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Sublingual-swallow immunotherapy (SLIT) in patients with asthma due to house-dust mites: a double-blind, placebo-controlled study

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A double-blind, placebo-controlled study was carried out in 85 patients with a well-documented history of perennial asthma caused by house-dust mites. Patients received either placebo or sublingual immunotherapy (SLIT) with a standardized *Dermaphagoides pteronyssinus* (DP)–*D. farinae* (DF) 50/50 extract. After a run-in period, patients received increasing doses up to 300 IR every day for 4 weeks and then three times a week for the following 24 months. The cumulative dose was about 104 000 IR, equivalent to 4.2 mg Der p 1 and 7.3 mg Der f 1. Symptom and medication scores and respiratory function were assessed throughout the trial. Serum specific IgE and IgG4 were determined before SLIT (t_0) and after 6 (t_1), 11 (t_2), 17 (t_3), and 25 months (t_4) of SLIT. Mite exposure was evaluated at t_0 , t_2 , and t_4 by semiquantitative guanine determinations. Patients aged 15 years and older were asked to assess their quality of life (QoL) by completing the SF20 (Short Form Health Status Survey) plus two items at t_0 , t_2 , and t_4 . Use of inhaled corticosteroids and β_2 -agonists was significantly decreased after 25 months of treatment in both groups ($P<0.03$). SLIT patients showed significant improvements in respiratory function at t_4 (% predicted FEV₁ ($P=0.01$), VC ($P=0.002$), morning ($P=0.01$) and evening ($P=0.03$) PEF, and reduction in daytime asthma score ($P=0.02$). In the SLIT group, the post-treatment PD₂₀ was 1.75 times higher than the baseline value. There was no change in PD₂₀ in the placebo group. Compared to the placebo group, the SLIT group showed a significant increase in specific IgE DP ($P=0.05$), IgE DF ($P=0.02$), IgG4 DP ($P=0.001$), and IgG4 DF

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($P=0.001$) levels after SLIT. QoL scores were similar in both groups at t_0 and t_2 . At t_4 , all scores were better in the SLIT group than in the placebo group, with the differences being most marked for the general perception of health ($P=0.01$) and physical pain ($P=0.02$). Adverse events were similar in the two groups. This study shows that SLIT in house-dust-mite-related asthma has a good safety profile and improves respiratory function, bronchial hyperreactivity, and QoL.

The efficacy of specific immunotherapy by the subcutaneous route has been assessed in many controlled studies. Allergen immunotherapy is effective in patients with allergic rhinitis, IgE-mediated asthma, and venom hypersensitivity (1). In contrast, opinions conflict as to the efficacy of immunotherapy when treatment is administered by the oral or sublingual route (2). Both positive (3–16) and disappointing (17, 18) results have been reported. Most often, oral or sublingual immunotherapy (SLIT) has been shown to be effective, although not always absolutely safe (12). The efficacy of sublingual treatment has been ascribed to the use of higher doses. It has been suggested that this route may be safer than others. However, most of the positive clinical studies assessing the sublingual route were performed with pollen-allergen extracts (3–7, 10, 13–16).

The present study compared sublingually administered house-dust-mite extract and placebo in patients with perennial asthma.

Material and methods

Patients

Eighty-five patients aged 7–42 years with at least a 1-year history of moderate or moderately severe asthma (Aas score 2 or 3 [19]) due to house-dust mites agreed to take part in this clinical study, which was approved by the ethics committee of the Lapeyronie and Saint-Eloi Hospital, Montpellier, France. All the patients (or parents of minors) freely gave their informed consent. The diagnosis of allergy to house-dust mites (*Dermatophagoides pteronyssinus* and/or *D. farinae*) was based on clinical history, positive skin tests using standardized extracts (Stallergènes SA, Antony, France), and the presence of specific IgE as shown by RAST (Pharmacia Diagnostics Uppsala, Sweden), with class 2 as the cutoff value. Sensitization to *Alternaria* or *Cladosporium* was an exclusion criterion. In the event of sensitization to animal danders, presence at home of a pet also led to

exclusion. Patients who were treated with oral or parenteral corticosteroids (more than 15 consecutive days), depot steroids, inhaled corticosteroids in a dosage greater than 1000 µg/day (beclomethasone dipropionate), inhaled β_2 -agonists more than four times/day, and/or oral β_2 -agonists or methylxanthines were excluded, as were those who had received immunotherapy for mite in the previous 2 years. Pregnant women were also excluded. Only patients with a FEV₁ greater than 70% of predicted were included.

Table 1 shows the characteristics of the two groups. They were comparable at baseline except for bronchial reactivity, which was significantly more severe in the SLIT group.

Treatment

Treatment consisted of graded courses of a standardized (20) house-dust-mite extract or placebo in the same glycerosaline diluent followed by maintenance therapy. The extract was graded in four strengths: 1, 10, 100, and 300 IR/ml. The in-house reference extract (labeled 100 IR) was defined as the concentration eliciting a wheal with a mean diameter 7 mm by skin prick test in 30 subjects sensitive to the corresponding allergen. Treatment was initiated between September 1993 and May 1994; the study ended in September 1996. The Der p 1 and Der f 1 contents of 1 ml 100 IR allergenic extract were 8 and 14 µg, respectively.

Patients received increasing doses from each vial, starting with one drop from the 1 IR/ml vial and increasing to 10 drops on day 4, taken sublingually in the morning before breakfast. The drops were kept in the mouth for at least 2 min and then swallowed. This process was repeated for the second vial (10 IR/ml) (from 1 drop to 10 drops on day 8). On days 9–15, 1–20 drops from the third vial (100 IR/ml) were given. Finally, on days 16–24, the patient took 5–20 drops from the last vial (300 IR/ml). Once the 20-drop dose was reached, the patient was switched to maintenance therapy consisting of 20 drops of 300 IR/ml every day for 4 weeks and then three times a week for the following 24 months.

Table 1. Characteristics of patients included in study

	SLIT	Placebo treatment
No. of subjects	42	43
Sex (male:female)	23:19	25:18
Age		
Mean age (years) $\pm$ SD	21 $\pm$ 10	22 $\pm$ 10
Age range	7–36	7–42
Rhinitis <i>n</i> (%)	18 (43%)	17 (40%)
Previous desensitization <i>n</i> (%)	11 (26%)	11 (26%)
Mean duration of asthma (years) $\pm$ SD	10 $\pm$ 8	8 $\pm$ 7
Asthma severity (Aas score)		
1– <i>n</i> (%)	0	0
2– <i>n</i> (%)	36 (86%)	34 (79%)
3– <i>n</i> (%)	6 (14%)	9 (21%)
4– <i>n</i> (%)	0	0
5– <i>n</i> (%)	0	0
Methacholine bronchial challenge	105	229
PD ₂₀ -geometric mean (μ g)		

The cumulative dose taken by the patients was about 104 000 IR, equivalent to 4.2 mg Der p 1 and 7.3 mg Der f 1.

Study design

The patients underwent a run-in period of 4 or 8 weeks before beginning immunotherapy, for assessment of asthma severity and inclusion criteria. They were then randomized to placebo or active treatment.

The patients were also assessed after week 1 of maintenance therapy and at 12-week intervals throughout the maintenance therapy. At each clinical visit, asthma severity was assessed and respiratory function tests were conducted to determine VC, FEV₁, and PEFr. In addition, a methacholine bronchial challenge test, a quality of life (QoL) evaluation, and an assessment of mite exposure were done during the run-in period and after 11 and 25 months of maintenance therapy.

Blood samples were taken before the beginning of treatment and after 6, 11, 17, and 25 months of maintenance therapy.

The patients were asked to give their impression of the degree of improvement after 11 and 25 months of treatment, on a 0–3 scale (0 indicating worsening and 3 remarkable improvement) and a 10-cm visual analog scale (VAS) (from 0 mm=no improvement to 10 mm=remarkable improvement). The investigators were also required to assess patient improvement from the run-in period, taking into account drug consumption, symptom scores, and FEV₁.

Diary card

The patients were requested to keep a daily record of their morning and evening PEFr (the best of three determinations) and of their nighttime and daytime asthma symptom scores during the run-in period, the graded dosing period, and the 3-week period before each clinical visit. Daytime and nighttime asthma symptoms were recorded on a four-point scale (0–3, with 0 indicating no symptoms and 3 severe symptoms). Thus, the maximum clinical score possible per patient per day was 6. PEFr was measured by a Mini-Wright peak flow meter, with the patient standing. In addition, drug intake (β_2 -agonists, anticholinergics, cromone, inhaled corticosteroids, and oral and parenteral corticosteroids) was recorded on the same diary card.

From these records, daily means for daytime and nighttime symptoms and for morning and evening PEFr were calculated for baseline, the graded dosing period, and each 3-week period of maintenance therapy. In the same way, daily means were calculated for each of the above periods for the following drugs: inhaled corticosteroids, β_2 -agonists, anticholinergics, cromones, and systemic corticosteroids. Doses were expressed in micrograms of beclomethasone, salbutamol, and ipratropium bromure and in milligrams of sodium nedocromil and prednisolone. Oral corticosteroid dosages were expressed in equivalents according to Hayes (21). Moreover, a cumulative symptom plus drug intake score was calculated by adding nighttime and daytime asthma scores to a specific score based on the class of drugs (2.5 points for 250 μ g of inhaled beclomethasone, 1 point for 100 μ g of salbutamol or 2 mg of sodium nedocromil, or 18 points for 20 mg of systemic prednisolone).

Skin tests

Before beginning treatment, all patients underwent cutaneous testing with the following allergens, provided by Stallergènes SA: *D. pteronyssinus* (DP), *D. farinae* (DF), cat dander, dog dander, *Alternaria*, *Cladosporium*, five-grass mix (orchard grass, meadow grass, ryegrass, sweet vernal grass, and timothy grass), *Ambrosia*, *Artemisia*, and birch.

House-dust-mite-avoidance measures

All the patients were asked to continue their normal cleaning activities. No additional environmental care was recommended at the time of enrollment. Avoidance measures, if any, were to remain unchanged throughout the study in order to maintain the same level of house-dust-mite exposure.

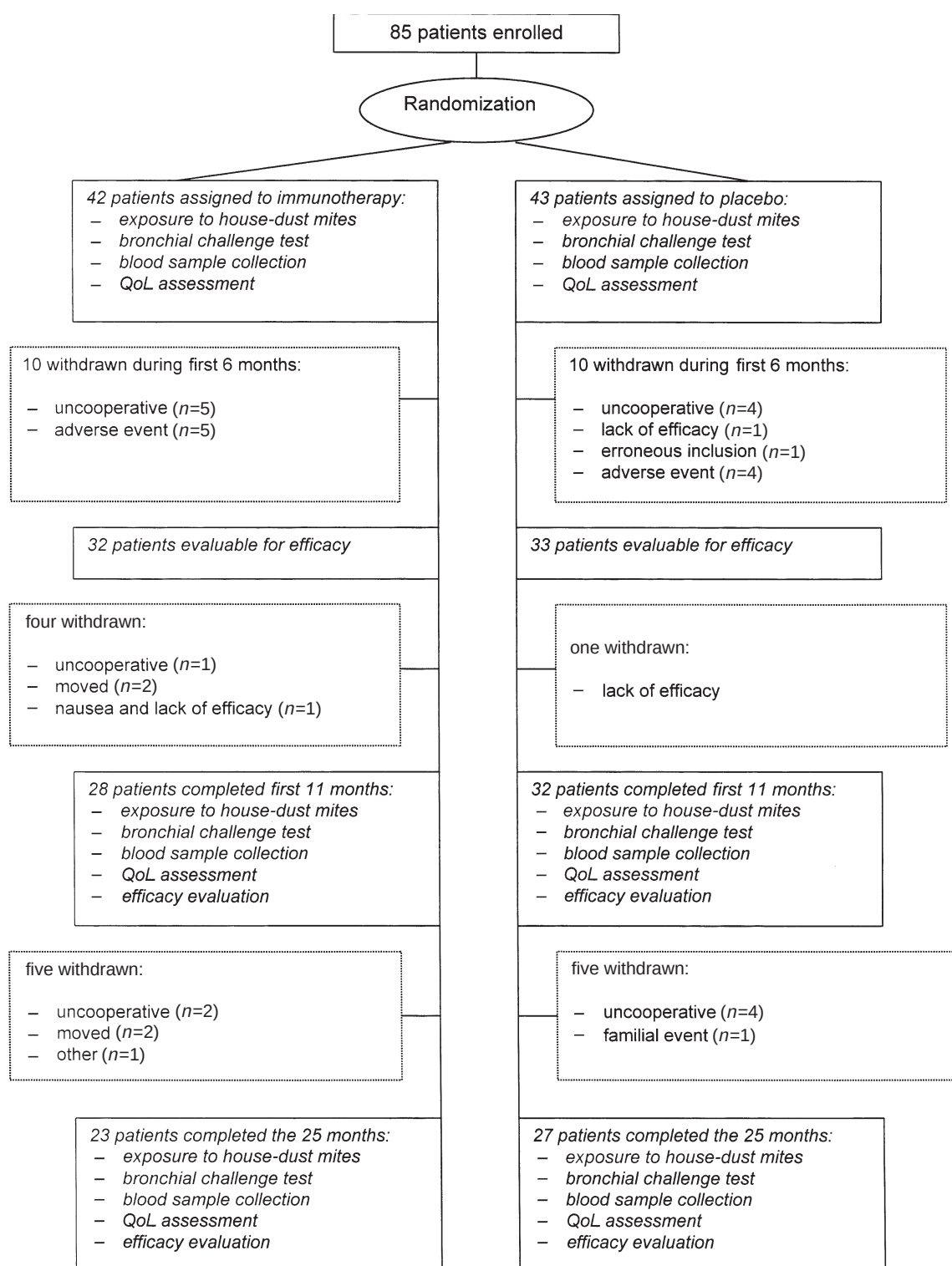


Figure 1. Study outline.

Assessment of exposure

The mite concentration of house dust was determined as an indicator of the exposure to house-dust-mite allergens. Patients were asked to collect dust (during the run-in period

and after 11 and 25 months of SLIT) from mattress surfaces with the same vacuum cleaner throughout the study (at least 800 W) and a constant vacuuming time of 2 min per square meter. Dust samples were stored at 4°C in sealed paper vacuum bags. Indoor mite exposure was evaluated by

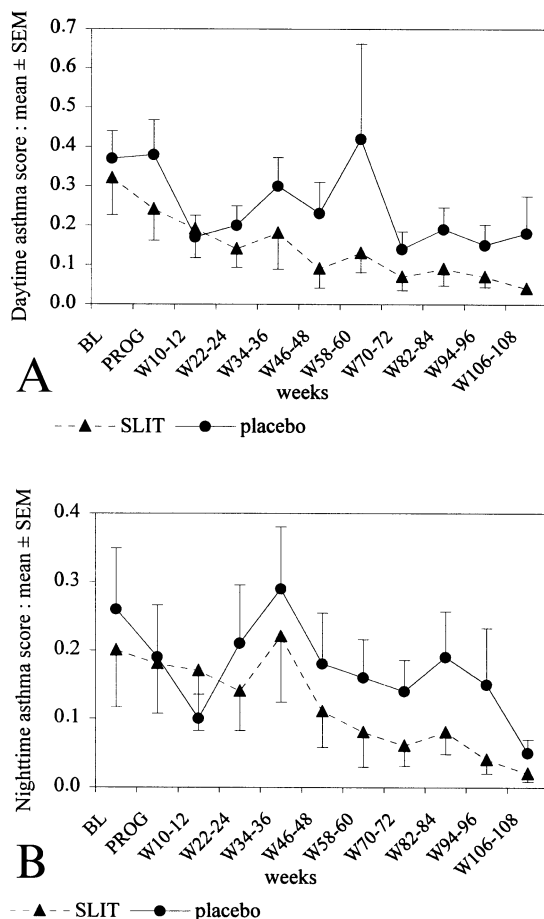


Figure 2. A) Daytime asthma scores (mean $\pm$ SEM). B) Nighttime asthma scores (mean $\pm$ SEM). SLIT group: $n=23$ at week 108 (25 months); placebo group: $n=26$ at week 108 (25 months). BL: baseline; PROG: progression; W: week.

semiquantitative guanine determinations (Acarex test[®]), and the results were expressed on a four-point scale: 0=guanine content <600 $\mu\text{g/g}$ of dust, 1= 600 – 2500 $\mu\text{g/g}$, 2= 2500 – $10\,000$ $\mu\text{g/g}$, and 3= $\geq 10\,000$ $\mu\text{g/g}$, respectively (22).

Laboratory tests

Ten-milliliter samples of venous blood were taken during the run-in period and throughout the maintenance therapy (after 6, 11, 17, and 25 months of SLIT). Blood samples were centrifuged, and sera were sent to a central laboratory for storage at -25°C . At the end of the trial, specific IgE (DP and DF) and specific IgG4 (DP and DF) were determined by the CAP System[®] (Pharmacia). Results were expressed in kIU/l for specific IgE and in percentages for IgG4 antibodies.

Bronchial challenge tests

Methacholine-challenge tests were conducted. The patients were instructed not to take any inhaled β_2 -agonists for 4 h

before the challenge and were required to have a FEV₁ of more than 80% of predicted at the time of testing. After a baseline FEV₁ recording, the subject inhaled saline. FEV₁ was determined after 1 min. Increasing doses of methacholine were then inhaled up to the dose that induced a reduction of at least 20% in the baseline FEV₁. The dose inducing a 20% fall in FEV₁ (PD₂₀) was read from the dose-response plot.

Quality of life assessment

Patients aged 15 years and older were asked to assess their QoL by completing the SF20 Short-Form Health Status Survey plus two items concerning vitality. The SF20+2 consists of 22 items divided into seven scales measuring physical functioning, limitations in role functioning due to physical health problems, social functioning, general mental health, general health perception, physical pain, and vitality. For each scale, a QoL score was assigned on a 0–100 scale (from 0=worst possible to 100=best possible) based on the patient's responses. This questionnaire has been used in many clinical trials and had been validated (23).

Statistical methods

An interim statistical analysis was conducted after the first 11 months of treatment. The final analysis was done after 25 months of maintenance therapy.

The Mann-Whitney *U*-test was used for between-group comparisons of asthma severity and exposure to house-dust mite at the various follow-up time points. Student's *t*-test was used to compare the daily means calculated from diary-card data, immunoglobulin levels, respiratory function and bronchial challenge test results, and the patients' VAS assessments of efficacy. Wilcoxon's nonparametric test was used for between-group comparisons of QoL data.

Results

Patients

Of the 85 patients included in the study, 20 (10 in each group) were withdrawn during the first 6 months of treatment for lack of cooperation (five in the active treatment group and four in the placebo group), lack of efficacy (one placebo), failure to meet inclusion criteria (one placebo), or adverse events (five active and four placebo); dropouts were included in the safety analysis but excluded

Table 2. Changes in outcome measures from baseline to last diary card or last follow-up visit (end point of SLIT)

	SLIT			Placebo		
	Baseline Means $\pm$ SD	Last diary card Means $\pm$ SD	P	Baseline Means $\pm$ SD	Last diary card Means $\pm$ SD	P
Nighttime asthma	0.20 $\pm$ 0.47	0.17 $\pm$ 0.51	NS	0.26 $\pm$ 0.51	0.11 $\pm$ 0.35	NS
Daytime asthma	0.32 $\pm$ 0.53	0.17 $\pm$ 0.50	0.02	0.37 $\pm$ 0.40	0.19 $\pm$ 0.44	NS
Morning PEFR (l/min)	404.1 $\pm$ 134.1	426.3 $\pm$ 136.7	0.01	415.7 $\pm$ 100.6	424.3 $\pm$ 114.5	NS
Evening PEFR (l/min)	414.1 $\pm$ 137.3	433.2 $\pm$ 139.1	0.03	429.6 $\pm$ 100.5	437.7 $\pm$ 117.4	NS
Use of inhaled β_2 -agonists (μ g salbutamol/day)	181 $\pm$ 192	122 $\pm$ 235	0.01	168 $\pm$ 172	79 $\pm$ 136	0.02
Use of inhaled corticosteroids (μ g beclomethasone/day)	555 $\pm$ 455	348 $\pm$ 410	0.01	495 $\pm$ 430	308 $\pm$ 408	0.02
Use of cromones (mg nedocromil/day)	0.66 $\pm$ 2.06	0.33 $\pm$ 1.36	NS	0.77 $\pm$ 2.30	0.70 $\pm$ 2.02	NS
Cumulative score (symptoms plus drugs)	8.10 $\pm$ 5.87	5.21 $\pm$ 5.88	0.006	7.64 $\pm$ 5.22	4.52 $\pm$ 4.89	0.003

	Baseline	Last visit	P	Baseline	Last visit	P
	Means $\pm$ SD	Means $\pm$ SD		Means $\pm$ SD	Means $\pm$ SD	
VC (l)	3.29 $\pm$ 1.45	3.52 $\pm$ 1.38	0.002	3.56 $\pm$ 1.42	3.89 $\pm$ 1.56	NS
FEV ₁ (l)	2.76 $\pm$ 1.13	2.88 $\pm$ 1.05	0.03	2.91 $\pm$ 0.99	2.97 $\pm$ 0.92	NS
FEV ₁ (% predicted)	95.9 $\pm$ 18.4	102.7 $\pm$ 24.6	0.01	95.2 $\pm$ 13.4	99.3 $\pm$ 18.5	NS

from the efficacy analysis because of their short treatment durations. Fig. 1 gives an outline of the study design according to CONSORT rules (24).

Of the 65 evaluable patients, 32 received SLIT and 33 placebo. At baseline, there were no statistically significant differences between these two groups except for greater sensitivity to methacholine in the active treatment group (active treatment mean PD₂₀=103; placebo 213 μ g, $P=0.03$, Student's *t*-test).

In the active treatment group, nine patients withdrew before the end of the trial (four moved, three failed to cooperate, one had nausea and lack of efficacy, and one was a woman planning a pregnancy); 28 completed the first 11 months of maintenance therapy and 23 completed the second year. In the placebo group, six patients withdrew before the end of the trial (one experienced a familial event, four failed to cooperate, and one had lack of efficacy); 32 completed the first 11 months of maintenance therapy and 27 completed the second year.

Efficacy evaluation by the patients and physicians

There were no statistically significant between-group differences in the efficacy evaluations by the patients and

physicians after 11 months of treatment. After 25 months of treatment, the efficacy evaluation by the patients was in favor of the active treatment, and the VAS difference approached statistical significance (67.2 mm in the active treatment group; 52.5 in the placebo group) ($P=0.07$, Student's *t*-test). Efficacy as assessed by the physicians was greater in the active treatment group (71% of patients improved) than in the placebo group (63.6%), but the difference was not significant.

Symptom scores

Analysis of the daily means for daytime and nighttime asthma symptom scores during the 3-week period before each visit (Fig. 2A and B) failed to disclose any statistically significant differences after 11 or 25 months of treatment. However, there was a significant decrease in the daytime asthma score at end point ($P=0.02$) compared to the run-in period in the SLIT group, but not in the placebo group (Table 2).

Rhinitis frequency was similar in both groups at baseline (43% and 40% in the SLIT and placebo groups, respectively). The two groups were significantly different (chi-square test) with respect to the presence of rhinitis after 11 months

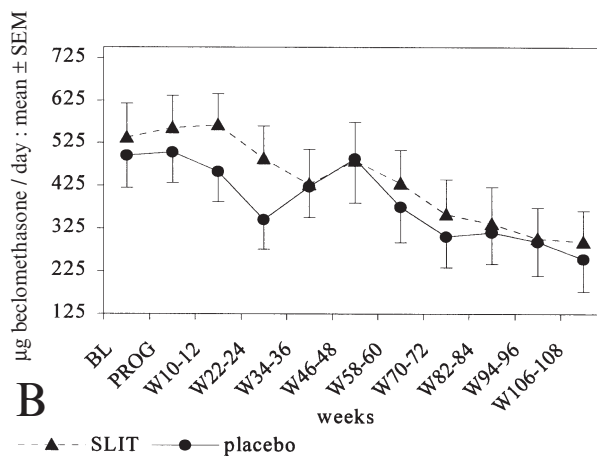
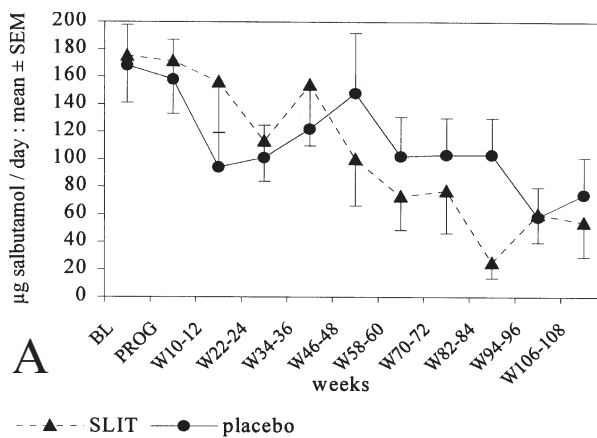


Figure 3. A) Use of inhaled β_2 agonists (mean $\pm$ SEM). B) Use of inhaled corticosteroids (mean $\pm$ SEM). SLIT group: $n=23$ at week 108 (25 months); placebo group: $n=26$ at week 108 (25 months). BL: baseline; PROG: progression; W: week.

(21.4% SLIT vs 50% placebo, $P=0.02$) and 19 months of treatment (16% vs 41.4%, $P=0.04$). Moreover, the SLIT group showed a significant reduction in rhinitis frequency at treatment completion (MacNemar test, $P=0.006$) from the run-in period.

Medication scores

The daily means for use of the main medications during the 3-week period before each visit (Fig. 3A and B) showed no statistically significant differences after 11 or 25 months of treatment for any drug (inhaled corticosteroids, β_2 -agonists, anticholinergics, cromones, and systemic corticosteroids). Use of inhaled steroids and β_2 -agonists was significantly reduced after 25 months of treatment in both groups (Table 2). The intragroup analysis did not show any significant difference for cromones (Table 2). Of the 65 evaluable patients, two took anticholinergics during a few

Table 3. Change in exposure to house-dust mites (mattress samples)

	Inclusion		11 months		25 months	
	SLIT $n=31$	Placebo $n=31$	SLIT $n=25$	Placebo $n=28$	SLIT $n=18$	Placebo $n=25$
Class 0= <600 $\mu\text{g/g}$ dust	2	1	3	1	1	0
Class 1= 600 – 2500 $\mu\text{g/g}$	10	16	13	16	8	13
Class 2= 2500 – $10\,000$ $\mu\text{g/g}$	14	9	7	6	8	10
Class 3= $\geq 10\,000$ $\mu\text{g/g}$	5	5	2	5	1	2
Median	2	2	1	1	1.5	1

days of the study period: mean doses used by these patients were 12 and 3.4 μg ipratropium bromure/day, respectively, during the corresponding 3-week period. None of the patients used systemic corticosteroids or inhaled anticholinergics during the last period. Use of systemic corticosteroids (oral or parenteral) was very low during the trial; 25 patients in the active group (78.1%) and 27 patients in the placebo group (81.8%) did not take any systemic corticosteroids from inclusion to treatment completion. Patients used fewer systemic corticosteroids in the SLIT group than in the placebo group (mean cumulative prednisolone doses per patient for the whole trial were 62.6 and 77.6 mg, respectively). Moreover, the prednisolone dose in milligrams in the placebo group was underestimated because of missing data for two patients (one injection of an unknown amount of methylprednisolone and one 8-day course of oral betamethasone in an unspecified dosage). However, the mean cumulative prednisolone dose showed no statistically significant differences after 11 or 25 months of treatment. Within both groups, the cumulative symptoms plus drug intake score decreased significantly after 25 months of treatment (Table 2). However, the difference between the groups was not significant.

Respiratory function

The SLIT group showed significant improvements (Student's t -test) in morning ($P=0.005$) and evening ($P=0.02$) PEFs after 11 months (data not shown) and after 25 months ($P=0.01$ and 0.03 , respectively) from the run-in period (Table 2, Fig. 4A and B). No such within-group differences were observed in the placebo group.

There were no significant between-group differences in asthma severity and respiratory function test results after 11 or 25 months of treatment. However, significant increases (Student's t -test) in FEV₁ (% predicted) ($P=0.01$) (Fig. 5), FEV₁ ($P=0.03$), and VC ($P=0.002$) were seen after 25 months in the

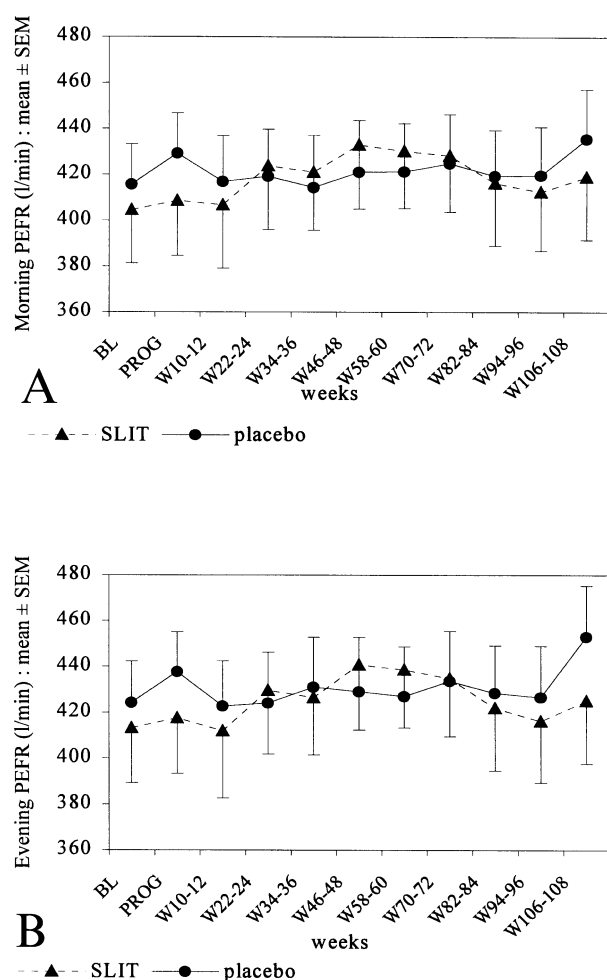


Figure 4. A) Morning PEFR (mean ± SEM). B) Evening PEFR (mean ± SEM). SLIT group: $n=23$ at week 108 (25 months); placebo group: $n=26$ at week 108 (25 months). BL: baseline; PROG: progression; W: week.

SLIT group, but not in the placebo group (Table 2). Previous immunotherapy courses had no significant effect on treatment efficacy.

Exposure to house-dust mites

The distributions of the guanine-test classes (0–3) in house-dust samples before, during, and after treatment are given in Table 3. At baseline, guanine-test results were comparable in the two groups (median=2). The concentrations decreased significantly ($P=0.01$, Wilcoxon T test) in both groups after 11 and 25 months of treatment (median=1 or 1.5).

Laboratory tests

Mean serum levels of specific IgE and IgG4 antibodies to *D. pteronyssinus* and *D. farinae* fell in the placebo group and

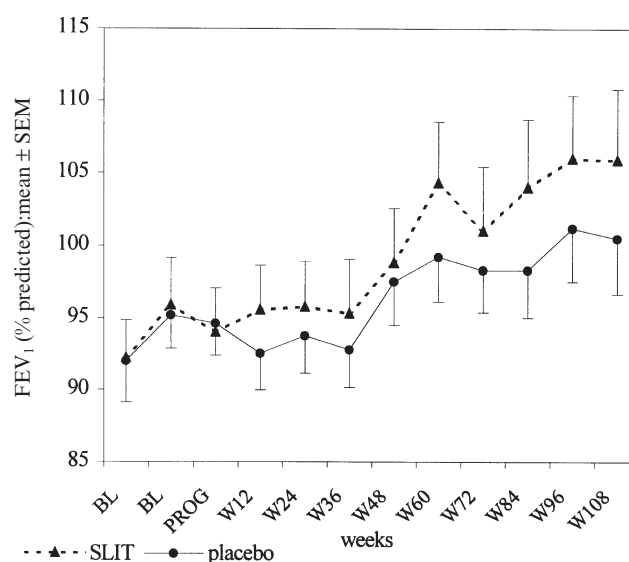


Figure 5. FEV₁ as % predicted (mean ± SEM). BL: baseline; PROG: progression; W: week.

increased in the SLIT group during the trial. Between-group comparisons of the within-group changes (post-treatment minus pretreatment) were conducted (Table 4). After 25 months of treatment, the difference between the two groups was significant (Student's *t*-test) for IgE DP ($P=0.05$), IgE DF ($P=0.02$), IgG4 DP ($P=0.001$), and IgG4 DF ($P=0.001$).

Bronchial challenge tests

In the SLIT group, methacholine bronchial challenge tests were conducted after 11 and 25 months in 23 and 17 patients, respectively, compared with 23 patients in the placebo group. The between-group comparisons of PD₂₀ were adjusted on the baseline data. Although there was no statistically significant between-group difference in post-treatment PD₂₀, it should be noted that, in the SLIT group, PD₂₀ after 11 and 25 months was 1.98 and 1.75 times higher than at baseline, respectively. The difference was significant after 11 months ($P=0.02$, Student's *t*-test) but was only of borderline significance ($P=0.09$) after 25 months of treatment because of the small number of patients. Changes in PD₂₀ were considerably smaller in the placebo group (1.31 and 1.07 times baseline after 11 and 25 months, respectively).

Quality of life (QoL) assessment

Thirty-eight patients were included in the QoL analysis; 18 in the SLIT group and 20 in the placebo group. QoL scores were similar in both groups before treatment and after 11 months. After 25 months, all QoL scores were higher in the

Table 4. Specific IgE and IgG4 levels

	IgE DP k IU/l						IgE DF k IU/l				IgG4 DP %				IgG4 DF %			
	SLIT			Placebo			SLIT		Placebo		SLIT		Placebo		SLIT		Placebo	
	n	Mean	SD	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BL	26	126.4	115.36	30	139.4	125.77	83.8	70.79	99.4	95.47	4.6	3.02	5.3	3.74	3.9	1.65	4.5	2.29
6 m	27	181.4	213.09	30	121.8	104.49	125.9	136.66	80.9	66.87	6.0	6.51	4.8	2.80	4.8	2.87	4.2	1.56
11 m	27	170.5	193.88	26	124.2	111.71	107.0	97.87	84.2	74.81	5.7	3.46	4.6	1.86	4.7	1.91	4.0	1.25
17 m	25	167.3	201.04	28	117.5	93.82	112.7	98.24	84.6	69.05	6.0	4.50	5.0	3.09	4.9	2.77	4.3	1.73
25 m	23	152.7	202.07	26	112.7	108.59	103.6	103.62	79.8	80.48	6.1	4.22	5.1	2.61	5.1	2.65	4.4	1.75
Difference	23	23.9	129.25	26	-37.5	70.20	21.3	84.11	-28.0	48.96	1.5	2.13	-0.4	1.79	1.2	1.61	-0.2	1.13
t-test	P=0.05						P=0.02				P=0.001				P=0.001			

BL: baseline; m: month; difference: post-treatment minus pretreatment.

SLIT group than in the placebo group, with differences being most marked for general mental health (79.7 and 60.7, respectively – $P=0.07$, Wilcoxon test), general perception of health (76.5 and 56.8, respectively – $P=0.01$), and physical pain (86.2 and 68.3, respectively – $P=0.02$). The changes from baseline to end point showed significant differences in favor of the SLIT group compared with the placebo for social functioning (+12.2 and -2.1, respectively – $P=0.04$, Wilcoxon T test) and bodily pain (+20.0 and -4.2, respectively – $P=0.01$).

Safety assessment

An adverse event occurred at least once in 15 of the 42 patients in the SLIT group (35.7%) and in 14 of the 43

Table 5. Adverse events

Symptoms	SLIT group	Placebo group
Dermatologic (urticaria)	3	2
ENT (total)	3	2
Throat itching	1	0
Buccal inflammation	1	0
Rhinitis	0	1
Sinusitis	1	1
Gastrointestinal (nausea, vomiting, gastralgia)	3	3
Respiratory (asthma)	5	4
CNS (total)	1	3
Headache	0	2
Dizziness	1	1
Total adverse events	15	14

ENT: ears, nose, and throat; CNS: central nervous system.

patients (32.6%) in the placebo group ($P=0.84$, chi-square test). The overall incidence was similar in both treatment groups. The events are reported in Table 5 for both groups.

Discussion

In the present study, we assessed the efficacy of SLIT with drops of a *D. pteronyssinus*-*D. farinae* 50/50 extract compared with placebo in adults and children with moderate asthma due to sensitization to house-dust mites. The doses used were 200 times higher than standard subcutaneous doses for a treatment period of similar length.

A number of controlled studies have reported the results of SLIT in allergic patients. Some studies showed that specific immunotherapy has prophylactic effects against the development of bronchial symptoms (3, 7, 12, 15) in patients with rhinitis or rhinoconjunctivitis. Tari et al. assessed the efficacy of SLIT vs placebo in 58 patients younger than 12 years of age with asthma and rhinitis due to mites (12). The treatment lasted 18 months. Rhinitis and asthma symptom scores decreased significantly after 12 and 18 months of treatment. Bronchial specific and aspecific sensitivity decreased significantly after 12 months. Similar results were obtained by Casanovas et al. (3) in a double-blind study in 15 patients allergic to *Olea europaea* pollen and undergoing SLIT before the beginning of the pollen season; the actively treated patients developed significantly less dyspnea at the pollinic peak than those on placebo. Another double-blind, placebo-controlled study of SLIT was carried out in 66 olive-tree-allergic patients (15) treated pre- and coseasonally for 2 consecutive years. The actively treated

patients had a significantly lower score for dyspnea during the two seasons.

In the present study, the diary-card data showed significant improvements in morning and evening PEF_R and daytime asthma in the SLIT group. Significant decreases in inhaled corticosteroid and β_2 -agonist use were observed in both groups. Bronchial sensitivity to methacholine decreased significantly after 11 months of treatment in the SLIT group, but, because of the small number of patients, this decrease was only of borderline significance at the end of the study. Although the primary objective of this study was not to evaluate the course of rhinitis, results showed that rhinitis symptoms were significantly less frequent in the SLIT group than in the placebo group at two time points. Finally, all the QoL scores assessed after 25 months were better in the SLIT group than in the placebo group, with significant differences for general health perception and physical pain. It has been shown that a 7% increase in FEV₁ is associated with perception of a clinical improvement by the patients (25). Taken in concert, these data explain why the efficacy evaluations by the investigators and patients were in favor of SLIT.

Several factors may explain the absence of statistically significant differences in symptom scores between the two groups. First, the number of patients may have been too small to detect such a difference. We expected a 60% improvement in the SLIT group vs 30% in the placebo group. On this assumption, we calculated that at least 70 patients were required in each group for two-tailed testing with an error of $\alpha=\beta=0.05$. At the end of the trial, the number of patients was less than half of this value.

Second, patient selection according to consensus conference recommendations may have yielded a population in which disease severity was not sufficient to allow the detection of treatment effects. At baseline, mean FEV₁ was about 95% of predicted in both groups. Asthma severity was moderate (Aas score 2) in 86% of the patients in the SLIT group and 79% in the placebo group. Mean nighttime and daytime asthma symptom scores were less than 0.4 at baseline in both groups.

Third, some patients may have been overmedicated (particularly with inhaled corticosteroids), and this may have masked the effect of SLIT. The study design was in accordance with recommendations of the international consensus on stepwise asthma therapy, which requires that physicians reduce medication once the patient's asthma is well controlled (26). Overall, 19 of the 49 patients who completed their diary card for month 25 of treatment were taking no medication at the end of the study, indicating that

the effort to reduce medication during the study was successful in both groups.

Fourth, the patients may have changed their normal cleaning activities during the trial. Changes in house-dust-mite exposure may have occurred if the patients vacuumed their mattress and bedroom carpets more frequently and washed their sheets and pillowcases more often. Consistent with this possibility, exposure to house-dust mites decreased after 11 and 25 months of treatment in both groups. This may have contributed to improve the patients' condition in both groups. It has been established that guanine content is correlated with Der p 1–Der f 1 allergen concentrations (22). A guanine class 2 or 3 corresponds to >10 μ g of mite group I allergen (considered to be a risk factor for the development of acute asthma) in 88% and 100%, respectively, of dust samples. A guanine class 1 is much more difficult to interpret: Quoix et al. (22) found more than 2 μ g of Der p 1 and Der f 1 (considered to be a risk factor for sensitization) in two-thirds of class 1 dust samples.

The patients included in our study were instructed in the management of asthma and were seen every 12 weeks for medication adjustments. They received standard asthma pharmacotherapy including inhaled corticosteroids in most cases. We believe that inclusion in the study may in itself have contributed to control the disease process. Patient education, self-management using symptoms or peak flow, and regular follow-up have been shown to induce significant improvements in asthma control and QoL (27).

For all these reasons, our results are not inconsistent with prior studies demonstrating the clinical efficacy of SLIT in patients with allergic asthma (12). At the end of the trial, we found large and significant between-group differences in DP- and DF-specific IgE levels and in DP- and DF-specific IgG4 levels, which were considerably increased in the SLIT group. The significant IgG4 increase in this trial and in other studies (4, 12, 13) is further evidence substantiating the effect of SLIT on the immune system. However, the mechanism of action of SLIT is not well known. One of the current hypotheses is induction of tolerance by Langerhans' cells that internalize the antigens in the sublingual mucosa before migrating to the regional lymph nodes, where they act as antigen-presenting cells. This may result in T-cell response modifications responsible for oral tolerance (28). The mechanisms underlying specific immunotherapy have been far more extensively studied for the subcutaneous route than for the sublingual route: a decrease in interleukin-4 and an

increase in interferon-gamma were demonstrated after immunotherapy in bee venom-sensitive patients [29, 30]. The efficacy of specific immunotherapy may involve a “switch” from a Th₂ to a Th₁ pattern.

Although further work is needed to evaluate SLIT, the present study demonstrated the good safety profile and efficacy of this route of administration.

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