

Clinical efficacy of sublingual-swallow immunotherapy: a double-blind, placebo-controlled trial of a standardized five-grass-pollen extract in rhinitis

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Sublingual-swallow immunotherapy (SLIT) using high doses of standardized allergen extracts has been found to be effective in reducing allergic symptoms and medication needs. A double-blind, placebo-controlled study was carried out in a large number of patients to determine whether medication needs can be reduced by SLIT. Some 136 patients with grass-pollen rhinitis with or without mild asthma were studied. Patients received either placebo or SLIT with a standardized grass-pollen extract administered daily with increasing doses up to 300 IR (index of reactivity) from January to the end of July 1994. During the grass-pollen season, patients were instructed to use medications as required and to visit their doctors in case of asthma. Symptom-medications scores were assessed during the pollen season, and serum-specific IgG4 was measured before and at the end of SLIT. In the SLIT group, drug consumption dropped significantly throughout the pollen season ($P < 0.02$). Moreover, at the peak of the pollen season, betamethasone consumption was significantly reduced in the SLIT group ($P < 0.02$). Only one patient in the SLIT group had an asthma attack compared to eight patients in the placebo group ($P < 0.02$). IgG4 levels increased significantly in the SLIT group ($P < 0.001$) but without correlation with symptoms. Side-effects were comparable in both groups. This study indicates that SLIT in grass-pollen rhinitis is well tolerated, improves overall clinical symptoms, and reduces drug consumption and the need for oral corticosteroids.

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A review of the medical literature shows that various local routes of immunization – sublingual, oral, or variations thereof – have been tried for treatment of allergic conditions. The interpretation of many of these studies has been equivocal because of the use of low doses and/or poorly characterized extracts. After the earlier study of van Niekerk & De-Wet (1) and within the past

5 years, many controlled studies using high doses of standardized extracts have shown that sublingual immunotherapy (SLIT) was more effective than placebo for pollen-induced rhinitis (2–4) or house-dust-mite-induced rhinitis (5), although no efficacy was demonstrated in cat allergy (6).

The treatment of allergic rhinitis is usually based on the use of antihistamines (H_1 -blockers) and topical nasal corticosteroids. Although most patients demonstrate significant improvement, there is a subset of patients who require oral corticosteroids for optimal control of symptoms. It has been previously observed that classic subcutaneous injection immunotherapy can significantly reduce the need for oral corticosteroids (7).

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This multicenter, double-blind, placebo-controlled trial aimed to determine the efficacy and safety of SLIT in the treatment of allergic rhinoconjunctivitis. The major outcome measure was a cumulative score in medication needs. Since the use of a topical steroid p.r.n. is considered inappropriate, because its effects may last for an undefined time, in this study, the need for p.r.n. use of oral corticosteroids (e.g., betamethasone) was considered a secondary outcome measure.

Material and methods

Patients

Some 136 patients (mean age: 27 ± 10 years; range: 8–55 years) with a history of pollen-induced rhinoconjunctivitis volunteered to participate in this clinical study. A small proportion ($n=26$) of the patients had had mild asthma during previous pollen seasons. The diagnosis of allergy to grass pollen was made by clinical history, positive skin prick tests with a standardized pollen extract (Stallergènes SA, Antony, France), and serum specific IgE measured by the CAP System® (Pharmacia Diagnostics AB, Uppsala, Sweden). An exclusion criterion was sensitization to *Alternaria*, the only major mold in the region, but sensitization to tree, weed, mite, and animal allergens were not exclusion criteria if symptoms were not perennial. In the case of sensitization to animal epithelia, the implicated animal was not permitted in the home.

All the patients, or both parents of the minors, gave their informed consent to participation in the study, which was approved by the Lyon Hospital ethics committee.

Allergen preparation

The same batch of purified, standardized allergen (Stallergènes SA, Antony, France) was used throughout the study. The extract consisted of a mixture of five major grass pollens (orchard grass, meadow grass, ryegrass, sweet vernal grass, and timothy grass) prepared by a modification of a previously described method (8). Control of the extract potency was done by the manufacturer by RAST inhibition, isoelectrofocusing, and immunoprinting, and compared with the internal standard by *in vivo* and *in vitro* tests. The biologic activity of the extract was expressed as index of reactivity (IR) defined by comparing skin tests induced by a 9% codeine phosphate positive control with three dilutions of the grass-pollen extract. A potency of 100 IR was designated as the strength of grass-pollen extract that elicited a geometric mean wheal size of 7 mm in diameter by skin prick test in 30 subjects sensitized to the corresponding allergen. The batch-to-

batch consistency of the allergenic extracts was accepted within a limit of variation of less than 50% of the potency. The level of the timothy major allergen Phl p 5 in 100 IR extract was $6.4 \mu\text{g/ml}$ (dosage due to the courtesy of Dr O. Cromwell, Allergopharma, Reinberg, Germany).

Treatment

Treatment with drops of the five-grass-pollen extracts, or placebo, in the same glycerosaline diluent, started on January 1994, and the study ended in July 1994.

Patients were instructed to hold the treatment extract under their tongue for 2 min and then swallow the solution. The progression of doses was made in 25 days according to the protocol presented in Table 1. When the dose of 300 IR/day (corresponding to 20 drops/day of the 300 IR/ml concentration vial) was reached, the maintenance treatment, at the same doses, was followed every day until the end of the study. The cumulative dosage received by the patients was therefore 40 700 IR, corresponding to 2.6 mg of the timothy pollen major allergen Phl p 5.

Design of the study

Patients were selected for the study at the end of autumn 1993, with no run-in period before the inclusion. Patients were randomly included, in a double-blind fashion, in the active-treatment or placebo group. Patients were asked to maintain their symptoms as low as possible by taking medication according to a standardized schedule. Mild symptoms were treated with either ocular or nasal 2% disodium cromoglycate, or both (Fisons, Loughborough, UK); more severe symptoms were treated by adding 10 mg o.d. cetirizine (UCB Pharma SA, Nanterre, France); and severe symptoms were treated by adding 0.50 mg betamethasone (Schering-Plough, Levallois-Perret, France). The

Table 1. Sublingual immunotherapy protocol

	Drops at 1 IR	Drops at 10 IR	Drops at 100 IR	Drops at 300 IR
Progression of doses	1	1	1	5
	3	3	2	6
	6	6	4	8
	10	10	6	10
			8	12
			12	14
			16	16
			20	18
				20

Maintenance treatment → 300 IR/day (20 drops/day).

dose of betamethasone was left to the patient's evaluation of symptoms in order to obtain the best control possible.

Medication scores

Long-acting antihistamines and topical corticosteroids were not allowed during the study. However, rescue treatment was authorized for insufficiency of effect of SLIT on the symptoms. As previously described (9), the concomitant treatment was noted daily and specified according to the following scale:

- score 1: administration of ocular cromoglycate
- score 1: administration of nasal cromoglycate
- score 2: one tablet of cetirizine
- score 3: tablets of betamethasone.

Mean rescue treatment scores were calculated each week. Between groups, comparison was also expressed as area under curve (AUC) during the 14 weeks of the pollen season.

Symptom scores

Symptom scores were recorded daily for rhinitis and conjunctivitis during the pollen season. Sneezes, nasal discharge and nasal pruritis, eye redness, itching of eyes, and tears were scored on a scale from 0 to 3; 0=absence of signs or symptoms; 1=signs or symptoms present but not disturbing; 2=symptoms disturbing; 3=very disturbing with impaired social or professional life, as previously described (9). Mean symptoms and total rhinitis and conjunctival scores were calculated each week. Between-group comparison was expressed as AUC during the 14 weeks of the pollen season. Associated adverse events (e.g., asthma attack) and all medical events were also recorded. In addition, overall clinical efficacy was assessed by the investigators and expressed as worsening, failure, moderate results, or complete success.

Specific IgE and IgG4

Blood was taken at the inclusion of the patients and at the end of the study. Serum was stored at -20°C before quantifying timothy-specific IgE and IgG4. The dosages were performed in duplicate with the RAST CAP System (Pharmacia Diagnostics, Uppsala, Sweden) for IgE and radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden) for IgG4 determinations.

Pollen counts

Pollen counts for the year 1994 of the area were obtained from the Laboratory of Aerobiology, Pasteur Institute, Paris, France.

Statistical methods

Qualitative data were analyzed by the chi-square test or Fisher's exact test. Quantitative data with normal distribution were compared by Student's *t*-test. AUC of symptom scores and medication scores, and IgE and IgG4 levels were compared by the Mann-Whitney *U*-test. Within-group comparisons of IgE and IgG4 were done by Wilcoxon's signed rank *t*-test. Scores were plotted as median $\pm 95\%$ confidence interval of the median. All the tests were two-tailed, and the level of significance was set at 0.05.

Results

Demographic characteristics

Table 2 shows the characteristics of the two groups of patients. Despite the lack of a run-in period and matching procedure before inclusion in the study, there were no statistical differences between the two groups in age, sex, duration of rhinitis, incidence of asthma, and other sensitizations.

Some 120 patients were eligible for analysis. Twelve were excluded for major deviance from the protocol (recent subcutaneous immunotherapy with grass-pollen extract). Four other patients were also excluded for various reasons: one accidental death, one patient who interrupted the treatment for personal reasons, one interruption due to viral hepatitis, and one patient of whom coauthors lost sight.

Symptom scores

As indicated in Fig. 1, symptoms were maintained at a low level throughout the pollen season (median value for the whole pollen season equal to 1.3 and 1.6 in the placebo and treated groups, respectively). The peak of symptoms was chronologically associated with the peak of the pollen season (Fig. 2). The AUC of each symptom and the

Table 2. Characteristics of patients eligible for analysis

	Active treatment	Placebo treatment
No. of subjects	62	58
Male/female	36/26	35/23
Mean age (years) $\pm$ SD	29 $\pm$ 13	26 $\pm$ 12
Age distribution (years)	9–55	8–55
Mean duration of rhinitis (years) $\pm$ SD	12 $\pm$ 8	11 $\pm$ 9
Asthma during previous pollen seasons	10	16
Sensitizations other than grass	45	49
Previous parenteral immunotherapy interrupted for more than 3 years	14	8

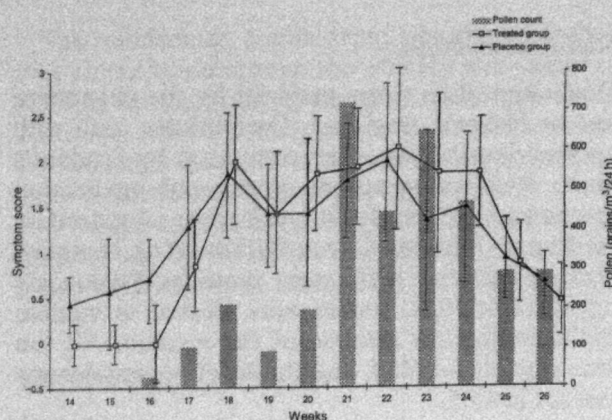


Fig. 1. Rhinitis scores (median $\pm$ 95% IC) and pollen counts.

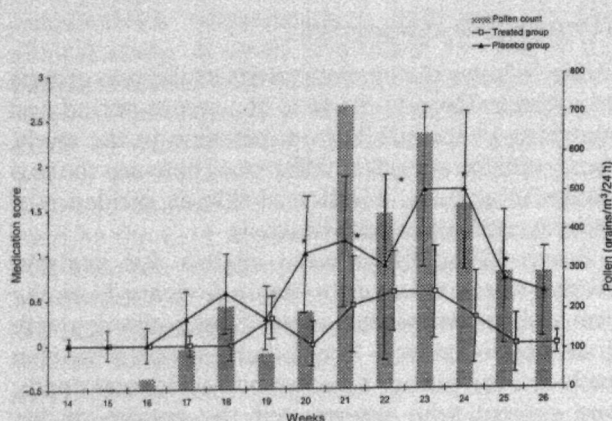


Fig. 2. Medication scores (median $\pm$ 95% IC) and pollen counts (* P <0.01).

total score of rhinitis and conjunctivitis did not differ significantly between groups (Table 3).

At the peak of the pollen season, 1/10 patients in the treated group who had asthma in previous seasons also presented asthma attacks, in comparison to 8/16 patients in the placebo group (P <0.02, chi-square test). Overall clinical efficacy assessed by the investigators indicated significant improvement (P <0.01) in the treated group.

Medication scores

The AUC of medication score was significantly (P <0.01) lower in the SLIT group than in the placebo group (Table 3). During the first 6 weeks of the pollen season, the medication scores in the SLIT group were lower than in the placebo group (Fig. 2). The use of antihistamines and nasal or ocular cromoglycate in the placebo group was relatively low despite the high pollen counts, indicating that the patients had rather mild disease. The analysis of the consumption of betamethasone, the primary rescue treatment indicated for severe

Table 3. Comparison of area under curve (AUC) analysis of symptom and medication scores between active and placebo group

		Active	Placebo	
<i>n</i>		62	58	
Nasal symptoms				
Sneezing	Median	48.5	40.5	NS*
	±95% IC	9.17	12.35	
Rhinitorrhea	Median	50	43.5	NS
	±95% IC	8.98	13.31	
Itching	Median	16.5	21.5	NS
	±95% IC	8.86	13.37	
Total score rhinitis	Median	131	116.5	NS
	±95% IC	22.71	36.68	
Ocular symptoms				
Redness	Median	20	16	NS
	±95% IC	8.54	6.14	
Itching	Median	18	19	NS
	±95% IC	8.92	10.60	
Total score conjunctivitis	Median	53	43	NS
	±95% IC	26.88	21.75	
Medication score	Median	30	91.5	<i>P</i> <0.01
	±95% IC	22.96	31.58	
Betamethasone intake (nb.tab.)	Median	0	5	<i>P</i> <0.05
	±95% IC	0.51	5.60	

NS: nonsignificant.

symptoms, demonstrated a significant difference (P <0.05) between the two groups (Table 3). The mean number of betamethasone tablets taken by the patients in the treated group was 4 ± 0.8 , compared to 17 ± 13 in the placebo group (P <0.02), indicating that the actively treated group required 80% less "rescue" oral betamethasone than the placebo group. The number of patients who required steroids at the peak of the pollen season in the active treatment group (9/62) was significantly lower than in the placebo group (21/58) (P <0.01).

Side-effects

SLIT was well tolerated in general, as well as by the 40 children studied. The rate of side-effects was not statistically different between the two groups (Table 4). Buccopharyngeal pruritis and wheezing were the main side-effects documented. Gastrointestinal effects were reported by three patients in

Table 4. Types and frequencies of side-effects

	Placebo group	Treated group
No. of patients	10	18
Skin pruritis	1	1
Buccopharyngeal pruritis	3	9
Wheezing	4	6
Spasmophilia	1	0
Gastrointestinal	0	3
Rhinitis	2	1

the actively treated group. No severe systemic reactions were reported during this study.

Immunoglobulin levels

As indicated in Fig. 3A, there was a significant increase of timothy-specific serum IgE levels in the treated group (preseasonal 19.5 ± 10.34 kUI/l vs postseasonal 57.5 ± 23.54 kUI/l, $P < 0.001$; median $\pm$ 95% CI). The increase of serum specific IgE in the placebo group was significantly lower than in the treated group ($P < 0.001$). The median postseasonal timothy-specific serum IgG4 level in the treated group was significantly higher than in the placebo group ($3.5\% \pm 1.45$ vs $2.0\% \pm 0.6$, $P < 0.03$; median $\pm$ 95% CI). Intragroup comparison of pre- and postseasonal levels showed a significant increase of specific IgG4 in the treated group ($P < 0.001$), but no difference in the placebo group (Fig. 3B). There was no correlation between the IgG4 levels and symptom and/or medications scores within both groups of patients. Moreover, the IgG4/IgE ratio did not correlate with symptom and medication scores (data not shown).

Discussion

This study had a specific design which utilized medication requirements, "rescue medication usage", as the primary outcome variable. Nasal and conjunctival symptoms were kept at a low level by adequate pharmacologic treatment. Thus, although lower in the actively treated group, these symptoms did not differ significantly between both groups. However, the investigators' assessment of the efficacy showed that they felt active SLIT was significantly better in controlling symptoms than placebo.

The patients included in the study presented mild asthma symptoms during the previous 2 years 1992 and 1993, probably due to low levels of pollination. Pollen counts during 1994 were significantly much higher, leading to an increase in the frequency and severity of asthma attacks. Despite the fact that this study was not specifically designed to evaluate the effect of SLIT in asthma, and the small number of asthmatic patients studied, the results showed that the frequency of asthma attacks was significantly reduced in the actively treated group as compared to the placebo group. However, these results need to be confirmed in a properly designed study on the efficacy of SLIT on seasonal asthma.

The major outcome measure was the reduction in medication needs. When pollen counts were low, as expected, there was no significant difference between both groups, but medication scores were lower in the actively treated group. On the other hand, during the last 6 weeks of the pollen season,

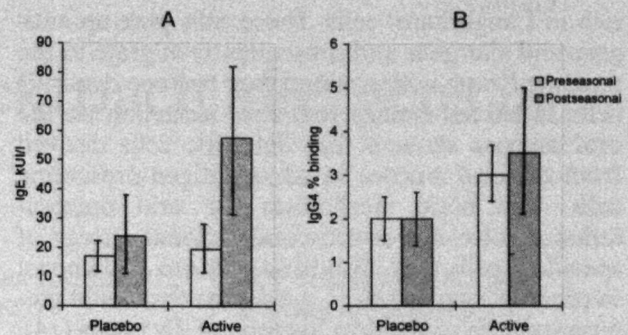


Fig. 3. Median ($\pm$ 95% IC) levels of timothy-specific serum IgE and IgG4 levels.

there was a highly significant difference between both groups. This observation confirmed our previous study showing that SLIT could reduce medication needs (2). Beside a significant reduction in medication consumption in the actively treated group, SLIT can reduce the needs for oral corticotherapy during pollinosis. The oral corticosteroid requirement was four times less in patients receiving SLIT. Other authors, using subcutaneous immunotherapy, showed a reduction in oral corticosteroid needs in the immunotherapy group (7).

An important point is the cumulative dose of allergen, 500 times greater than that usually administered in subcutaneous immunotherapy. Despite these larger doses of allergens, the rate of side-effects was not significantly higher in the actively treated group, and side-effects mainly consisted of local oral pruritus. Moreover, the incidence of gastrointestinal effects, which were specifically sought, was very low when compared to other SLIT studies (1, 3–5) or to oral immunotherapy (10–12).

The positive clinical effects observed in this study agree with other studies where the efficacious doses were 3–50-fold higher than that used for subcutaneous immunotherapy (2–5). These data confirm the need for administration of large cumulative doses of allergens in SLIT. However, the lack of published placebo-controlled, dose-ranging studies does not permit the precise definition of an optimal amount of allergen needed to obtain effective SLIT. The immunologic effect of sublingual administration of allergen extracts was demonstrated again by the increase in serum allergen-specific IgE and IgG4. It is not known whether this immunologic effect is due to the sublingual or to the oral administration of allergens. As for the subcutaneous immunotherapy, there was no relationship between the therapeutic efficacy of SLIT and the variation of IgG4 levels.

A hypothetical mechanism might explain the efficacy of SLIT. The mucosa of the mouth is particularly

rich in Langerhans' cells. These cells pick up antigens and allergens and subsequently migrate to the draining lymph nodes, where they become dendritic cells. In animal studies, tolerance induction via the oral mucosa showed that dendritic cells derived from the oral mucosa acted as antigen-presenting cells. The basic mechanism for oral mucosa-induced tolerance is subsequent clonal anergy of specific T cells (13). Dendritic cells are also known to produce interleukin-12, a cytokine that could preferentially drive the development of Th 1 cells (14).

In summary, this study shows that a SLIT regimen with a standardized grass-pollen extract was well tolerated and resulted in a significant reduction of the need for oral corticosteroids and other medication and an improvement in pollen-induced asthma symptoms.

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