

Sublingual-swallow immunotherapy with standardized 3-grass pollen extract: a double-blind, placebo-controlled study

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Background: Sublingual immunotherapy (SLIT) is accepted as a safe and effective route for the treatment of grass pollen allergy, but clarification of its clinical and biological efficacy requires more study.

Objective: To evaluate the efficacy, safety, and compliance of SLIT with a standardized 3-grass pollen extract in patients with grass pollen seasonal allergic rhinoconjunctivitis, with or without mild asthma.

Methods: This multicenter, randomized, double-blind study included 127 patients (aged 12–41 years; mean age, 24.9 years) with grass pollen seasonal allergic rhinoconjunctivitis, with or without mild asthma. They received either SLIT with a high-dose, standardized, 3-grass pollen extract or placebo for 10 months before and during the grass pollen season. The efficacy evaluation compared weekly clinical scores (defined as the sum of the symptom score and rescue medication score) to measure rhinoconjunctivitis and asthma for the first 8 weeks of the pollen season. We also evaluated safety and compliance and measured changes in anti-*Dactylis* specific IgG4 antibody levels.

Results: There was a trend in favor of the study group in the mean adjusted clinical score. The groups were not comparable on inclusion ($P = .02$): the SLIT group included more subjects with asthma and had a higher mean IgG4 serum level. Additional exploration according to subgroups with and without asthma found that among the patients without asthma, the SLIT group had a significantly better clinical score ($P = .045$). Anti-*Dactylis* specific IgG4 levels increased significantly in the SLIT group.

Conclusion: SLIT with a standardized, high-dose, 3-grass pollen extract is safe and significantly improves the clinical score in patients with hay fever and without asthma during the pollen season.

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INTRODUCTION

Allergic rhinitis is the most common manifestation of immediate hypersensitivity in adolescents and young adults.¹ Although a benign ailment, allergic rhinitis can alter patients' quality of life (QoL) considerably. It is often associated with conjunctivitis and asthma, of which it is a frequent precursor.²

The Allergic Rhinitis and Its Impact on Asthma guidelines recommend that seasonal allergic rhinoconjunctivitis be managed by symptomatic treatment, combined with specific immunotherapy to modulate the immune system. These guide-

lines advise high doses of standardized allergen extracts to achieve efficacy, and they validated sublingual immunotherapy (SLIT) for routine clinical use in adults and children.¹

Despite the many articles published on SLIT during the past 5 years, numerous aspects still require clarification. The Cochrane review by Wilson et al³ demonstrated that SLIT is a safe and effective treatment for allergic rhinitis, but the magnitude of its clinical efficacy on rhinoconjunctivitis and asthma, compared with other treatments, including injection immunotherapy, remains uncertain. Reported immunological changes are also controversial. Still another question about this self-administered treatment is compliance, which has yet to be evaluated.

This multicenter study was designed to compare the efficacy, safety, and compliance of a high-dose, standardized, 3-grass pollen extract with placebo, administered by sublingual swallowing before and during the grass pollen season in patients with grass pollen allergy. Efficacy was evaluated by a clinical score, defined as the sum of the symptom score and the rescue medication score.

METHODS

Study Design

This double-blind, placebo-controlled, multicenter study ($n = 15$) was conducted in accordance with good practice guide-

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lines and approved by the Ethics Committee for Strasbourg, France. Patients were selected during the 2001 grass pollen season and subsequently randomized to receive the study treatment or placebo. The study included 7 visits for evaluation. Subjects took the allocated study product for 10 months, 8 before and 2 during the grass pollen season. This duration of treatment was chosen to obtain a cumulative dose of 3-grass pollen extract of approximately 32,000 index of reactivity (IR) at the end of the study. They were asked to record their symptoms and use of rescue medication during the 2002 pollen season.

The primary efficacy outcome was measured by the mean clinical score in the intention-to-treat (ITT) population of the SLIT and placebo groups during the first 8 weeks of the 2002 grass pollen season. Secondary outcomes included analysis of subscores and specific items composing the clinical score in the ITT and per-protocol populations, measurement of nasal obstruction, QoL evaluated by miniquestionnaire by Juniper et al,⁴ serum concentration of *Dactylis* grass pollen specific IgG4 before and after treatment, compliance, and safety.

Patients

The study included 69 male and 49 female patients aged 12 to 41 years (mean $\pm$ SD age, 24.9 ± 7.6 years). Adults gave written informed consent for participation, and both parents provided written informed consent for minors.

The inclusion criteria were as follows: (1) seasonal allergic rhinoconjunctivitis for at least the past 2 years, with or without intermittent asthma (forced expiratory volume in 1 second, $>80\%$ of the predicted value on enrollment into the study); (2) sensitization to grass pollen with positive skin prick test results with a 3-grass pollen extract (*Dactylis*,

Phleum, or *Lolium*); and (3) specific IgE anti-*Dactylis* antibodies (class 2 or higher) (CAP System; Pharmacia, Uppsala, Sweden). Exclusion criteria were sensitization to perennial allergens or to animal allergens if the animal was present in the patient's immediate environment, sensitization to weed and tree pollen with clinical manifestations, immune diseases, and previous specific immunotherapy for grass pollen. Use of drugs that might interfere with evaluation of the clinical score was also grounds for exclusion.

Treatment

The patients were randomized at the second visit to receive SLIT with either a standardized 3-grass pollen extract (33.3% *Dactylis glomerata* [orchard grass], 33.3% *Phleum pratense* [timothy grass], and 33.3% *Lolium perenne* [rye grass], Allergbio, Varennes-en-Argonne, France in 50% glycerin) or placebo (phenol-free saline solution in 50% glycerin). The treatments (active product or placebo) were packaged similarly. Patients took the study product sublingually, on an outpatient basis. They were instructed to keep the drops under their tongue for 2 minutes before swallowing them. After a buildup of 5 weeks, they reached the maintenance dose, a 300 IR 3-grass pollen extract 3 times a week. Dose was not reduced during the pollen season. By the end of the study, each patient in the active treatment group had received a mean cumulative dose of 31,800 IR of the 3-grass pollen extract, corresponding to 2.75 mg of group 5 grass pollen major allergen (Fig 1).

Clinical Score

The clinical score was equal to the sum of the symptom (rhinitis plus conjunctivitis) and rescue medication scores.

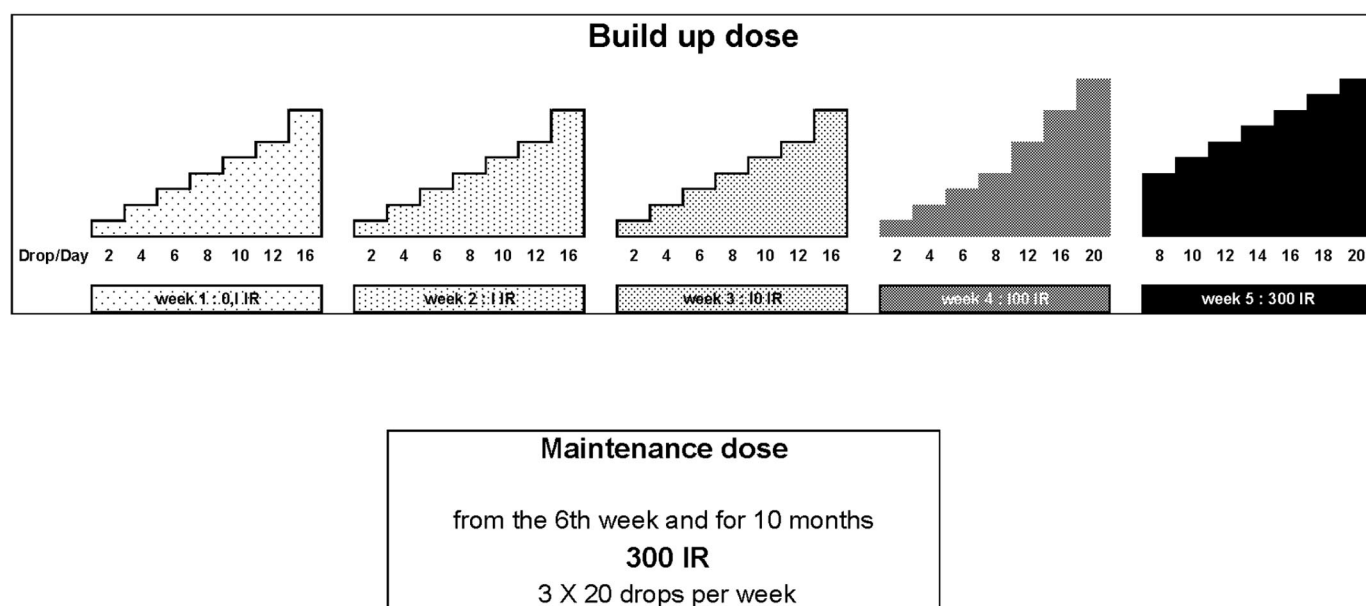


Figure 1. Updosing schedule and maintenance treatment. IR indicates index of reactivity.

The weekly clinical score was calculated from the data recorded by the patient in self-evaluation diaries during the 8-week evaluation period. Symptoms of rhinitis (nasal obstruction, rhinorrhea, sneezing, and nasal itching), conjunctivitis (tearing, eye redness, and ocular itching), and asthma (coughing, difficulty in breathing, and wheezing) were scored according to a 4-point scale: 0, no symptom; 1, mild symptoms and no discomfort for the patient; 2, moderate symptoms not affecting activities or sleep; and 3, severe symptoms, affecting activities or sleep. The maximum score was 30. Each patient had to have at least 1 symptom of rhinitis or a conjunctivitis score of 2 or greater.

Only specific rescue medications were allowed. Patients were instructed to use them if needed and to record them in their diaries. Medication was scored as follows: antihistamine eyedrops, 0.5 point per drop; antihistamine or corticosteroid nasal spray, 0.5 point per spray (ie, for 4 intranasal inhalations, $0.5 \times 4 = 2$ points); oral H_1 -antihistamine, 2 points per tablet; short-acting bronchodilators, 1 point per puff; inhaled corticosteroids, 2 points per 200- μ g puff of budesonide; and oral corticosteroids, 4 points per 20-mg prednisolone tablet. A weekly score was calculated from the data in each patient's diary.

Evaluation of Nasal Obstruction, QoL Evaluation, and Compliance Evaluation

Mean peak nasal inspiratory flow was measured in all patients at each visit with an electronic peak flow meter with an appropriate facial mask (One Flow; Clement Clarke International, Essex, England).

Patients evaluated their QoL by completing the mini-questionnaire by Juniper et al⁴ in the course of the 3 visits before and during the pollen season. The score per category was calculated as the unweighted mean of its items and the overall score as the unweighted mean of all 14 items.

We measured compliance by comparison of the number of allergen units (expressed in IR) reported and the number planned in the protocol. Furthermore, the investigator recorded the number of days patients missed treatment and scored compliance as follows: 4, excellent (0 days missing); 3, good (<2 days of interruption); 2, mediocre (3–6 days missing); poor, 1 (>6 days missing); and 0, not interpretable. The number of allergen units expressed in IR was calculated based on the compliance scale, knowing the intake of drops and the concentration of treatment for each day, and was indicated on the patient's diary. Furthermore, overall compliance was evaluated according to medication intake and with the 14-item questionnaire tested by Malet et al,⁵ which allowed patients to give their opinions of SLIT and of the protocol as a whole.

Laboratory Tests and Safety Evaluation

Anti-*Dactylis* specific IgG4 antibody levels were measured in serum, collected before and at the end of treatment, by an immunoenzyme fluorometric assay (CAP System). Serum

samples were stored at -20°C until the end of the study, and all were tested under the same conditions.

All adverse events (respiratory, skin, gastrointestinal, and general) collected by physician inquiries or spontaneously reported by patients were recorded and classified according to the World Health Organization terminology.

Statistical Analyses

To determine the number of patients necessary to be included in the study to show a significant difference between the groups for the primary outcome, we used data from published studies based on a similar score. The number of patients for $\alpha = .05$ and $\beta = .20$ was 58 in each group. The data analysis took into account the grass pollen count measured at the National Aerobiological Surveillance Network, Saint-Clément les Places, France. When data were missing from a patient's diary, the last observation was carried forward. Between-group mean score values (clinical, symptoms, and medications) were analyzed using an analysis of variance adjusted for the weekly pollen count (according to the location of the patient's study center), the week of evaluation (up to 8 weeks), interactions between these variables, and their interactions with the treatment group.

Before performing any analyses, we realized that the pollen counts in various study regions were not the same, which is why we decided to introduce the grass pollen effect in the model. The *t* test and the Wilcoxon rank sum nonparametric test were used to compare normally distributed and nonnormally distributed quantitative variables, respectively. The Cochran-Mantel-Haenszel test with rdit transformation was applied to qualitative ordinate variables, and the χ^2 test compared qualitative variables. All results were given as mean $\pm$ SD. SAS statistical software, version 8.2 (SAS Institute Inc, Cary, North Carolina) was used to perform the statistical analyses. Safety was evaluated with a descriptive analysis of the adverse events observed. Because the groups were not comparable on inclusion, a post hoc additional exploration was performed according to subgroups with and without asthma.

RESULTS

Patients

Of the 127 patients enrolled, 9 withdrew during the first month. The remaining 118 patients were randomly assigned to the active treatment (SLIT) group ($n = 61$) or the placebo group ($n = 57$). Figure 2 presents a flowchart of the study.

Group comparisons at baseline (before treatment) showed that the SLIT group contained more subjects with asthma and had significantly higher mean serum levels of IgE and IgG4 (Table 1).

Efficacy Evaluation

In the ITT population, the adjusted clinical score averages from the analysis of variance did not differ significantly between the SLIT group and the placebo group (least-square mean $\pm$ SD values were 4.8 ± 0.6 and 5.4 ± 0.6 , respec-

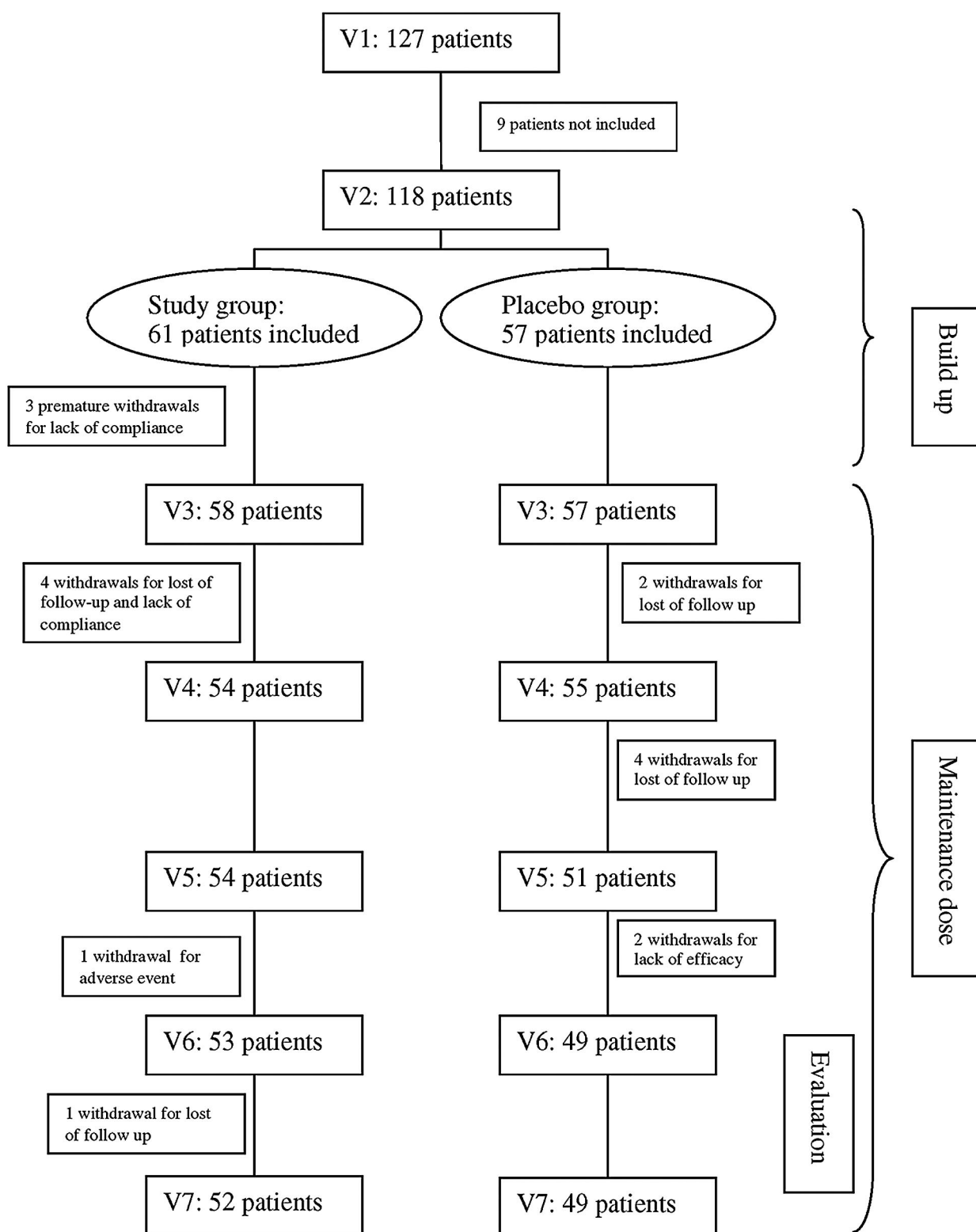


Figure 2. Study outline. V1 through V7 indicate visit 1 through visit 7.

Table 1. Main Demographic and Characteristic Features in Randomized Patients^a

ITT population	SLIT group (n = 53)	Placebo group (n = 51)	P value
Age, y	23.5 ± 7.2	26.5 ± 7.8	.05 ^b
Adults ^c	46 (86.8)	47 (92.2)	.37 ^d
Male sex ^c	34 (64.2)	35 (68.6)	.63 ^d
Duration of rhinitis, mo	71.9 ± 32.6	75.8 ± 32.9	.59 ^b
Rhinoconjunctivitis score evaluated by the patient	14.2 ± 4.4 ^e	13.5 ± 3.8	.25 ^b
Patients with conjunctivitis ^c	51 (96.2)	51 (100.0)	.16 ^d
Patients with asthma ^c	20 (37.7)	9 (17.6)	.02 ^d
Anti-Dactylis IgE level, kU/L	56.7 ± 36.8	42.7 ± 35.6	.03 ^b
Anti-Dactylis IgG4 level, mg/L	0.42 ± 0.48	0.27 ± 0.32	.03 ^b

Abbreviations: ITT, intention to treat; SLIT, sublingual immunotherapy.

^a Data are given as mean ± SD unless otherwise indicated. All patients in the SLIT and the placebo groups had a positive skin prick test result to grass pollen. Results were considered positive when the wheal size of the grass pollen extract was 75% or more of the positive control.

^b Using the Wilcoxon rank sum test.

^c Data are given as number (percentage) of each group.

^d Using the χ^2 test.

^e On enrollment into the study.

tively; $P = .22$) (Table 2). Even though the difference was not significant, there is a trend in favor of the SLIT group.

In a separate post hoc analysis of the patients without asthma ($n = 75$), the SLIT group had significantly better clinical scores than the placebo group (least-square mean ± SD values, 3.0 ± 0.7 vs 4.7 ± 0.6 ; $P = .045$). The between-group differences were not significant for the rhinitis and conjunctivitis subscores, although the trend was always in favor of the SLIT group (Table 3). In contrast, the subscore medication differed significantly between the treatment groups (least-square mean ± SD value for the SLIT group vs the placebo group, 0.34 ± 0.18 vs 0.83 ± 0.16 ; $P = .02$); furthermore, the Spearman rank correlation between the med-

ication score and the clinical score at the end of the study is $r = 0.66$ ($P < .001$) (Fig 3).

Evaluation of Nasal Obstruction and QoL Evaluation

The 3 peak nasal inspiratory flow measurements during pollen season did not differ significantly between the 2 groups (SLIT vs placebo group): 168 ± 67 vs 160 ± 53 ($P = .72$) at visit 5, 164 ± 58 vs 157 ± 62 ($P = .57$) at visit 6, and 160 ± 62 vs 148 ± 59 ($P = .43$) at the last visit. Mean values were slightly higher in the SLIT group.

At the end of the study, overall QoL scores were better for the SLIT group than the placebo group (least-square mean value, 1.35 vs 1.80 ; $P = .07$). The QoL score for “nasal

Table 2. Comparison of the Areas Under the Curve of the Efficacy of the Treatment in the ITT Population

Type of score	SLIT group (n = 53)	Placebo group (n = 51)	P value ^a
Clinical score ^b			
Mean	39.31	45.31	.22
SD	± 32.30	± 33.98	
Min-max	3.1–138.8	0–143.3	
Rhinitis score			
Mean	22.26	23.12	.67
SD	± 16.55	± 17.50	
Min-max	2.00–64.10	0–83.60	
Conjunctivitis score			
Mean	7.79	11.18	.08
SD	± 9.28	± 10.82	
Min-max	0–38.80	0–43.60	
Rescue medication score			
Mean	7.18	9.15	.11
SD	± 11.59	± 10.79	
Min-max	0–66.36	0–59.29	

Abbreviations: ITT, intention to treat; Min-max, minimum-maximum; SLIT, sublingual immunotherapy.

^a Using the Wilcoxon rank sum test.

^b This score included the symptom score plus the rescue medication score.

Table 3. Comparison of the Areas Under the Curve of the Efficacy of the Treatment in the Nonasthmatic ITT Population

Type of score	SLIT group (n = 33)	Placebo group (n = 42)	P value ^a
Clinical score ^b			
Mean	32.39	42.18	.045
SD	± 26.21	± 33.52	
Min-max	3.1–102.0	0–38.6	
Rhinitis score			
Mean	20.55	23.49	.128
SD	± 15.88	± 18.76	
Min-max	2.0–64.1	18.4–83.6	
Conjunctivitis score			
Mean	7.70	9.93	.115
SD	± 9.30	± 9.90	
Min-max	0–36.0	0–43.6	
Rescue medication score			
Mean	3.48	7.57	.016
SD	± 5.37	± 8.23	
Min-max	0–20.7	0–28.3	

Abbreviations: ITT, intention to treat; Min-max, minimum-maximum; SLIT, sublingual immunotherapy.

^a Using the Wilcoxon rank sum test.

^b This score included the symptom score plus the rescue medication score.

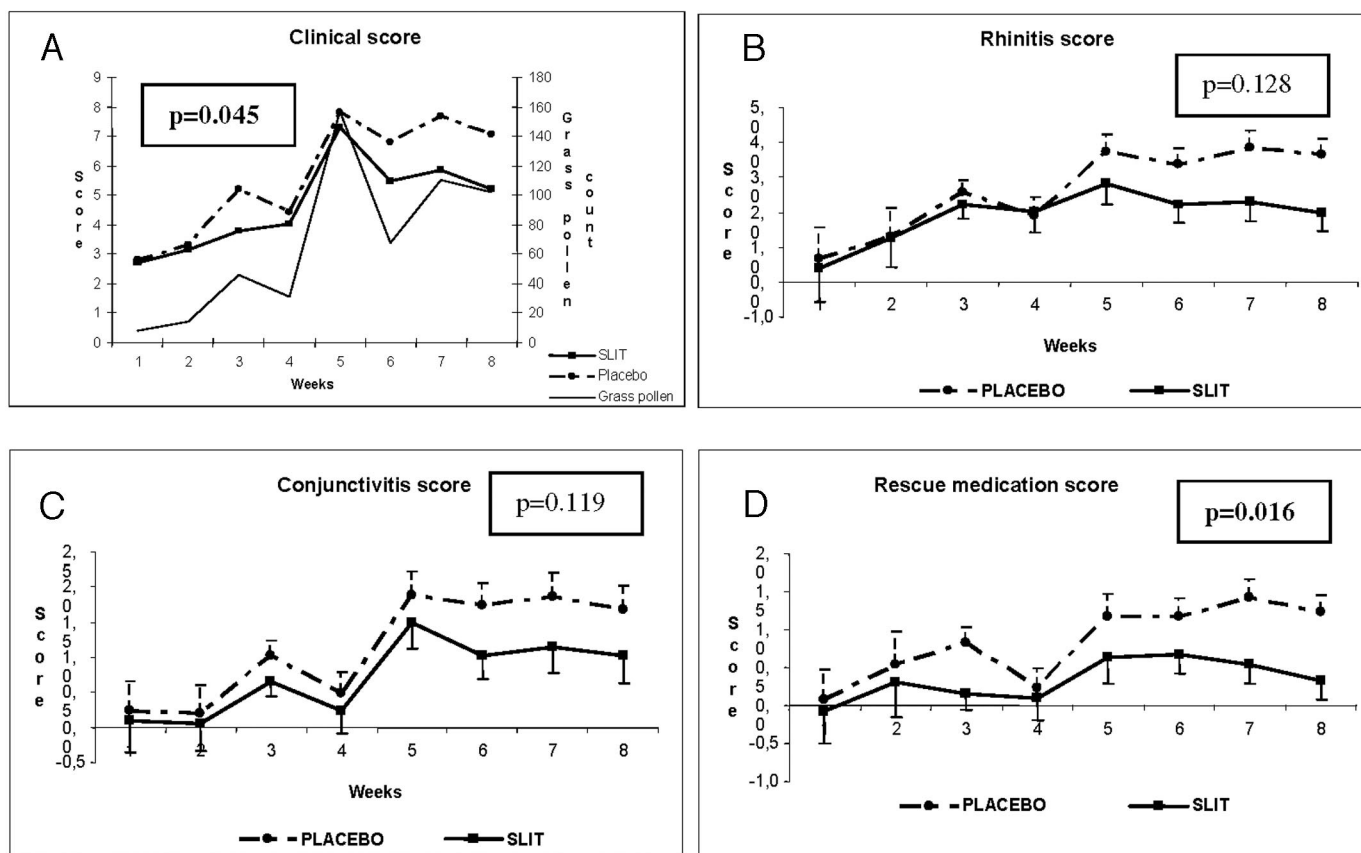


Figure 3. Mean clinical score with the grass pollen count theoretical curve, which takes the patients' locations and their inclusion period into account (A) and rhinitis score (B), conjunctivitis score (C), and rescue medication score (D), all adjusted to the grass pollen count in nonasthmatic patients during the 8 weeks of evaluation (grass pollen season 2002). P values were determined by variance analysis. SLIT indicates sublingual immunotherapy.

discomfort” at the last visit was also better for the SLIT group (least-square mean value, 1.82 vs 2.37; $P = .08$), although not significantly.

Laboratory Tests

Measurement of anti-*Dactylis* specific IgG4 antibodies at inclusion showed a significantly ($P = .03$) higher baseline level for the SLIT group (Table 1). This difference was more marked at the end of the study. The anti-*Dactylis* specific IgG4 antibody level increased from a mean $\pm$ SD of 0.42 ± 0.48 to 0.80 ± 0.92 mg/L for the SLIT group and remained unchanged (0.27 ± 0.32 vs 0.26 ± 0.27 mg/L) for the placebo group ($P < .001$) (Fig 4).

The Spearman rank correlation between the anti-*Dactylis* specific IgG4 level at the end of the study and the cumulative IR dose during the treatment period approached significance for the SLIT group ($r = 0.26$, $P = .08$) but not for the placebo group ($r = 0.02$, $P = .87$).

Compliance Evaluation

The number of IRs reported taken and the number planned in the protocol showed a mean $\pm$ SD compliance rate of $99.6\% \pm 7.7\%$ during the buildup phase, $99.6\% \pm 4.0\%$ during the maintenance phase, and $99.6\% \pm 3.8\%$ for the study as a whole. The compliance evaluated by the investigator found that 48 of 101 patients (47.5%) forgot to take their treatment from time to time; all 48 were able to resume the treatment plan easily. Furthermore, 14 of 104 patients (13.5%) had mediocre, poor, or uninterpretable treatment compliance.

Safety Evaluation

Safety was evaluated in 118 patients who took at least 1 treatment dose during the study. The mean duration of exposure was 260.5 days. Most patients tolerated the study treatment well, and no serious adverse events were reported. At least 65 of the 118 patients (55.1%) reported an adverse event for which a link with the study products could not be ruled out, and significantly more of them were in the SLIT than the

placebo group (42 of 61 patients [68.9%] vs 23 of 57 patients [40.4%]; $P = .003$). The principal adverse events were mouth and throat irritation, which were more common in the SLIT group than in the placebo group. Other notable adverse events were lip edema and abdominal pain (Table 4). Of 9 patients who interrupted their treatment for adverse events, only 3 were related to SLIT. One patient in the placebo group dropped out because of its lack of efficacy.

DISCUSSION

This multicenter study was designed to compare the efficacy, safety, and compliance of a standardized 3-grass pollen extract and of placebo in patients with grass pollen allergic rhinoconjunctivitis. The pollen extract and placebo were administered by sublingual swallowing for a long period before and during the allergy season.

Patients were selected according to strict inclusion criteria during the grass pollen season and evaluated during the next pollen season. The study population corresponds to the age range (12–40 years) among whom the prevalence of rhinoconjunctivitis is highest.¹

In this study, the primary outcome compared the clinical score among the ITT population. However, the 2 groups were not comparable at the beginning, because the active product group contained significantly more subjects with asthma. Obtaining groups comparable at baseline is known to be a major difficulty in desensitization studies. As Malling⁶ points out, the problem of randomization is a major concern in most studies evaluating the efficacy of SLIT. Only a few studies^{7,8} have analyzed disease severity in their patients during a run-in period before randomization, to ensure balance in that respect.

Although the 2 groups were not comparable and there were more subjects with asthma in the SLIT group, the adjusted mean clinical score showed a trend in that group’s favor. Furthermore, the lack of a significant difference between the

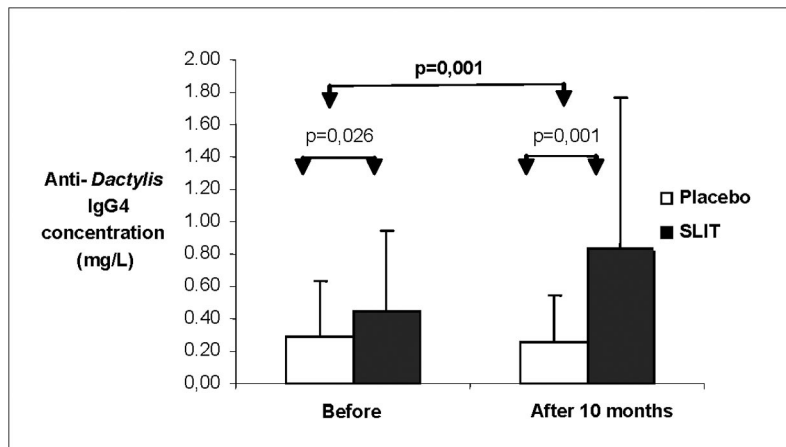


Figure 4. Anti-*Dactylis* specific IgG4 antibodies in the placebo and sublingual immunotherapy (SLIT) group (intention-to-treat population). P values were determined by the Wilcoxon rank sum test.

Table 4. Main Adverse Events^a

Adverse event	SLIT group (n = 61)	Placebo group (n = 57)	Total (N = 118)
Oral irritation	27	1	28
Throat irritation	22	7	29
Oral or lip edema	10	0	10
Abdominal pains	7	2	9
Diarrhea	5	2	7
Headache	5	5	10
Mouth ulcers	0	3	3

Abbreviation: SLIT, sublingual immunotherapy.

^a Data are given as number of patients affected in each group.

2 groups in the number of subjects with asthma at the end of the study suggests a clinical effect (data not shown). We then conducted a supplementary secondary analysis in the patients without asthma and found that the clinical score in the SLIT group was significantly better than in the placebo group.

These results are consistent with those of other studies.^{3,7,8} Our primary objective was to compare patients according to a global clinical score. Clinical efficacy was measured in all randomized patients (by an ITT analysis). These 2 characteristics allow us to evaluate the true treatment effect.

During the past 15 years, several randomized, placebo-controlled, clinical trials have demonstrated the safety of SLIT with grass pollen extracts in adults and children.⁹ To our knowledge, the literature contains reports of any severe systemic adverse event.¹⁰ The most frequently reported adverse effects in the SLIT group are oral and sublingual itching after taking the dose. High doses of allergen administered sublingually may also cause the gastrointestinal adverse effects, sometimes significant, that have been reported.¹¹ In our study, the individual allergen dose administered was approximately 350 times the dose usually administered during subcutaneous injection immunotherapy (SCIT), but no severe adverse events were noted in the active treatment group. The principal adverse events were mild oral reactions that did not require treatment. These local adverse events were, however, more common in the active treatment group. These safety results seem good despite the high cumulative doses of allergenic extracts administered during the treatment period: a mean cumulative dose during the 10-month treatment period of 31,800 IR (ie, 2.75 mg of major grass allergen group 5 [Dac g 5, Lol p 5, and Phl p 5]). This dose is more than 300 times the dose of grass pollen allergens administered during SCIT of similar duration. A few other studies^{8,12,13} using similar standardized grass pollen extracts expressed as IR units reached similar cumulative doses. It is likely that the total dose of allergen administered is related to treatment efficacy, but the wide range of allergen preparations used and the absence of standardization of the enzyme-linked immunosorbent assay measuring grass pollen major allergens make it impossible to compare and analyze this question.

Specific immunotherapy is frequently associated with an increase in allergen specific IgG4 antibodies, although this is

not always correlated with clinical efficacy.^{12–17} Not all studies report induction of IgG4.^{5,18,19} The levels of *Dactylis* grass pollen specific IgG4 at inclusion and at the end of the study showed a significant increase in the SLIT group ($P < .001$), indicating that the local immune system of the oral mucosa was strongly exposed to pollen antigens. The IgG4 serum levels approximately doubled, in accordance with Smith et al,⁸ who reported that allergen specific IgG4 concentrations approximately tripled in the active treatment groups after 1 year of SLIT. These increased concentrations were sustained after 2 years of treatment but moved back toward baseline in the group that received only 1 year of SLIT. This result seemed to indicate an immunological effect of the dose of SLIT.

Self-administration of the treatment at home may pose compliance problems. Adherence to SLIT was recently evaluated by a quantitative evaluation in a real-life setting, in which patients were randomly asked to count the remaining quantity of SLIT treatments at a given moment. This study²⁰ found adherence to be 97.3%. Studies assessing compliance in SCIT show discontinuation rates between 10% and 34%,²¹ with as many as half of all patients not compliant because of adverse effects.²²

In conclusion, 10 months of high-dose grass pollen SLIT improved the clinical score in patients with hay fever and without asthma.

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