN- γ ratio were significantly decreased in the good responders group relative to the intermediate responders group ($P = .03$ and $P = .04$; Mann-Whitney test). No significant differences were found in the absolute levels of IL-13, IL-4, or IL-10 released in any of the subgroups at 24 months compared with baseline or between the subgroups at 24 months.

Spontaneous cytokine release was below the limits of detection or low compared with the HDM-stimulated release in most cases. Spontaneous proliferation was low relative to the HDM-stimulated proliferation in most cultures. No significant changes in HDM-stimulated proliferation were found.

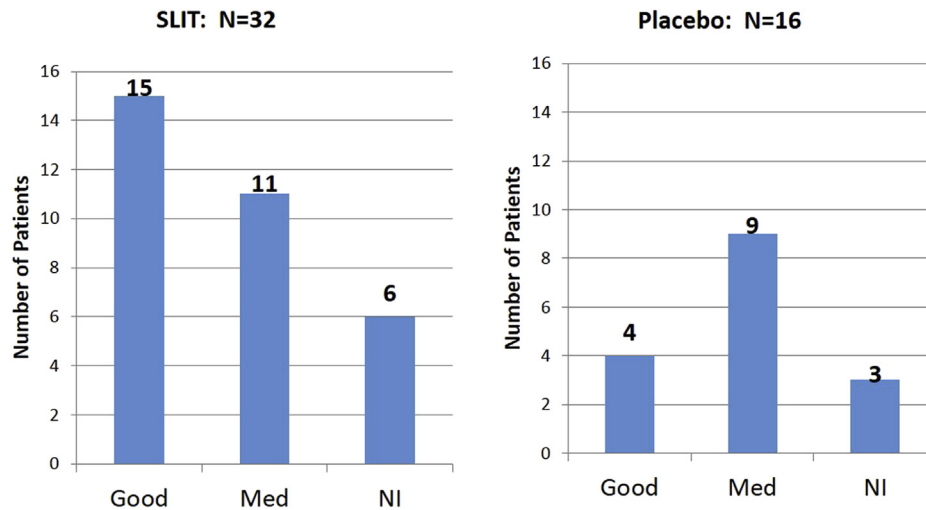


Figure 3. Profile of responses. On the basis of clinical improvement at 24 months, the patients were divided into 3 groups: good responders, 60% or greater improvement on both the Total 5 Symptom Score and quality of life scores; intermediate (med), 30% to 59% improvement on either or both scores; and no improvement (NI), 0% to 29% on both scores. There was a higher proportion of good clinical improvers in the active SLIT group (47%) than in the placebo group (25%; $P = .07$). IL indicates interleukin; IFN- γ , interferon γ .

Discussion

We have investigated the clinical improvement during 2 years in patients with house dust mite sensitive persistent rhinitis and have found that both actively treated and placebo treated patients had variable, clinical, and immunologic responses to the treatment received. We have also found that a subgroup of actively treated patients who had a very good clinical response improvement of more than 60% had a significant progressive decrease in IL-5/IFN- γ ratio during the treatment period. Significant progressive decreases in the IL-5/IFN- γ ratio accompanying clinical improvement in patients responding to SLIT have not been previously reported.

In the patients who completed the study (2 years of active or placebo treatment), there was significant clinical improvement (indicated by highly significant decreases in the T5SS and QOL) in both the active ($n = 32$) and placebo ($n = 16$) patients between baseline and end point (Table 1). Good clinical improvements in placebo-treated groups have also been found by others⁸ and are often the subject of speculation. It may represent spontaneous remission of symptoms or a placebo “care effect” of being part of a clinical study, the use of rescue medications, specific allergen avoidance, regular follow-up, education, and self-management. Patients enrolled in our study were not provided with specific instructions for intensive HDM avoidance.

To explore possible explanations for this variability particularly in relation to the immunologic markers we had measured, we divided the patients into 3 categories of clinical improvement: good responders, intermediate responders, and no improvement group.

Using this grouping, we found 15 (47%) of 32 good responders in the active group, but only 4 (25%) of 16 in the placebo group. The frequency of nonresponders or no improvement patients was the same in the active group (6 [18.7%]) of 32 compared with the placebo group (3 [18.7%] of 16).

Our study has found that a significant clinical improvement after 2 years of treatment with HDM immunotherapy is associated with a shift in the HDM-stimulated cytokine response from a T_H2 -type cytokine response toward a more T_H1 -dominated response as reflected by significant decreases in the IL-5/IFN- γ , IL-13/IFN- γ , and IL-4/IFN- γ ratios. An excess of T_H2 cytokines relative to the T_H1 cytokine IFN- γ (expressed as a ratio), rather than the absolute amounts of these cytokines, is associated with the expression of atopic disease.¹²

There is increasing acceptance that subcutaneous immunotherapy (SCIT) and SLIT may share common mechanisms.^{6,13,14} Although variable results have been reported in investigations of T-cell responses after HDM and pollen immunotherapy,^{6,13–15} some of this reported variability could be due to the small number of patients studied in the studies reported.

Two recent small studies of HDM immunotherapy,^{16,17} which investigated both SCIT and SLIT and reported good therapeutic outcomes, found no significant T-cell changes. Antúnez et al¹⁶ found no significant increase in the percentage of IFN- γ^+ /IL-4 $^+$ T cells during 2 years in either the SCIT or SLIT groups (1112 individuals per group), and Eifan et al¹⁷ found no change in the release of IL-4, IL-5, IL-13, or IFN- γ in the SLIT, SCIT, or pharmacotherapy groups after 12 months (14–15 individuals per group). Another investigation of HDM SLIT¹⁸ with significant clinical improvement in the active group did find evidence of a shift away from T_H2 polarization: in the SLIT group, the mean IL-5 release was significantly decreased at 12 months ($n = 13$) and 24 months ($n = 9$), whereas IFN- γ , which was significantly decreased at 12 months, tended to normalize at 24 months. Two studies of HDM SCIT for asthma¹⁹ and rhinitis²⁰ with significant clinical improvement in the immunotherapy groups reported evidence for a T_H2 to T_H1 shift: Tsai et al¹⁹ found a significant decrease in the percentage of IL-4 $^+$ T cells and a significant increase in the percentage of IFN- γ^+ T cells in 30 asthmatic children, compared with baseline, after 1 year of immunotherapy, and Gardner et al²⁰ found that the proportion of IL-4 $^+$ T cells significantly decreased, whereas there was no change in IFN- γ^+ T cells in 9 adults with rhinitis after 9 months of treatment. A previous study of 27 asthmatic children²¹ found significantly decreased IL-4 and increased IFN- γ in the SIT group compared with the untreated group after 1.5 to 2 years of treatment; however, changes in clinical symptoms were not reported. None of these studies have examined differences in cytokine changes in responder or nonresponder subgroups.

Similar to the findings for HDM immunotherapy, some^{22–24} but not all^{25–27} investigations of 1 to 2 years of pollen immunotherapy, using allergen-stimulated PBMCs, found evidence of a T_H2 to T_H1 cytokine shift. Notably, a previous study found that a decrease in IL-5 production, representing T_H2 cytokines, accompanies maintenance immunotherapy with grass pollen antigens, whereas

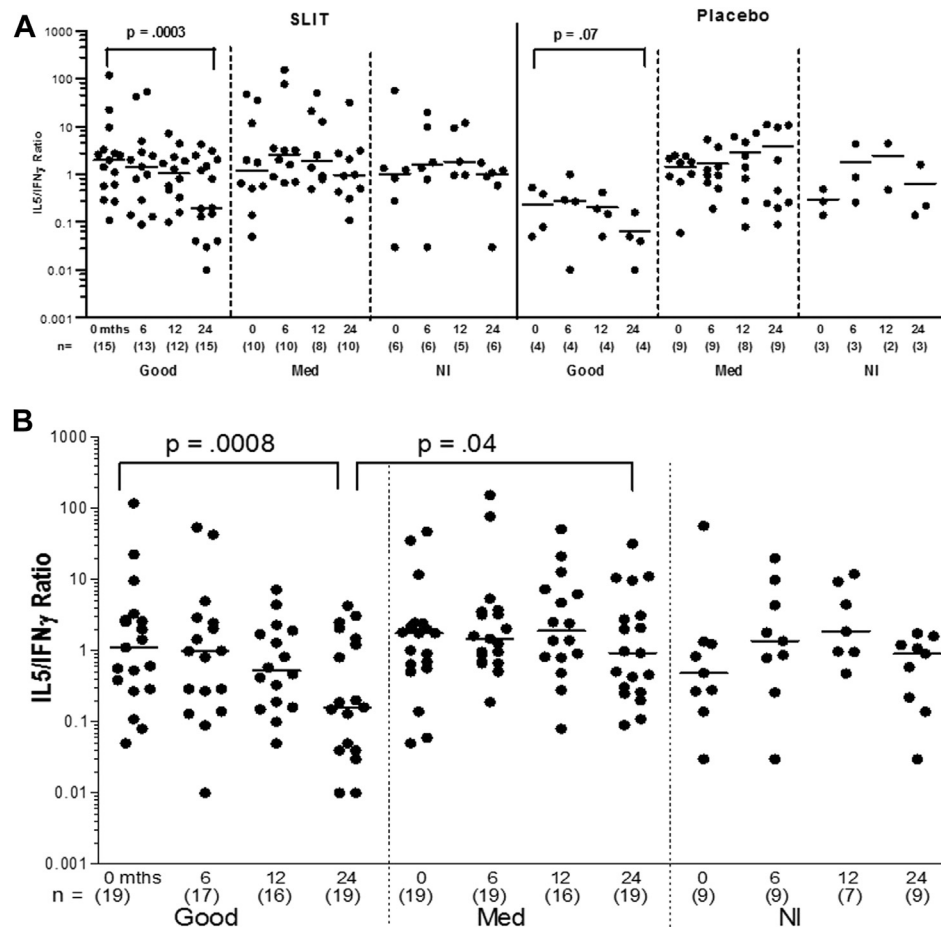


Figure 4. Interleukin (IL) 5/interferon γ (IFN- γ) release from house dust mite-stimulated peripheral blood mononuclear cells at 0, 6, 12, and 24 months in patients with good, intermediate (Med), and no (NI) clinical improvement at 24 months. A, Sublingual immunotherapy (SLIT) and placebo patients. B, All patients divided by their clinical response only. At 24 months, the IL-5/IFN- γ ratio in the good responder group was significantly decreased relative to 0 months ($P < .001$; Wilcoxon matched pairs test) and relative to the intermediate responder group at 24 months ($P = .04$; Kruskal-Wallis test).

changes in IL-10 tend to be transient events associated with early specific immunotherapy phases.²⁸ One of these studies²⁷ found that grass pollen immunotherapy for rhinitis was associated with increases in local nasal but not peripheral T_H1:T_H2 cytokine ratios. It has also been suggested that a T_H2 to T_H1 cytokine shift during allergen immunotherapy is subsequent due to the induction of regulatory T cells.^{15,22}

With regard to the induction of T-cell tolerance to inhalant allergens assessed by proliferation in culture, some studies of SCIT have found significant suppression of HDM-^{21,29}, or grass pollen-^{29,30}-stimulated proliferation of PBMCs or T cell clones, whereas other studies have reported no changes to HDM²⁰ or grass pollen.^{26,27} Some studies of SLIT have found significantly decreased HDM-,¹⁸ birch pollen-,²⁴ or grass pollen^{31,32}-stimulated proliferation of PBMCs, whereas other studies found no significant effect to HDM³³ or grass pollen.²⁴ In most of these studies, individuals were receiving treatment for a year or longer,^{18,21,24–27,29–31} but most previous studies had only 9 to 12 individuals in the immunotherapy groups, and many did not report on any clear clinical improvement or have control or placebo groups. In our serial investigation of 48 individuals who completed 2 years of SLIT, we found no significant changes in HDM-stimulated proliferation overall or in the good clinical responders.

Although our study sample is comparatively small, it is larger than most other published studies on cytokine release from antigen-stimulated lymphocytes before and after immunotherapy. Studies of lymphocyte culture and cytokine

determination are labor intensive. Patients have a wide variability in cytokine responses, which may depend on the stage of the disease, allergen exposure, or immune responsiveness, as they do for IgE levels.

This study has confirmed that the clinical and immune responses to HDM SLIT is heterogeneous and indicates that grouping based on a mean of the clinical responses of all patients in an active or placebo arm may obscure the identification of a responder group to a given SLIT vaccine. Furthermore, inclusion of nonresponders in an analysis may mask the detection of benefit in a subgroup within an active treatment group. However, when separating those individuals who respond in the way we have in this study, it is possible to see clinical and immunologic heterogeneity within both active and placebo groups in studies with a smaller numbers of participants. This analysis of our study may for explain why there have been several studies indicating SLIT with HDM vaccines, both published⁸ and unpublished, when patients in active and placebo groups are not stratified for degree of response.

In this study 18.7% of the individuals in the placebo and active groups were nonresponders (ie, having no clinically significant improvement). Clinical factors that would contribute to non-response to a vaccine would include lack of adherence, poor technique, unreliable reporting of symptoms on diary card, inadequate refrigeration of the vaccine, poor selection of the study participants (eg, polysensitive patients), and psychosocial problems in the study participants.³⁴ Intermediate responses may result from variability in the above or a combination of or a lesser degree of the above

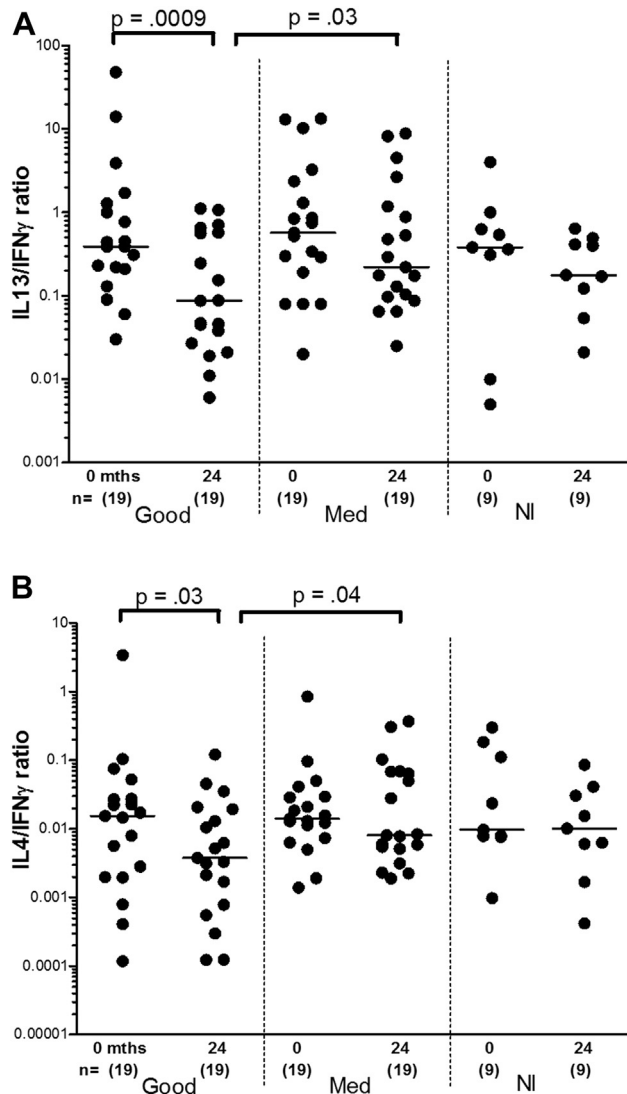


Figure 5. A, Interleukin (IL) 13/interferon γ (IFN- γ). B, IL-4/IFN- γ release from house dust mite-stimulated peripheral blood mononuclear cells at 0 and 24 months in patients with good, intermediate (Med), and no (NI) clinical improvement. In the good responder group, the IL-13/IFN- γ and IL-4/IFN- γ ratios were significantly decreased at 24 months compared with 0 months ($P < .001$ and $P = .03$, respectively; Wilcoxon matched pairs test) and compared with the intermediate responder group at 24 months ($P = .03$ and $P = .04$; Mann-Whitney test).

factors. In our study, the group had higher IgE levels as a group, which may have contributed to their nonresponses (eg, if they were polysensitive to allergens not present in the vaccine).

Little is published on the natural history of patients with HDM-induced persistent allergic rhinitis, but clinical observation suggests that an as yet undefined number of these patients tend to spontaneously get better on their own during a 2-year period (in our study, 25% in the placebo group) for reasons that are as yet also unknown. One would not expect the level of spontaneous improvement to be as high in short-term studies of seasonal or intermittent rhinitis. It would appear that the longer the study is conducted, the higher the possibility that more patients would improve or remit spontaneously. This could also explain why statistically better results have been obtained with longer-term HDM studies and in those with more severe symptoms.^{7,35,36}

We found no statistical differences at baseline in T5SS, QOL, Der p specific IgE levels, or cytokine release in the good, intermediate, or no improvement subgroups described. At the 2-year follow-up, we did

not observe an elevated *D pteronyssinus* specific IgE in the good responder group or a change in skin prick test responses perhaps because we used a relatively lower dose of vaccine 300 IR on alternate days for this study. The cumulative dose of mite vaccine received by the patients in the study during 2 years was estimated to be 96,600 IR. Currently, the recommended dose is 300 IR daily. A higher vaccine dose would be more likely to stimulate IgE specific HDM antibodies. Our finding of significant clinical improvement even with a lower dosage regimen in 47% of the actively treated group compared with 25% in the placebo group indicates that the dose required to effect a good clinical response may be lower in some and may vary in different allergic patients for reasons currently not clear.

Recent studies of larger samples of patients using a grass allergy tablet sublingual vaccine at 300 IR and 500 IR revealed short-term benefit.³⁷ In a long-term study³⁸ of another grass tablet given sublingually, progressive immunologic changes were also observed during a 2-year period. Clinical improvement was accompanied significant changes in allergen specific IgG4 in the active but not placebo groups. We have not investigated allergen specific IgG4 changes in this study.

Further studies elucidating clinically relevant allergens for vaccines, enhanced by recombinant allergens, at higher doses or increased frequency of administration are also be important in this regard.^{38,39} In our study, it is also possible that the dose and regimen (3 times weekly) used for maintenance during the 2 year period were insufficient. The optimum dosage, duration of treatment, and frequency of administration of SLIT have not yet been established.⁴ Investigation of the effect of SLIT in patients with ragweed sensitive rhinitis^{39,40} found that patients receiving the highest dose of immunotherapy (3 tablets 3 times a week) had a highly significant response for rhinitis and conjunctivitis total scores compared with those receiving lower dosages. Furthermore, it is known whether the vaccine extracts used contain essential antigens required to induce a response in all study participants with mite allergy in our study.

Currently, there is uncertainty in the literature on the strength of recommendations for SLIT vaccines for allergens other than grass pollen.⁴¹ It is, however, clear from a recent systematic review by Rudalovic et al³ that SLIT remains safe and effective for reduction in symptoms and medication use.

To assess the efficacy and underlying mechanisms of improvement for patients receiving HDM SLIT, in future studies it may be necessary to profile these HDM sensitive patients more carefully with respect to baseline immune responsiveness to the different HDM allergens (which may vary in different ecosystems regions, eg, the tropics [Singapore] vs Mediterranean regions), duration of disease, severity of symptoms at baseline, levels and seasons of mite exposure, and mite antigens in the vaccine and medication required to control symptoms.¹⁰ Unless these variables are standardized, patients with HDM allergy seen in the usual routine clinic environment may be too heterogeneous for inclusion in clinical trials with crude mite extracts, which are not standardized for these parameters. Large regional studies may rather than large multicenter global studies across different continents may be necessary in view of the diversity and heterogeneity of mite species found in different regions of the globe and of the immune responder phenotype in different populations.

Further studies should investigate whether the changes we have observed in IL-5/IFN- γ , IL-13/IFN- γ , and IL-4/IFN- γ ratios consistently take place during long-term SLIT, with larger numbers of patients in active and placebo arms, because determination and documentation of these changes may prove to be a reliable marker of efficacy or lack of response to a SLIT vaccine.

The results of this study have emphasized the need for stratification of clinical responses when assessing responses to immunotherapy treatment. We found that a good clinical response

(improvement of ≥ 60 in both rhinitis symptom scores and QOL) is associated with significant decreases in HDM-stimulated release from PBMCs of IL-5, IL-13, and IL-4 relative to IFN- γ during 2 years of SLIT therapy for HDM allergic rhinitis. This finding is consistent with a shift in the HDM-stimulated cytokine response from a T_H2 -type cytokine response toward a more T_H1 dominated response being associated with significant clinical improvement in allergic rhinitis.

Acknowledgments

We acknowledge Dr Diane Hawarden for clinical assistance and Dr Najmeeyah Brown and Ms Sumayah Salie for their technical assistance with the laboratory studies. Dr Anne Combebias is also acknowledged posthumously for her assistance with the study, and Dr Riad Fadel (Stallergenes) is thanked for assistance with the analysis.

References

- [1] Wilson DR, Torres Lima M, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy*. 2005;60:4–12.
- [2] Compalati E, Passalacqua G, Bonini M, Canonica GW. The efficacy of sublingual immunotherapy for House dust mites respiratory allergy: results of a GA²LEN meta-analysis. *Allergy*. 2009;64:1570–1579.
- [3] Radulovic S, Wilson D, Calderon M, Durham S. Systematic reviews of sublingual immunotherapy (SLIT). *Allergy*. 2011;66:740–752.
- [4] Walker SM, Durham SR, Till SJ, et al. Immunotherapy for allergic rhinitis. *Clin Exp Allergy*. 2011;41:1177–1200.
- [5] Penago M, Compalati E, Tarantini F, et al. Efficacy for sublingual immunotherapy in the treatment of allergic rhinitis in paediatric patients 3–18 years of age: a meta analysis of randomised controlled double blind trials. *Ann Asthma Immunol*. 2006;97:141–148.
- [6] Canonica GW, Bousquet J, Casale T, et al. Sublingual immunotherapy: World Allergy Organization Position Paper 2009. *Allergy*. 2009;64(suppl 91):1–59.
- [7] Mortemousque B, Bertel F, De Casamayor J, Verin P, Colin J. House dust mite sublingual swallow immunotherapy in perennial conjunctivitis: a double blind placebo controlled study. *Clin Exp Allergy*. 2003;33:464–469.
- [8] Passalacqua G, Pasquali M, Anano R, et al. Randomised double blind controlled study with sublingual carbamylated allergoid immunotherapy in mild rhinitis due to mites. *Allergy*. 2006;61:849–864.
- [9] Guez S, Vatrinet C, Fadel R, Andre C. House dust mite sublingual swallow immunotherapy in perennial rhinitis: a double blind placebo controlled study. *Allergy*. 2000;55:369–375.
- [10] Canonica GW, Baena Cagnani CE, Bousquet J, et al. Recommendations for standardisation of clinical trials with allergen specific immunotherapy for respiratory allergy: a statement of a World Allergy Organization (WAO) Task Force. *Allergy*. 2007;62:317–324.
- [11] Juniper EF, Guyatt GH. Development and testing of a new measure of health status for clinical trials in rhino conjunctivitis. *Clin Exp Allergy*. 1991;21:71–83.
- [12] Nurse B, Puterman AS, Haus M, Berman D, Weinberg EG, Potter PC. PBMC's from both atopic asthmatic and non-atopic children show a $TH2$ cytokine response to house dust mite allergen. *J Allergy Clin Immunol*. 2000;106:84–91.
- [13] Moingeon P, Batard T, Fadel R, Frati F, Sieber J, Van Overtvelt L. Immune mechanisms of allergen-specific sublingual immunotherapy. *Allergy*. 2006;61:151–165.
- [14] Maggi E. T cell responses induced by allergen-specific immunotherapy. *Clin Exp Immunol*. 2010;161:10–18.
- [15] Shamji MH, Durham SR. Mechanisms of immunotherapy to aeroallergens. *Clin Exp Allergy*. 2011;41:1235–1246.
- [16] Antúnez C, Mayorga C, Corzo JL, Jurado A, Torres MJ. Two year follow-up of immunological response in mite-allergic children treated with sublingual immunotherapy: comparison with subcutaneous administration. *Pediatr Allergy Immunol*. 2008;19:210–218.
- [17] Eifan AO, Akkoc T, Yildiz A, et al. Clinical efficacy and immunological mechanisms of sublingual and subcutaneous immunotherapy in asthmatic/rhinitis children sensitized to house dust mite: an open randomized controlled trial. *Clin Exp Allergy*. 2010;40:922–932.
- [18] O'Hehir RE, Gardner LM, de Leon MP, et al. House dust mite sublingual immunotherapy: the role for transforming growth factor-beta and functional regulatory T cells. *Am J Respir Crit Care Med*. 2009;180:936–947.
- [19] Tsai Y-G, Chien J-W, Chen W-L, Shieh J-J, Lin C-Y. Induced apoptosis of $TH2$ lymphocytes in asthmatic children treated with *Dermatophagoides pteronyssinus* immunotherapy. *Pediatr Allergy Immunol*. 2005;16:603–608.
- [20] Gardner LM, Thien FC, Douglass JA, Rolland JM, O'Hehir RE. Induction of T 'regulatory' cells by standardized house dust mite immunotherapy: an increase in CD4+ CD25+ interleukin-10+ T cells expressing peripheral tissue trafficking markers. *Clin Exp Allergy*. 2004;34:1209–1219.
- [21] Wei W, Liu Y, Wang Y, et al. Induction of CD4+CD25+Foxp3+IL-10+ T cells in HDM-allergic asthmatic children with or without SIT. *Int Arch Allergy Immunol*. 2010;153:19–26.
- [22] Möbs C, Slotsch C, Löffler H, Jakob T, Hertl M, Pfützner W. Birch pollen immunotherapy leads to differential induction of regulatory T cells and delayed helper T cell immune deviation. *J Immunol*. 2010;184:2194–2203.
- [23] Nieminen K, Laaksonen K, Savolainen J. Three-year follow-up study of allergen-induced in vitro cytokine and signaling lymphocytic activation molecule mRNA responses in peripheral blood mononuclear cells of allergic rhinitis patients undergoing specific immunotherapy. *Int Arch Allergy Immunol*. 2009;150:370–376.
- [24] Bohle B, Kinaciyan T, Gerstmayr M, Radakovics A, Jahn-Schid B, Ebner C. Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance and immune deviation. *J Allergy Clin Immunol*. 2007;120:707–713.
- [25] Rolinck-Werninghaus C, Kopp M, Liebke C, Lange J, Wahn U, Niggemann B. Lack of detectable alterations in immune responses during sublingual immunotherapy in children with seasonal allergic rhinoconjunctivitis to grass pollen. *Int Arch Allergy Immunol*. 2005;136:134–141.
- [26] Francis JN, Till SJ, Durham SR. Induction of IL-10⁺CD4⁺CD25⁺ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol*. 2003;111:1255–1261.
- [27] Schulten V, Tripple V, Sidney J, et al. Association between specific timothy grass antigens and changes in T1- and T2-cell responses following specific immunotherapy. *J Allergy Clin Immunol*. 2014;134:1076–1083.
- [28] Wachholz PA, Nouri-Aria KT, Wilson DR, et al. Grass pollen immunotherapy for hayfever is associated with increases in local nasal but not peripheral $Th1$: $Th2$ cytokine ratios. *Immunology*. 2002;105:56–62.
- [29] Jutel M, Akdis M, Budak F, et al. IL-10 and TGF- β cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol*. 2003;33:1205–1214.
- [30] Ebner C, Siemann U, Bohle B, et al. Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from $TH2$ to $TH1$ in T cell clones specific for Phi p 1, a major grass pollen allergen. *Clin Exp Allergy*. 1997;27:1007–1015.
- [31] Fanta C, Bohle B, Hirt W, et al. Systemic immunological changes induced by administration of grass pollen allergens via the oral mucosa during sublingual immunotherapy. *Int Arch Allergy Immunol*. 1999;120:218–224.
- [32] Burastero SE, Mistrello G, Falagiani P, et al. Effect of sublingual immunotherapy with grass monomeric allergoid on allergen-specific T-cell proliferation and interleukin 10 production. *Ann Allergy Asthma Immunol*. 2008;100:343–350.
- [33] Cosmi L, Santarlasci V, Angeli R, et al. Sublingual immunotherapy with *Dermatophagoides* monomeric allergoid down-regulates allergen-specific immunoglobulin E and increases both interferon-gamma- and interleukin-10-production. *Clin Exp Allergy*. 2006;36:261–272.
- [34] Ippoliti F, De Santis W, Volterrani A, et al. Psychological stress affects response to sublingual immunotherapy in asthmatic children allergic to house dust mite. *Pediatr Allergy Immunol*. 2006;17:337–345.
- [35] Passalacqua G, Albano M, Fregonese L, et al. Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite induced rhinoconjunctivitis. *Lancet*. 1998;351:629–632.
- [36] Bousquet J, Scheinmann P, Guinépain MT, et al. Sublingual swallow immunotherapy (SLIT) in patients with asthma due to house dust mites: a double blind placebo controlled study. *Allergy*. 1999;54:249–260.
- [37] Didier A, Malling HJ, Worm M, et al. Optimal dose, efficacy and safety of once daily sublingual immunotherapy with a 5 grass pollen tablet for seasonal allergic rhinitis. *J Allergy Clin Immunol*. 2007;120:1338–1345.
- [38] Dahl R, Kapp A, Colombo G, et al. Sublingual grass allergen tablet immunotherapy provides sustained clinical benefit with progressive immunologic changes over 2 years. *J Allergy Clin Immunol*. 2008;121:512–518.
- [39] Bieber T, Simon HU. Allergen-specific immunotherapy: current concepts and future directions [editorial]. *Allergy*. 2011;66:709–712.
- [40] André C, Perrin-Fayolle M, Grosclaude M, et al. A double-blind placebo-controlled evaluation of sublingual immunotherapy with a standardized ragweed extract in patients with seasonal rhinitis evidence for a dose-response relationship. *Int Arch Allergy Immunol*. 2003;131:111–118.
- [41] Frew A. Sublingual immunotherapy. *N Engl J Med*. 2008;358:2259–2264.