

A novel approach in allergen-specific immunotherapy: Combination of sublingual and subcutaneous routes

Sevgi Keles, MD,^a Elif Karakoc-Aydiner, MD,^a Ahmet Ozen, MD,^b Ayse Gul Izgi, MS,^a Ayzer Tevetoglu, MS,^a Tunc Akkoc, PhD,^a Nerin N. Bahceciler, MD,^a and Isil Barlan, MD^a *Istanbul, Turkey*

Background: Subcutaneous allergen-specific immunotherapy (SIT) has an early onset of action, whereas repeated injections and safety concerns have limited its use in the pediatric age group. Meanwhile, the improved safety profile of the sublingual route has been accepted as an alternative despite its relatively late onset of action.

Objective: We sought to improve the efficacy and safety of SIT with a combination of the subcutaneous route in the build-up phase and sublingual maintenance in comparison with the sublingual or subcutaneous routes alone.

Methods: Fifty-one house dust mite-sensitized children with mild-to-moderate asthma were randomized into one of 4 groups to receive either (1) subcutaneous immunotherapy (SCIT), (2) sublingual immunotherapy (SLIT), (3) SCIT plus SLIT, or (4) pharmacotherapy. Clinical parameters were evaluated at baseline and months 1, 4, 12, and 18. Allergen-specific immunoglobulin levels and allergen-induced IL-5, IL-10, IL-13, IL-17, TGF- β , and IFN- γ levels were evaluated as well.

Results: In the SCIT and SCIT plus SLIT groups, the number of asthma attacks and inhaled corticosteroid dosage decreased compared with baseline values at the months 4, 12, and 18 but only at month 12 in the SLIT group. The improvement in visual analog scores for rhinitis was significant only in the SCIT plus SLIT group. Increases in the levels of regulatory and T_H1 cytokines were observed both in the SCIT and SLIT groups, with some differences in dynamics. Antigen-specific IgG₄ levels increased in the SCIT and SCIT plus SLIT groups but not in the SLIT group. Clinical symptom scores were correlated positively with IL-5 levels and negatively with antigen-specific IgG₄, IFN- γ , and TGF- β levels.

Conclusion: Our novel regimen of immunotherapy, SCIT plus SLIT, appeared promising in that it successfully combined the advantages of the 2 alternatives: rapid onset and potency in SCIT and safety and avoidance of injections in SLIT. (*J Allergy Clin Immunol* 2011;■■■:■■■-■■■.)

Key words: Children, cytokine, Der p 1, subcutaneous immunotherapy, sublingual immunotherapy

From ^athe Division of Pediatric Allergy and Immunology, Marmara University, Istanbul, and ^bthe Division of Pediatric Allergy and Immunology, Yeditepe University, Istanbul. Supported by the Marmara University Scientific Research Committee (SAG-C-TUP-030408-0072).

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication October 13, 2010; revised March 20, 2011; accepted for publication April 11, 2011.

Reprint requests: Sevgi Keles, MD, Marmara University, Division of Pediatric Allergy and Immunology, Tophanelioglu cad No 13-15, Altunizade 34660, Istanbul, Turkey. E-mail: sevgi_keles@yahoo.com.

0091-6749/\$36.00

© 2011 American Academy of Allergy, Asthma & Immunology
doi:10.1016/j.jaci.2011.04.033

Abbreviations used

ASNPT: Allergen-specific nasal provocation test
HDM: House dust mite
ICS: Inhaled corticosteroid
MSA: Medication score for asthma
MSR: Medication score for rhinitis
NSBPT: Nonspecific bronchial provocation test
SCIT: Subcutaneous immunotherapy
SIT: Allergen-specific immunotherapy
SLIT: Sublingual immunotherapy
SSA: Symptom score for asthma
SSR: Symptom score for rhinitis
TMS: Total medication score
VAS: Visual analog scale

Allergen-specific immunotherapy (SIT) is a widely used therapeutic tool for the management of respiratory allergies and is recognized as the only modifier of the biological responses to offending allergens. Subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT) are the 2 commonly recognized SIT approaches in daily practice.¹ Induction of T-cell tolerance to the specific allergen in addition to decreased T_H2 cytokine responses and development of allergen-specific IgA, IgG₁, and IgG₄ blocking-type antibodies with an increased IgG₄/IgE ratio are the cardinal features of SIT.² Several studies demonstrating the early onset of action and favorable clinical efficacy of SCIT, even after the cessation of the treatment, have led SCIT to be accepted as a therapeutic option for the management of respiratory allergic disorders.³⁻⁵ However, SCIT has limitations in the pediatric age group because of the discomfort of repeated injections and unwanted side effects.^{6,7} As a consequence, the interest in a safer alternate modality inspired the research into noninjection routes of SIT.^{8,9} After this attempt, delivery of allergens through the mucosal route was proposed in 1986.⁹ The sublingual area, where antigen-presenting cells are abundant, has been proved to be convenient for allergen administration to induce mucosal tolerance. The prolonged contact of the allergen without stability problems has provided a great advantage for the introduction of allergen to the immune system.¹⁰⁻¹² As a result, SLIT is accepted as an effective therapeutic strategy for the modulation of the ongoing immunopathologic response in patients with allergic disorders.¹¹⁻¹³

Novel approaches for the improvement of both the efficacy and safety of SIT, especially in children, are still under investigation. In this study we aimed to analyze the clinical effects and cytokine responses of a novel approach for SIT in which SCIT is administered in the build-up phase and SLIT in the maintenance

phase in a randomized, controlled, prospective manner in house dust mite (HDM)–sensitive asthmatic children.

METHODS

Study population

The study included 60 consecutive children (5–12 years of age) with mild persistent/moderate asthma/rhinitis according to Global Initiative for Asthma guidelines who were monosensitized to HDM. These patients were followed up at our pediatric allergy and immunology outpatient clinic and received inhaled/intranasal steroids for at least 2 years with no reduction in symptoms. Patients were randomized into one of 4 groups receiving SCIT, SLIT, SCIT plus SLIT, or pharmacotherapy alone by using a computer-generated randomization method. The diagnosis and severity of asthma were based on medical history, physical examination findings, and postbronchodilator changes in FEV₁, as described in the Global Initiative for Asthma consensus report.¹⁴ The diagnosis and severity of rhinitis was based on the Allergic Rhinitis and its Impact on Asthma criteria.¹⁵ The study protocol was approved by the local ethics committee of Marmara University Hospital, and written informed consent was obtained from the parents.

Treatment

The standardized extract used throughout the study was a 1:1 mixture of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* administered as glycerinated solution (SLIT; ALK-Abelló, S.A., Madrid, Spain) or adsorbed on aluminum hydroxide (SCIT; Alutard SQ, ALK-Abelló, S.A.). The schedules of immunotherapy are summarized in Table E1 in this article's Online Repository at www.jacionline.org. SLIT was self-administered at home and included a 1-month induction phase followed by maintenance of 5 drops 3 times a week. Drops were given early in the morning before breakfast and held under the tongue for at least 2 minutes before swallowing. SCIT was administered in our clinic and included a 16-week (weekly injection) induction phase followed by a monthly maintenance phase. The dose of Der p 1 that was given in the build-up and maintenance phases was 1.5 and 52.8 µg of Der p 1 and 1.5 and 52.8 µg of Der f 1 in the SLIT group, 16.2 and 44.12 µg of Der p 1 and 22.9 and 62.1 µg of Der f 1 in the SCIT group, and 16.2 and 43.2 µg of Der p 1 and 22.9 and 43.2 µg of Der f 1 in the SCIT plus SLIT group, respectively. Doses were reduced by 20% to 40% in patients with allergic reactions. Also, all 4 groups were allowed to use their rescue medications (β₂-agonists and antihistamines) as needed and inhaled/intranasal corticosteroids in a stepwise fashion depending on the persistence and severity of the symptoms, as recommended.¹⁴

Study design

All eligible patients underwent an 8-week run-in period to evaluate their baseline clinical status based on symptom, medication, and visual analog scores and skin prick testing. By using the table randomization method, patients were randomized into one of 4 parallel groups to receive either SCIT (n = 15), SLIT (n = 15), SCIT in the build-up and SLIT in the maintenance phase (SCIT plus SLIT, n = 15), or pharmacotherapy (n = 15). On each clinic visit (–1, 0, 1, 4, and 12 months of follow-up), peripheral blood samples were drawn; data on medications, symptoms, visual analog scale (VAS) score, number of asthma attacks, dose of inhaled corticosteroid (ICS), and side effects were collected from diary cards; and physical examinations were performed for all patients. Additionally, total serum IgE and Der p 1– and Der p 2–specific IgE levels were measured annually by using the Immulite 2000 method (Euro/DPC, Llanbeic, United Kingdom), and serum Der p 1– and Der p 2–specific IgG₄ levels were evaluated by means of ELISA by ALK-Abelló in a blinded manner (0, 1, 4, and 12 months of follow-up). PBMCs were isolated from whole blood and cultured with or without Der p 1. Culture supernatants were analyzed for T_H1 (IFN-γ), T_H2 (IL-5 and IL-13), regulatory T (IL-10 and TGF-β), and T_H17 (IL-17) cell cytokines by means of ELISA. Patients were re-evaluated in terms of clinical parameters, including the number of asthma attacks and the dose of ICS at the 18-month follow-up. The study design is summarized in Fig E1 in this article's Online Repository at www.jacionline.org.

All patients were evaluated by categoric symptom scores for asthma (SSAs) and symptom scores for rhinitis (SSRs), medication scores for asthma

TABLE I. Demographic characteristics and median percentage improvement in symptom and medication scores and percentage of adverse reactions

Clinical evaluation	SCIT group (n = 11)	SLIT group (n = 13)	SCIT plus SLIT group (n = 14)	Pharmacotherapy group (n = 12)
Age (y)	7.1 ± 1.8	8.55 ± 2.1	8.25 ± 1.4	7.9 ± 2.8
Sex (F/M)	4/7	7/6	9/5	5/7
δ Improvement				
SSA	100%	97%	100%	
SSR	88%	36%	94%	
TSS	92%	52%	95%	
MSA	94%	94%	100%	
MSR	100%	38%	100%	
TMS	96%	74%	100%	
Adverse reaction, no. (%)	2 (15)	0	0	

δ Percentage of improvement was defined as the estimated difference of the SCIT or SLIT groups at 12 months in relation to the median of the pharmacotherapy group at 12 months.

TSS, Total symptom score.

and rhinitis, VAS scores for asthma and rhinitis, nonspecific bronchial provocation tests (NSBPTs), allergen-specific nasal provocation tests (ASNPTs), pulmonary function tests, skin prick tests, and PBMC isolation and culture, details of which are presented in the Methods section of this article's Online Repository at www.jacionline.org.

Statistical analysis

Statistical analyses were carried out with the SPSS program (version 16.0; SPSS, Inc, Chicago, Ill). The Wilcoxon signed-rank test was used in comparison of clinical and immunologic parameters, such as the number of asthma attacks, ICS dosage, cytokine results, specific IgG₄ levels, specific IgE levels, total serum IgE levels, SSAs, SSRs, total symptom scores, medication scores for asthma (MSAs), medication scores for rhinitis (MSRs), total medication scores (TMSs), and VAS scores for asthma and rhinitis, and Kruskal-Wallis and Mann-Whitney *U* tests were used for intergroup analysis of the same parameters. Spearman correlations were used to assess the relationships between the specific immunoglobulin levels and the symptom/medication scores, and Pearson correlations were used for antigen-specific immunoglobulin levels and cytokines. The χ² test was used where indicated. A *P* value of less than .05 was considered statistically significant.

RESULTS

Patients

Sixty patients between 5 and 12 years of age (31 female and 29 male patients) were included in the study. Two of the patients in the SCIT group withdrew their consent before the initiation of therapy; 2 patients from the SLIT group and 3 from the pharmacotherapy group were noncompliant to follow-up. One patient from the SCIT plus SLIT group discontinued her therapy (SLIT) after switching to SLIT due to noncompliance. In addition, 2 patients from the SCIT group were withdrawn because of adverse effects. Finally, 50 patients (11 from the SCIT group, 13 from the SLIT group, 14 from the SCIT plus SLIT group, and 12 from the pharmacotherapy group) completed the study protocol (see Fig E1). Demographic, clinical, and laboratory features of those patients are summarized in Table I and Table E2 in this article's Online Repository at www.jacionline.org.

All treated groups were comparable in terms of age, sex, total serum IgE levels, Der p 1– and Der p 2–specific IgE levels,

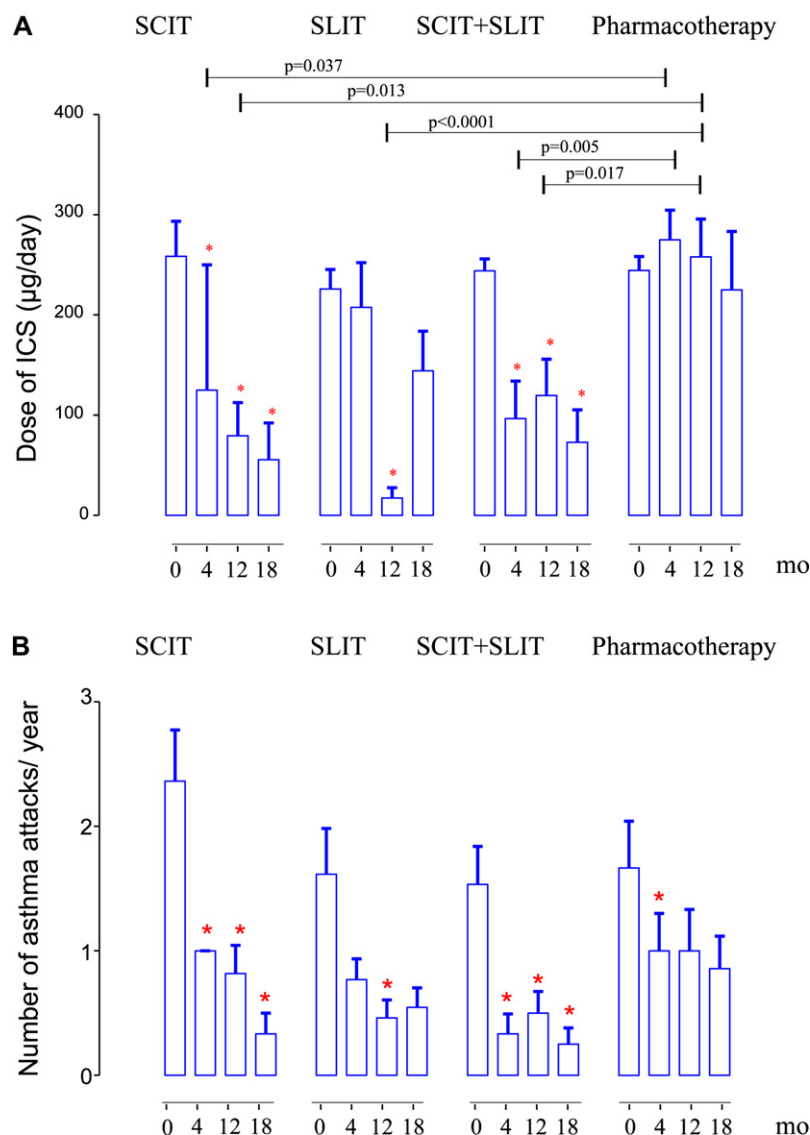


FIG 1. A, Dose of ICSs at baseline and 4, 12, and 18 months after treatment. **B,** Number of asthma attacks at baseline and 4, 12, and 18 months after treatment. *Within-group difference: values at months 4, 12, or 18 are significantly lower than the baseline value. The decreases in the number of attacks at months 4 and 18 in the SLIT group ($P = .062$ and $P = .065$, respectively) and at month 12 in the pharmacotherapy group ($P = .054$) were close to statistical significance.

NSBPT and ASNPT results, duration of asthma/rhinitis symptoms, total symptom/medication scores, and VAS scores at baseline (Table 1 and see Table E2).

Clinical evaluation

Reductions in both ICS dosage and number of asthma attacks were detected at months 4, 12, and 18 in the SCIT and SCIT plus SLIT groups, whereas they occurred at month 12 in the SLIT group (Fig 1, A and B).

Medication and symptom scores. The median percentage improvement in total symptom scores and TMSs was 92% and 96% in the SCIT group, 52% and 74% in the SLIT group, and 95% and 100% in the SCIT plus SLIT group, respectively, when compared with the pharmacotherapy group at month 12 (Table I). Both SCIT and SCIT plus SLIT resulted in similar decreases in

symptom and medication scores at months 4, 12, or both, except for SSRs, which decreased at month 12 only in the SCIT plus SLIT group. SLIT resulted in a significant decrease only in MSAs at month 12 (Fig 2).

VAS scores of asthma and rhinitis. Although a reduction in the VAS score for asthma was observed in all immunotherapy groups at months 4 and 12, the improvement in the VAS score for rhinitis was significant only in the SCIT plus SLIT group (see Fig E2 in this article's Online Repository at www.jacionline.org).

Adverse effects. No adverse effects were observed in the SLIT and SCIT plus SLIT groups, whereas 2 subjects from the SCIT group experienced dyspnea and wheezing during the build-up phase within 10 minutes after injection of vial 4. Although the allergen dose was decreased subsequently by 20% to 40%, immunotherapy was discontinued on recurrence of reactions.

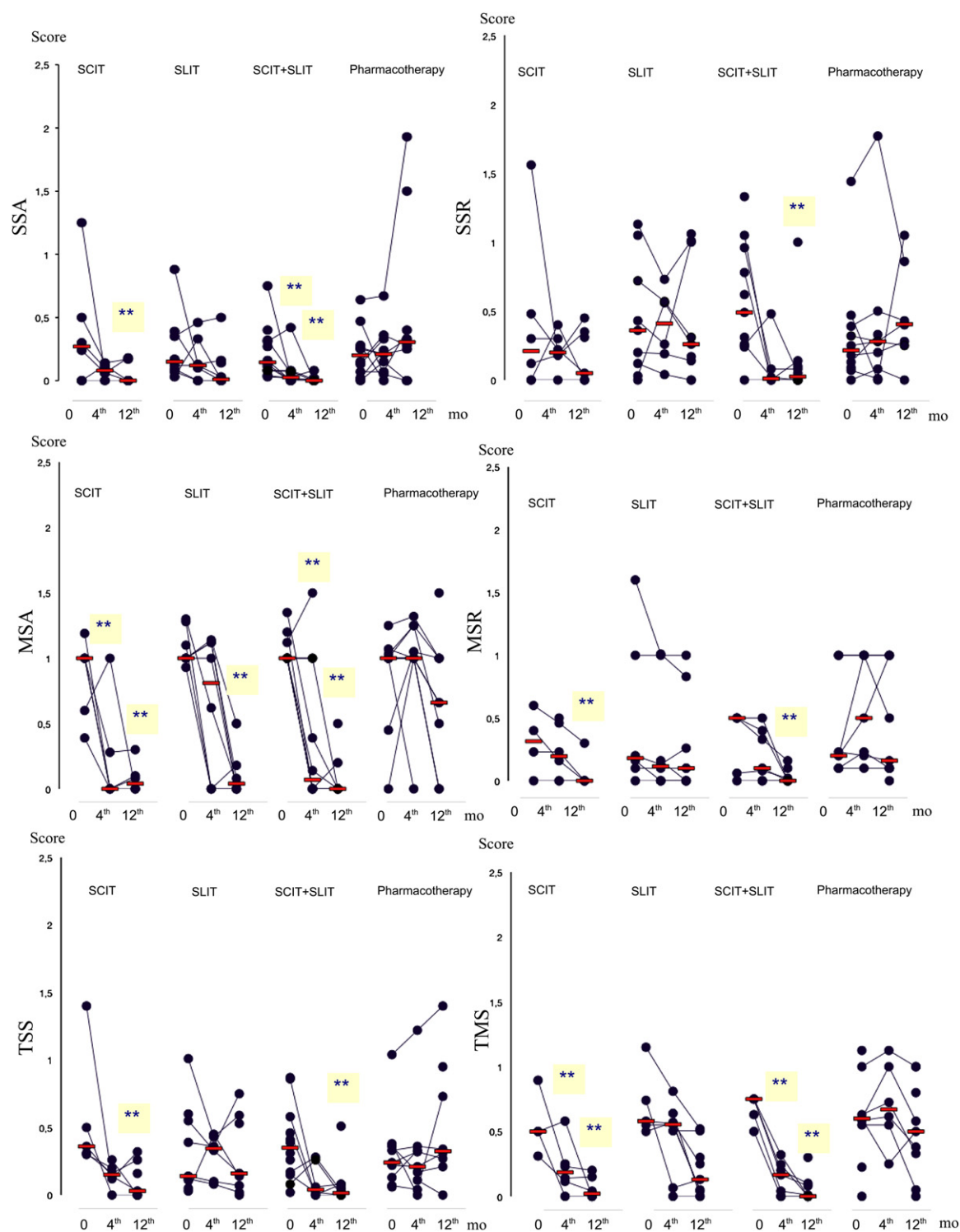


FIG 2. Symptom and medication scores of groups at baseline and 4 and 12 months after treatment.

**Between-group difference: the score of an immunotherapy group at a particular assessment is statistically significantly lower compared with that of the pharmacotherapy group.

Laboratory evaluation

In all immunotherapy groups, ASNPT results showed a significant decrease at month 12 compared with those of the pharmacotherapy group (Fig 3, A). Although no significant change in PC₂₀ values was detected in any of the

groups, 7 of 23 patients with positive bronchial hyper-reactivity at baseline were found to have no reactivity at month 12: 2 of 7 in the SLIT group, 4 of 7 in the SCIT plus SLIT group, and 1 of 5 in the pharmacotherapy group (Fig 3, B).

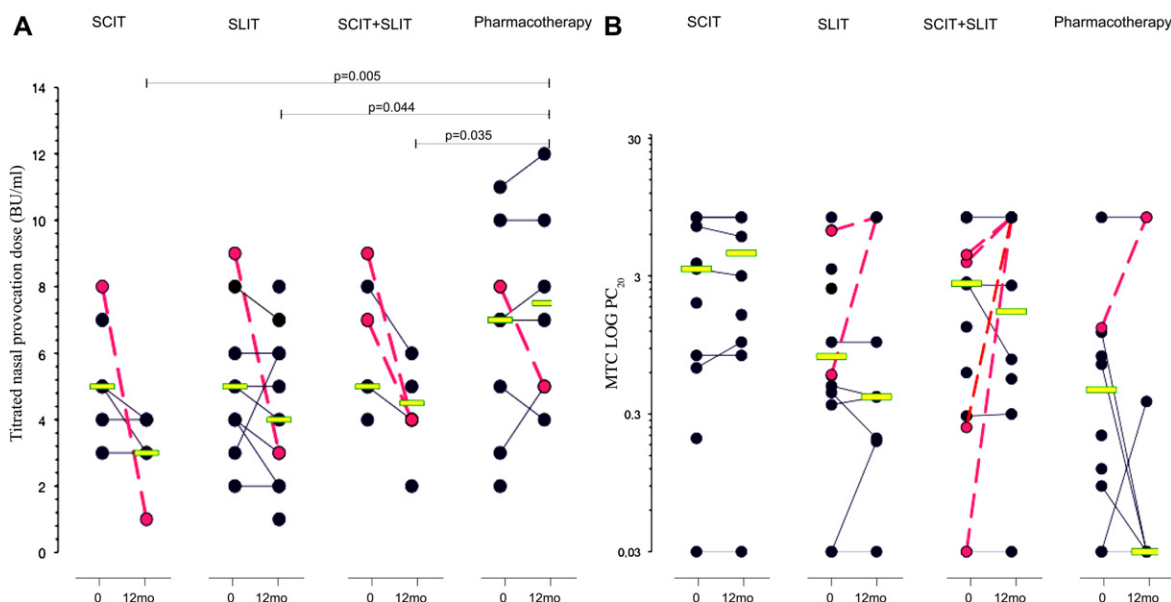


FIG 3. A, Titrated allergen-specific nasal provocation dose (in BU per milliliter) at baseline and 12 months after treatment. The 5 patients noted with red dashed lines had a positive ASNP test result at baseline and a negative test result after 12 months. B, NSBPT results at baseline and 12 months after treatment. The 7 patients noted with red dashed lines had positive bronchial hyperreactivity test results at baseline and negative test results at month 12. MTC LOG PC₂₀, Methacholine-induced provocative concentration log-transformed values.

Pulmonary function tests revealed a significant increase in FEV₁ at month 12 in the SCIT plus SLIT group compared with that seen in the pharmacotherapy group.

Diameters of wheals induced by *D pteronyssinus* and *D farinae* were significantly lower in the SCIT group at month 12 when compared with those at baseline and in the pharmacotherapy group (*D pteronyssinus*: $P = .009$ and $P = .006$, respectively; *D farinae*: $P = .004$ and $P < .0001$, respectively). No difference was detected in the other immunotherapy groups.

Immunologic evaluation

Total and specific immunoglobulin levels. There was no significant difference between any of the immunotherapy groups in terms of total serum IgE levels (see Table E2) and Der p 1-specific IgE levels and levels seen in the pharmacotherapy group at month 12 of therapy.

Der p 1-specific IgG₄ levels were significantly higher in the SCIT and SCIT plus SLIT groups at months 4 and 12 compared with both those in the pharmacotherapy group and baseline values but not those in the SLIT group (Fig 4). Der p 2-specific IgG₄ levels were significantly higher in the SCIT group at months 4 and 12 compared with those in the pharmacotherapy group ($P = .03$ and $P = .02$, respectively). Both the SCIT and SCIT plus SLIT groups revealed a significantly higher ratio of Der p 1-specific IgG₄/IgE levels at months 4 and 12 compared with levels in the pharmacotherapy group (for SCIT: $P = .008$ and $P = .008$, respectively; for SCIT plus SLIT: $P = .008$ and $P = .03$, respectively) and baseline values (for SCIT: $P = .04$ and $P = .04$, respectively; for SCIT plus SLIT: $P = .04$ and $P = .04$, respectively).

Cytokine responses. In the SCIT group, Der p 1 induced TGF- β levels that were significantly increased at month 1 compared

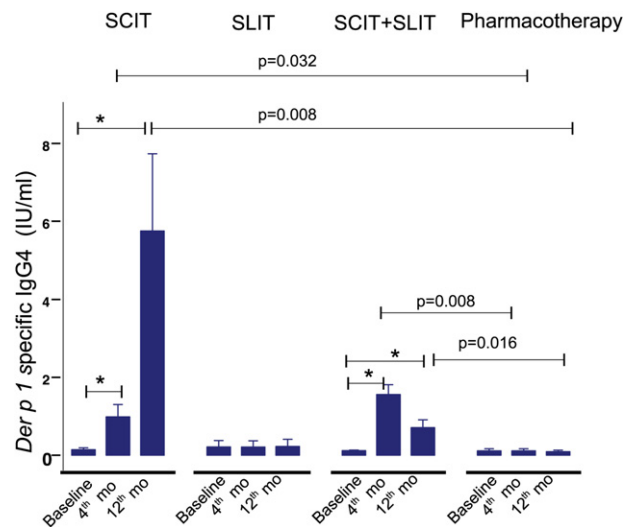


FIG 4. The median values for Der p 1-specific IgG₄ levels at baseline and months 4 and 12. * $P < .05$.

with baseline levels ($P = .02$), which then continued to increase at month 4, with a peak at month 12 (Fig 5). IL-10 levels increased at month 4, reaching a plateau at 4 to 12 months. IFN- γ levels increased at month 4, followed by a decrease to baseline levels at month 12. IL-13 levels increased from month 4 ($P = .25$) and peaked at month 12. Meanwhile, SLIT resulted in increased levels of Der p 1-induced TGF- β and IL-10 at month 4, which were maintained until month 12. In this group IFN- γ levels showed a transient increase at month 4, followed by a reduction at month 12. In the SCIT plus SLIT group, the cytokine responses were mostly similar to those of the SCIT group at months 4 and 12 (Fig 5). Lower levels

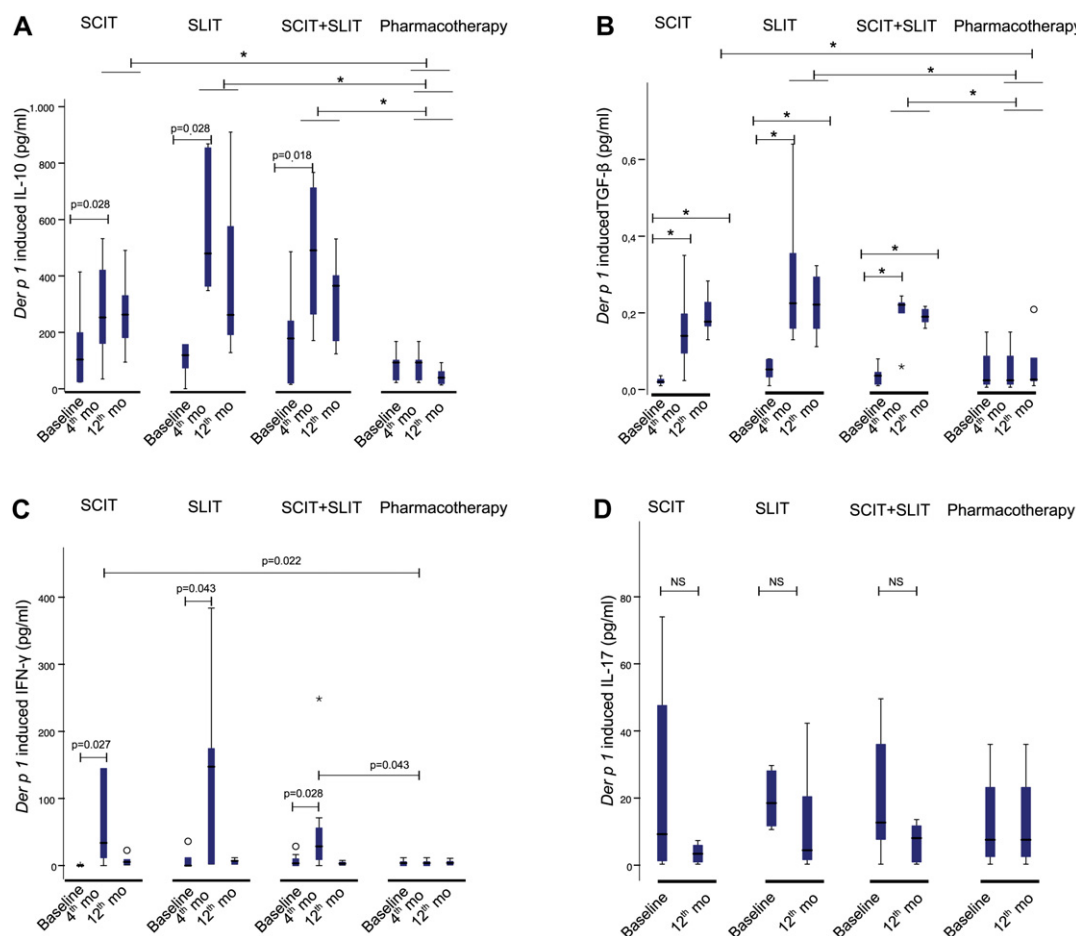


FIG 5. Levels of secreted cytokines at baseline and 4 and 12 months after treatment in the SCIT, SLIT, SCIT plus SLIT, and pharmacotherapy groups. PBMCs were cocultured with Der p 1 (allergen used in treatment). Secreted cytokines were measured in cultured supernatants by means of ELISA. **A**, Der p 1-induced IL-10. **B**, TGF- β . **C**, IFN- γ . **D**, IL-17. * $P < .05$. In Fig 5, C, the difference between the SLIT and pharmacotherapy groups at month 4 was close to statistical significance ($P = .063$). NS, Not significant.

of IL-17 were detected at the end of year 1 in all immunotherapy groups compared with those seen in the pharmacotherapy group, which did not reach statistical significance (Fig 5). IL-5 levels increased in the SCIT plus SLIT group at month 4 ($P = .03$) and the pharmacotherapy group at month 12 ($P = .04$).

Correlation between the symptom-medication scores, antigen-specific and total IgE levels, and cytokine responses

There were significant and positive correlations between Der p 1-induced IL-5 levels and SSAs, TMSs, and VAS scores for rhinitis ($r = 0.647$ and $P = .031$, $r = 0.823$ and $P = .006$, and $r = 0.598$ and $P = .031$, respectively) at baseline; IL-5 levels and VAS scores for rhinitis and TMSs at month 12 ($r = 0.724$ and $P = .003$ and $r = 0.602$ and $P = .014$, respectively); and IL-17 levels and MSRs at month 12 ($r = 0.566$ and $P = .044$).

Negative correlations were detected between IFN- γ /IL-5 ratios and SSAs, SSRs, and VAS scores for rhinitis ($r = -0.682$ and $P = .02$, and $r = -0.706$ and $P = .02$, and $r = -0.584$ and $P = .04$, respectively) at baseline; IFN- γ levels and TMSs at month 4 ($r = 0.573$ and $P = .03$); TGF- β levels and VAS scores for asthma at month 12

($r = -0.607$ and $P = .01$); IFN- γ levels and TMSs and MSAs at month 12 ($r = -0.618$ and $P = .02$ and $r = -0.672$ and $P = .008$); and the IFN- γ /IL-5 ratio and TMSs and VAS scores for rhinitis at month 12 ($r = -0.574$ and $P = .02$ and $r = -0.54$ and $P = .04$).

There were significant positive correlations between the IFN- γ /IL-5 ratio and the Der p 1-specific IgG₄/IgE ratio at month 12 ($r = 0.701$ and $P = .001$) and IFN- γ levels and Der p 1-specific IgG₄ levels at month 12 ($r = 0.784$ and $P < .0001$).

When Der p 1-induced cytokine levels were assessed in association with antigen-specific IgG₄ values, an obvious trend was observed in that IL-10 and TGF- β levels closely followed the changes in Der p 1-specific IgG₄ levels (see Fig E3 in this article's Online Repository at www.jacionline.org). Likewise, a similar trend was observed in symptom scores and antigen-specific IgG₄ levels; the decrease in scores was followed by increases in IgG₄ levels.

DISCUSSION

In a group of children followed over a period of 18 months, all 3 regimens of SIT, namely SCIT, SLIT, and SCIT plus SLIT, proved effective for the treatment of asthma and rhinitis, each with

different degrees of efficacy and onset of activity. Beginning with SCIT at the build-up phase provided rapid onset, not only in terms of clinical improvement at month 4 but also by eliciting a simultaneous surge in the production of TGF- β from month 1 and IFN- γ , IL-10, IL-13, and the blocking IgG antibodies at month 4. Meanwhile, the clinical efficacy of SLIT was less and resulted in similar increases in IFN- γ , TGF- β , IL-10, and IL-13 at month 4 but no influence on production of blocking IgG₄ antibodies. The novel regimen (ie, SCIT at the build-up phase followed by SLIT in the maintenance phase) proved satisfactory. It was as effective as SCIT for asthma and even more so for rhinitis and evoked favorable immunologic responses comparable with those of SCIT while providing the advantage of avoiding injections and related side effects during the maintenance phase. Because of ethical considerations in children, a placebo group was not included in the design of our study.

Comparative studies on the clinical effectiveness of SCIT and SLIT have been carried out on adults, with heterogeneous results.³⁻⁵ One of 2 double-blind studies demonstrated that both routes were almost equally effective in reducing symptom and medication scores,⁵ whereas the other found SCIT more effective than SLIT, with the SCIT group having a disease severity reduced to one third and the SLIT group to half the severity observed in placebo-treated subjects.³ In the study by Mungan et al,⁴ SCIT proved effective for both rhinitis and asthma, whereas SLIT proved effective for only rhinitis. Even better, the efficacy of SCIT plus SLIT for rhinitis symptoms in our study might also suggest that maintenance through the mucosal route might potentiate the efficacy of SCIT in controlling rhinitis symptoms.

To our knowledge, the current study is the first trial combining SCIT and SLIT in HDM-sensitized pediatric patients with respiratory allergy. We hypothesized that combining the 2 alternative modes of SIT, namely mucosal and systemic, might offer better clinical outcomes by integrating their individual advantages to potentiate mechanisms of tolerance. With all 3 immunotherapy regimens, ICS dosage and the number of asthma attacks were reduced during treatment, with earlier and more sustained decreases in the SCIT and SCIT plus SLIT groups. Likewise, those 2 groups proved efficacious in terms of decreasing symptom and medication scores for asthma starting from month 4 and rhinitis at month 12, with SCIT plus SLIT even more effective than SCIT for rhinitis. The efficacy of SLIT was limited only to VAS scores for asthma. Significantly lower ICS doses at months 4 and 18 in the SCIT and SCIT plus SLIT groups as opposed to the SLIT group only suggest that the former 2 groups are superior in terms of steroid-sparing effect compared with SLIT. However, the sublingual route proved safer than the subcutaneous because no patients receiving SLIT experienced significant adverse reactions, whereas 2 patients receiving SCIT had to discontinue treatment because of side effects.

SIT has been reported to decrease serum IL-4 levels or release of IL-4 and IL-13 from allergen-stimulated peripheral blood lymphocytes or increase numbers of circulating CD4⁺ T lymphocytes producing IFN- γ or the IFN- γ /IL-4 ratio.^{16,17} Whereas earlier studies emphasized a switch from T_H2 to T_H1 cells in peripheral blood,^{18,19} more recent studies on grass pollen immunotherapy have shown increased expression of mucosal and peripheral T-cell IL-10 and TGF- β .^{20,21} The induction of peripheral T-cell tolerance represents an essential step in SIT and is initiated by the increased autocrine action of allergen-specific regulatory T cells.^{22,23} A recent study of sublingual

HDM immunotherapy highlighted the pivotal role of TGF- β as the mediator of early SLIT-induced suppression of the T-cell responses to allergen.²⁴ There are few studies comparing the immunologic effects of SLIT and SCIT in the long-term.³⁻⁵ A unique aspect of the current study is the opportunity to evaluate the dynamics of antigen-induced cytokine responses in different modes of immunotherapy, including the novel route. All 3 modalities of SIT induced the Der p 1-specific regulatory cytokines IL-10 and TGF- β when compared with the pharmacotherapy group. In our novel route, SCIT plus SLIT, cytokine responses resembled those of SCIT at the build-up phase, which then was maintained at the increased level by using SLIT during the maintenance phase. As for T_H1/T_H2 cytokines, although IFN- γ levels increased in all immunotherapy groups at month 4, the ratio of IFN- γ to IL-13 or IL-5 remained unchanged throughout the study period.

Previous studies have shown that SIT induces a transient increase in serum specific IgE levels, followed by a gradual decrease over months or years²⁵; meanwhile, production of allergen-specific IgG₄ and IgG₁ increases, with 10- to 100-fold increases in sera.^{26,27} The resultant increase in IgG₄/IgE ratio reflects a change from allergen-specific T_H2 to regulatory T-cell predominance.²⁸ In our study no significant change in specific IgE levels was detected after 12 months in any of the groups. In contrast, Der p 1-specific IgG₄ levels increased significantly after 4 months in the SCIT and SCIT plus SLIT groups, peaking at month 12 in the SCIT group and decreasing slightly in the combination group. On the other hand, no significant change in Der p 1-specific IgG₄ levels was detected in the SLIT group, indicating the role of systemic (SCIT) but not mucosal (SLIT) delivery of antigen in inducing production of blocking IgG antibodies, as had previously been demonstrated by Rolinck-Werninghaus et al.²⁹ In our study the increase in Der p 1-specific IgG₄ levels throughout the study period with immunotherapy correlated best with increased TGF- β levels and to a lesser extent with IL-10 levels.

Symptom scores were correlated positively with Der p 1-induced IL-5 and negatively with IFN- γ levels, IFN- γ /IL-5 ratios, TGF- β levels, and Der p 1-specific IgG₄ levels. These data suggest that enhancement of both T_H1 and regulatory T-cell cytokines might account for the clinical improvement in all immunotherapy groups, whereas better clinical outcomes with SCIT and SCIT plus SLIT are most likely related to the more potent induction of IgG blocking antibodies with the systemic route of allergen administration.

In conclusion, this prospective, randomized, controlled study revealed that the systemic route of immunotherapy appeared more efficacious compared with the sublingual route in that it offered favorable clinical efficacy with earlier induction of regulatory cytokines, eliciting the production of blocking IgG₄ antibodies. The major advantage of SLIT was its safety profile and lack of injections. The novel regimen proposed seems to successfully combine the advantages of both routes without loss of clinical benefit and might be a promising alternative in children undergoing SIT. Longer-term studies will be required to establish whether this approach would be useful for immunotherapy in children.

We thank Aysegul Yildiz, MSc, for contributing to the study. We also thank ALK-Abelló for supplying allergen extracts for nasal provocation tests and for studying IgG₄ levels.

Key messages

- Both SCIT and SLIT are more effective than pharmacotherapy in HDM-sensitized children with asthma.
- The combination of SCIT with SLIT is more effective than SLIT and safer than SCIT.
- Der p 1-specific IgG₄ antibodies were induced in the SCIT and SCIT plus SLIT groups.

REFERENCES

- Halken S, Lu S, Valovirta E. New visions in specific immunotherapy in children: an iPAC summary and future trends. *Pediatr Allergy Immunol* 2008;19(suppl 19):60-70.
- Moingeon P, Batard T, Fadel R, Frati F, Sieber J, Van Overtvelt L. Immune mechanisms of allergen specific sublingual immunotherapy. *Allergy* 2006;61:151-65.
- Khinchin MS, Poulsen LK, Carat F, André C, Hansen AB, Mallin HJ. Clinical efficacy of sublingual and subcutaneous birch pollen allergen specific immunotherapy: a randomized placebo-controlled, double blind, double dummy study. *Allergy* 2004;59:45-53.
- Mungan D, Misirligil Z, Gurbuz L. Comparison of the efficacy of subcutaneous and sublingual immunotherapy in mite-sensitive patients with rhinitis and asthma- placebo controlled study. *Ann Allergy Asthma Immunol* 1999;82:485-90.
- 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;26:1253-61.
- Amin HS, Liss GM, Bernstein DI. Evaluation of near-fatal reactions to allergen immunotherapy injections. *J Allergy Clin Immunol* 2006;117:169-75.
- Ragusa VF, Massolo A. Non-fatal systemic reactions to subcutaneous immunotherapy: a 20-year experience comparison of two 10-year periods. *Allerg Immunol (Paris)* 2004;36:52-5.
- Penagos M, Passalacqua G, Compalati E, Baena-Cagnani CE, Orozco S, Pedroza A, et al. Metaanalysis of the efficacy of sublingual immunotherapy in the treatment of allergic asthma in pediatric patients, 3 to 18 years of age. *Chest* 2008;133:599-609.
- Bosquet J, Lockey RF, Mallin H-J. World Health Organization position paper: allergen immunotherapy: therapeutic vaccines for allergic diseases. *Allergy* 1998;53(suppl 44):1-42.
- Allam JP, Niederhagen B, Bucheler M, Appel T, Betten H, Bieber T, et al. Comparative analysis of nasal and oral mucosa dendritic cells. *Allergy* 2006;61:166-72.
- Akdis CA, Barlan IB, Bahceciler N, Akdis M. Immunological mechanisms of sublingual immunotherapy. *Allergy* 2006;61(suppl 81):11-4.
- Bousquet PJ, Cox LS, Durham SR, Nelson HS, Passalacqua G, Ryan DP, et al. Sublingual immunotherapy: World Allergy Organization position paper. *Allergy* 2009;64(suppl 91):1-59.
- Akdis CA, Barlan IB, Bahceciler N, Akdis M. Immunological mechanisms of sublingual immunotherapy. *Allergy* 2006;61(suppl 81):11-4.
- Guideline for asthma. Available at: <http://www.ginasthma.com/Guidelineitem.asp?i1=2&i2=1&intld=1561>. Accessed May 18, 2011.
- Passalacqua G, Durham SR. Global Allergy and Asthma European Network. Allergic rhinitis and its impact on asthma update: allergen immunotherapy. *J Allergy Clin Immunol* 2007;119:881-91.
- Ohashi Y, Nakai Y, Okamoto H, Ohno Y, Sakamoto H, Sugiura Y, et al. Serum level of interleukin-4 inpatients with perennial allergic rhinitis during allergen specific immunotherapy. *Scand J Immunol* 1996;43:680-6.
- Majori M, Caminati A, Corradi M, Brianti E, Scarpa S, Pesci A. T-cell cytokine pattern at three time points during specific immunotherapy for mite-sensitive asthma. *Clin Exp Allergy* 2000;30:341-7.
- Varney VA, Hamid QA, Gaga M, Ying S, Jacobson M, Frew AJ, et al. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest* 1993;92:644-51.
- Jutel M, Pichler WJ, Skrbic D, Urwyler A, Dahinden C, Muller UR. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-gamma secretion in specific allergen-stimulated T cell cultures. *J Immunol* 1995;154:4187-94.
- Nouri-Aria KT, Wachholz PA, Francis JN, Jacobson MR, Walker SM, Wilcock LK, et al. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J Immunol* 2004;172:3252-9.
- Pilette C, Nouri-Aria KT, Jacobson MR, Wilcock LK, Detry B, Walker SM, et al. Grass pollen immunotherapy induces an allergen-specific IgA2 antibody response associated with mucosal TGF-beta expression. *J Immunol* 2007;178:4658-66.
- Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. *J Clin Invest* 1998;102:98-106.
- Francis JN, Till SJ, Durham SR. Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol* 2003;111:1255-61.
- O'Hehir RE, Gardner LM, de Leon MP, Hales BJ, Biondo M, Douglass JA, et al. House dust mite sublingual immunotherapy: the role for transforming growth factor-beta and functional regulator T cells. *Am J Respir Crit Care Med* 2009;180:936-47.
- Muller U, Helbling A, Bischof M. Predictive value of venom-specific IgE, IgG and IgG subclass antibodies in patients on immunotherapy with honey bee venom. *Allergy* 1989;44:412-8.
- van der Giessen M, Homan WL, van Kernbeek G, Aalberse RC, Dieges PH. Subclass typing of IgG antibodies formed by grass pollen-allergic patients during immunotherapy. *Int Arch Allergy Appl Immunol* 1976;50:625-40.
- Jutel M, Jaeger L, Suck R, Meyer H, Fiebig H, Cromwell O. Allergen-specific immunotherapy with recombinant grass pollen allergens. *J Allergy Clin Immunol* 2005;116:608-13.
- Akdis M, Akdis CA. Therapeutic manipulation of immune tolerance in allergic disease. *Nat Rev Drug Discov* 2009;8:645-60.
- 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;136:134-41.

METHODS

Categoric SSAs and SSRs

Cough, wheezing, breathlessness, and dyspnea were recorded on a 3-point scale (0, no symptoms; 1, mild symptoms; 2, moderate symptoms; and 3, severe symptoms), and daily total categoric symptom scores for asthma (0-12) were calculated by adding the 4 scores together. Rhinorrhea, itching, sneezing, and nasal blockage were also recorded on a 3-point scale (0, no symptoms; 1, mild symptoms; 2, moderate symptoms; and 3, severe symptoms), and daily total categoric symptom scores for rhinitis (0-12) were calculated by using the same method above. The final total symptom score per day was calculated as the mean of the scores for asthma and rhinitis.

Medication scores for asthma and rhinitis

Daily prophylactic and rescue drug intake, including β_2 -agonists and inhaled/intranasal corticosteroids, was recorded on the same diary card. One point was given if β_2 -agonist rescue medication was needed on that day and 0 if not. Daily doses of ICSs used in the run-in period and during follow-up (0, 4, 12, and 18 months) were scored. Two points were given for daily ICS/nasal steroid use. The MSA was calculated by summing used ICSs and β_2 -agonist rescue medication, and the MSR was calculated based on inhaled nasal steroid use. The TMS per day was calculated as the mean of the medication score for asthma and rhinitis.

NSBPTs, ASNPTs, and pulmonary function tests

Pulmonary function tests and NSBPTs were performed by means of computerized spirometry (Zan Flowhandy II; Zan Messgerate GmbH, Oberthulba, Germany) at baseline and at the end of the first year of therapy, as described elsewhere. All children had baseline FEV₁ values of greater than 80% of predicted value. Nonspecific challenge was started from a dose of 0.0625 mg methacholine dose. The methacholine dose was doubled until a 20% decrease in FEV₁ (PC₂₀) was detected.

ASNPTs were performed by spraying the vehicle into both nostrils once, which was then followed by increasing doses of HDM extract: 2, 4, and 8 BU/mL (ALK-Abelló) in vehicle, respectively. These concentrations corresponded, respectively, to about 0.016, 0.032, 50, and 0.064 mg per puff of the major allergen. One point was given for each of the following parameters: 3 to 4 sneezes, anterior or posterior rhinorrhea, difficulty in nasal breathing, and pruritus of the nose. Two points were given for blockage of 1 nostril and pruritus of the palate or ear. Three points were given for anterior and posterior rhinorrhea, 5 or more sneezes, blockage of both nostrils, and the presence of conjunctivitis. In consecutive doses of allergen, a total score of greater than or equal to 6 was considered a positive challenge result. Cases with scores of less than 6 points at 8 BU/mL were considered unprovoked.

Skin prick tests

Skin prick tests were performed with 14 common aeroallergens: mites, molds, pollens, and animal dander, as described previously. Histamine and dihydrochloride saline were used as positive and negative controls, respectively. A wheal size of 3 mm or greater was considered positive in the presence of a negative saline response.

PBMC isolation and culture

PBMCs were isolated by means of Ficoll-Hypaque density gradient centrifugation and then suspended in RPMI 1640 with 2 mmol/L L-glutamine (Gibco, Invitrogen, Grand Island, NY) and 100 U/mL penicillin/streptomycin and 10% FCS (Sigma Chemical Co, St Louis, Mo). PBMCs (6×10^5) were incubated with and without 5 μ g/mL rDer p 1 (Indoor Biotechnologies Ltd, Cardiff, United Kingdom) in 500 μ L of each in 48-well plates (Costar Corp, Cambridge, Mass) at 37°C with 5% CO₂ for 5 days. The supernatants were collected and stored at -20°C until tested. IL-5, IL-10, IL-13, IFN- γ , TGF- β (Endogen, Rockford, Ill), and IL-17 (Invitrogen, Camarillo, Calif) levels of the culture supernatants were determined by using a commercial human ELISA kit, according to the manufacturer's instruction.

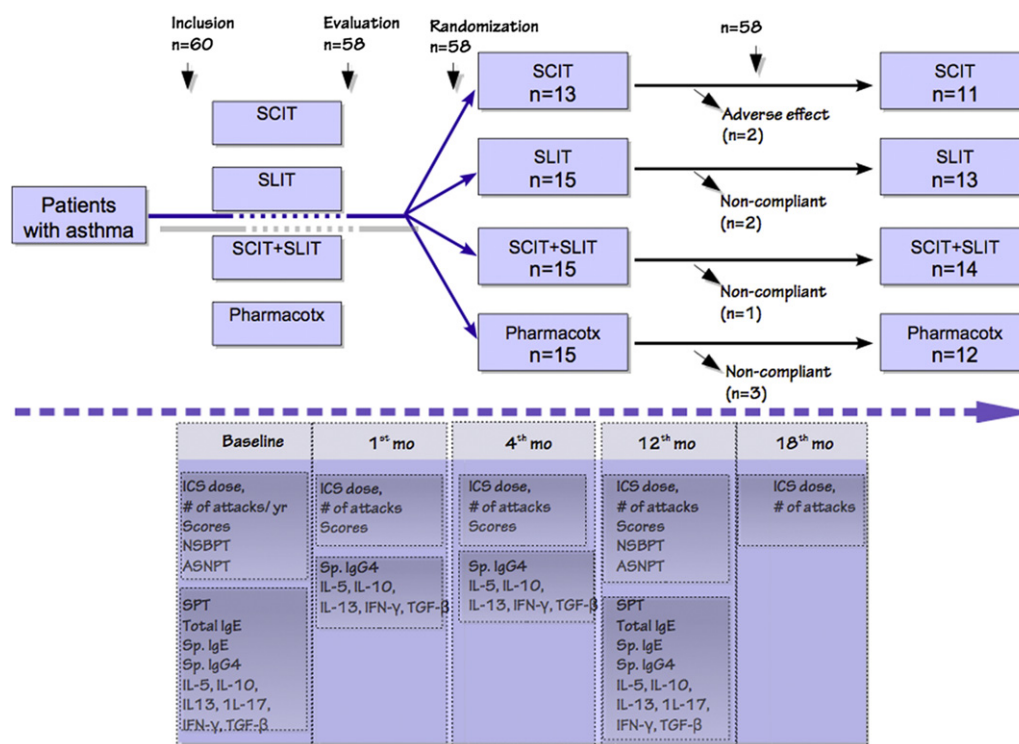


FIG E1. Study design.

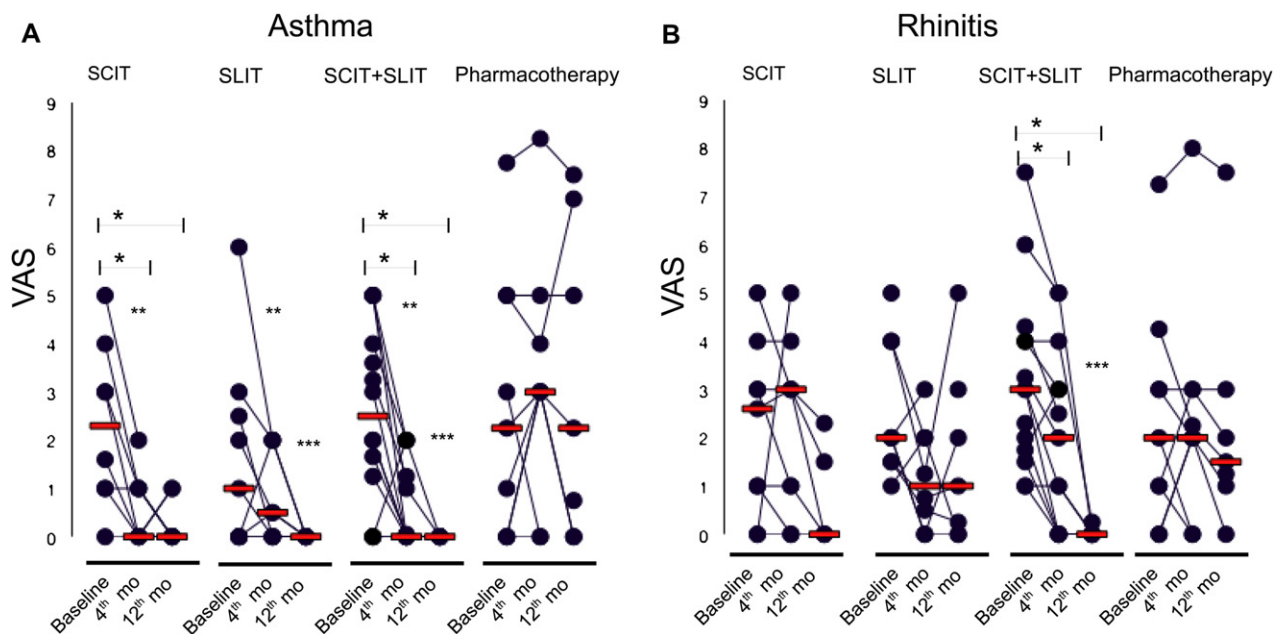


FIG E2. VAS scores for asthma (**A**) and rhinitis (**B**) in the SCIT, SLIT, SCIT + SLIT, and pharmacotherapy groups before treatment and 4 and 12 months after treatment. * $P < .05$ for the reduction in the VAS score compared with baseline values. ** $P < .05$ for the SCIT, SLIT, or SCIT plus SLIT groups versus pharmacotherapy at 4 months (Mann-Whitney U test). *** $P < .05$ for the SCIT, SLIT, or SCIT plus SLIT groups versus the pharmacotherapy group at 12 months.

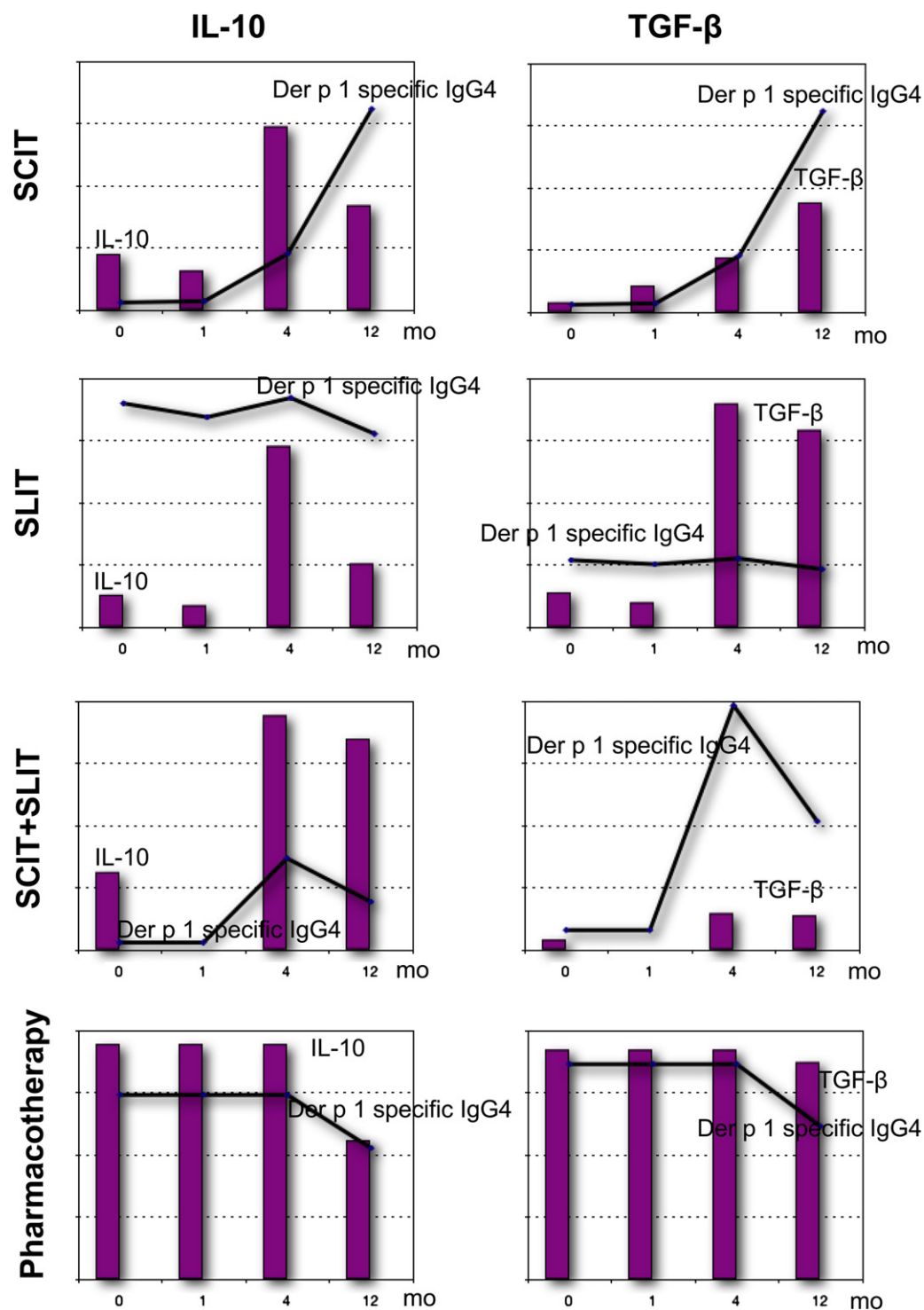


FIG E3. Der p 1–induced IL-10 and TGF- β (bars) and Der p 1–specific IgG₄ (lines) levels at baseline and months 1, 4, and 12. Lines and bars represent proportional changes during the study period.

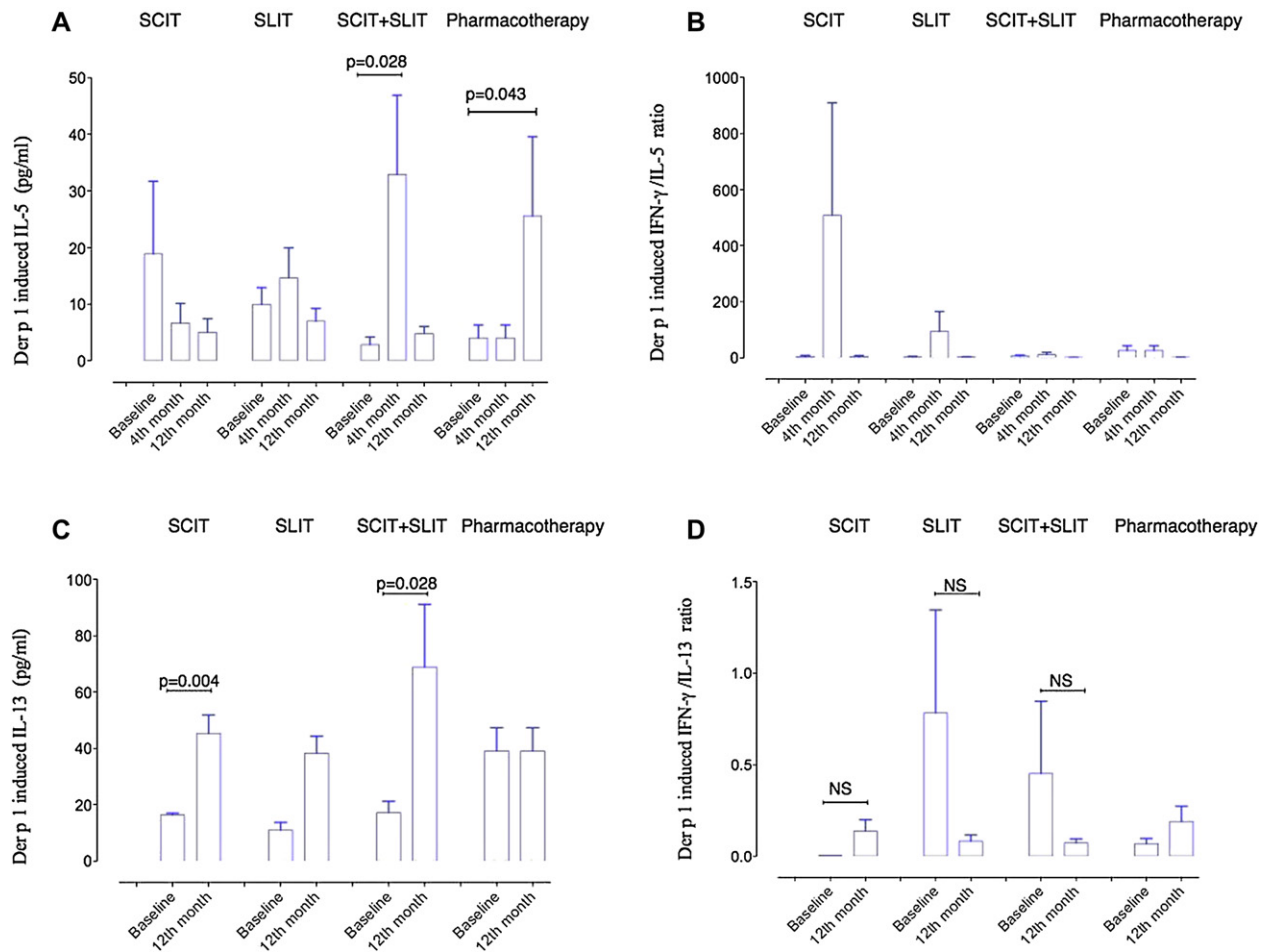


FIG E4. Levels of secreted cytokines at baseline (before immunotherapy) and 4 and 12 months after treatment in the SCIT, SLIT, SCIT plus SLIT, and pharmacotherapy groups. **A**, Der p 1-induced IL-5. **B**, IFN- γ /IL-5 ratio. **C**, IL-13. **D**, IFN- γ /IL-13 ratio.

TABLE E1. Immunotherapy schedule of the groups for 1 year

		SLIT group	SCIT group	SCIT + SLIT group
Build-up phase	Dose scheduled	Vial 0: 1-5 drops	Vial 1: 0.2, 0.4, 0.8 mL	Vial 1: 0.2, 0.4, 0.8 mL
		Vial 1: 1-5 drops	Vial 2: 0.2, 0.4, 0.8 mL	Vial 2: 0.2, 0.4, 0.8 mL
		Vial 2:1-5 drops	Vial 3: 0.2, 0.4, 0.6, 0.8 mL	Vial 3: 0.2, 0.4, 0.6, 0.8 mL
		Vial 3: 1-5 drops	Vial 4: 0.1, 0.2, 0.4, 0.6, 0.8, 1 mL	Vial 4: 0.1, 0.2, 0.4, 0.6, 0.8, 1 mL
		Vial 4: 1-5 drops		
	Duration	30 d	16 wk	16 wk
	Cumulative dose (Der p 1)	1.5 µg	16.2 µg	16.2 µg
	Cumulative dose (Der f 1)	1.5 µg	22.9 µg	22.9 µg
		750.7 STU	331.540 SQ-U	331.540 SQ-U
Maintenance phase	Dose scheduled	5 drops of vial 4 three times a week	1 mL of vial 4 per month	5 drops of vial 4 three times a week
		Cumulative dose (Der p 1)	44.1 µg	43.2 µg
		Cumulative dose (Der f 1)	62.1 µg	43.2 µg
		26.400 STU	900.000 SQ-U	21.600 STU

SQ-U, Standard quality unit; *STU*, skin test unit.

TABLE E2. Laboratory features

	SLIT group (n = 13)	SCIT group (n = 11)	SCIT + SLIT group (n = 14)	Pharmacotherapy group (n = 12)	<i>P</i> value*	<i>P</i> value†
ASNPT						
Baseline	5.1 ± 2.2	3 ± 1.22	6.1 ± 1.8	6.7 ± 3.0	NS	NS
Month 12	4.2 ± 2.2†	5.29 ± 1.70†	4.1 ± 1.3†	7.0 ± 3.0	<.05	<.05
NSBPT (mg)						
Baseline	2.5 ± 2.9	3.73 ± 3.17	3.9 ± 3.2	1.4 ± 2.5	NS	NS
Month 12	3.2 ± 3.9	4.52 ± 3.27	5.2 ± 3.6	2.7 ± 4.0	NS	NS
FEV ₁ (%)						
Baseline	99 ± 14	99 ± 14	107 ± 13	95 ± 4	NS	NS
Month 12	104 ± 6	104 ± 5.6	108 ± 10†	91 ± 15	<.05	<.05
Total serum IgE (IU/mL)						
Baseline	512 ± 471	829 ± 1142	503 ± 306	329 ± 211	NS	NS
Month 12	709 ± 629	1096 ± 1065	644 ± 698	352 ± 262	NS	NS
Allergen-specific immunoglobulins						
Der p 1-specific IgE (IU/mL)						
Baseline	62 ± 52	67 ± 33	83 ± 27	73 ± 37	NS	NS
Month 12	61 ± 53	44 ± 32	85 ± 34	75 ± 41	NS	NS
Der p 1-specific IgG ₄ (UA/mL)						
Baseline	0.21 ± 0.37	0.14 ± 0.1	0.11 ± 0.03	0.11 ± 0.11	NS	NS
Month 1	0.20 ± 0.35	0.34 ± 0.44	0.11 ± 0.03		NS	NS
Month 4	0.20 ± 0.36	0.98 ± 0.70†	1.56 ± 0.56†		<.05	<.05
Month 12	0.22 ± 0.41	5.74 ± 4.43†	0.70 ± 0.45†	0.09 ± 0.08	<.05	<.05

All values in the table are presented as means ± SDs. Statistically significant *P* values are presented in bold.

NS, Not significant.

*Comparison of the groups with the Kruskal-Wallis test.

†Comparison of the immunotherapy groups with the pharmacotherapy group, Mann-Whitney *U* test.