

## Double-blind placebo-controlled evaluation of sublingual-swallow immunotherapy with standardized *Parietaria judaica* extract in children with allergic rhinoconjunctivitis

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**Background:** Sublingual-swallow immunotherapy was recently recognized in the World Health Organization Position Paper (Allergen immunotherapy: therapeutic vaccines for allergic diseases) "as a viable alternative to parenteral injection therapy to treat allergic diseases" in adults. More controlled studies were required to assess the efficacy and safety of this treatment in children.

**Objective:** This study was carried out to assess the clinical efficacy and safety profile of sublingual-swallow immunotherapy with high-dose allergen in children with allergies.

**Methods:** We used a double-blind placebo-controlled design. Forty-one children with *Parietaria*-induced rhinoconjunctivitis were randomized to receive sublingual standardized *Parietaria judaica* extract (n = 20) or placebo (n = 21) for 2 years. The cumulative dose of allergen was 375 times higher than that used in parenteral immunotherapy and the cumulative dose of Par j 1 major allergen was 52.5 mg over 2 years. The main efficacy assessment criteria were symptoms and rescue medication scores recorded on the patients' diary cards. Secondary criteria were changes in skin and conjunctival specific reactivity as well as blood parameters, analyzed after 1 and 2 years of immunotherapy. The safety of the treatment was assessed by evaluating the frequency and severity of adverse effects.

**Results:** A significant reduction in rhinitis symptoms was observed in the active treatment group during the second season ( $P = .02$ ), with no difference in medication scores. A significant decrease in skin reactivity ( $P = .002$  after 2 years of treatment) and an increase in the threshold dose for conjunctival allergen provocation test ( $P = .02$ ) were observed in the active treatment group compared with the group receiving placebo. A significant increase in specific IgG<sub>4</sub> levels ( $P = .02$ ) was also observed in the active group. Immunotherapy was well tolerated.

**Conclusion:** Sublingual-swallow immunotherapy in *Parietaria*-allergic children provided a clinical benefit and a decreased specific reactivity to the allergen. The safety profile of this treatment, which constitutes an important issue, indicated good tolerance and compliance. (J Allergy Clin Immunol 1999;104:425-32.)

**Key words:** Seasonal rhinoconjunctivitis, *Parietaria*, children, sublingual-swallow immunotherapy

*Parietaria*-induced pollinosis is an important clinical feature in Europe, especially in the Mediterranean area.<sup>1</sup> *Parietaria* also appears to be an unrecognized cause of respiratory allergy in the United States<sup>2</sup> and Australia.<sup>3</sup>

The extraordinarily long persistence of *Parietaria* pollen in the atmosphere in the Mediterranean area is often responsible for almost perennial symptoms.

Specific immunotherapy represents the cornerstone of allergy treatment because, together with allergen avoidance (when possible), it is the only etiologic treatment available to patients with allergies. Under optimal conditions, for example, demonstration of the predominant role played by allergen sensitization in eliciting symptoms, the availability of high-quality allergen extracts, and correct patient monitoring, injection specific immunotherapy constitutes an effective treatment for allergic diseases. The recent World Health Organization (WHO) Position Paper concerning allergen immunotherapy<sup>4</sup> states that other routes of allergen administration, such as sublingual and intranasal routes, can represent "a viable alternative to parenteral injection therapy" for allergic diseases in adults. More studies assessing the efficacy and safety of sublingual immunotherapy are required before this treatment can be routinely recommended in children. Sublingual immunotherapy can be delivered by either the sublingual-spit or sublingual-swallow method. The clinical efficacy of the sublingual-swallow method has been validated.

One open<sup>5</sup> and 2 double-blind placebo-controlled studies<sup>6,7</sup> with sublingual immunotherapy with use of *Parietaria* extract demonstrated a clinical benefit in adult

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**Abbreviations used**

IR: Index of reactivity

WHO: World Health Organization

**TABLE I.** Demographic data

| Characteristics of patients               | Active group | Placebo group |
|-------------------------------------------|--------------|---------------|
| No. of subjects included                  | 20           | 21            |
| Sex (male/female ratio)                   | 13:7         | 12:9          |
| Age (y)                                   |              |               |
| Mean age                                  | 10           | 10            |
| Mean range                                | 6-14         | 7-13          |
| Mean duration of rhinitis (y [ $\pm$ SD]) | 3 $\pm$ 2    | 4 $\pm$ 3     |
| Polysensitized patients                   |              |               |
| Mites                                     | 1            | 1             |
| <i>Alternaria</i>                         | 1            | 3             |
| Grass                                     | 4            | 1             |
| Patients eligible for efficacy analysis   | 16           | 17            |

patients, but no study has been conducted with this allergen in children.

The current double-blind placebo-controlled study investigated the clinical efficacy and safety of sublingual-swallow immunotherapy with high-dose standardized *Parietaria judaica* extract in children with *Parietaria*-induced pollinosis in Italy, where this allergen has an important impact all over the country.

## METHODS

### Study design

This was a double-blind, placebo-controlled, parallel-group study.

Forty-one patients with a positive history of rhinoconjunctivitis, with or without mild intermittent asthma, caused by *Parietaria* pollen sensitization documented by positive skin tests (wheal diameter >4-mm) and positive radioallergosorbent test results (class II and above) were enrolled between November 1995 and March/April 1996. Patients with uncontrolled asthma, symptomatic polysensitization or who were treated with  $\beta$ -blockers or long-acting corticosteroids were excluded. Patients with cats at home were also excluded. Without any baseline assessment, the patients were randomized to receive either active (20) or placebo (21) sublingual therapy for 2 consecutive years (Table I).

At the inclusion visit skin tests with other allergens (mites, *Alternaria*, cockroach, and grass) were performed to evaluate cosensitization with *Parietaria* pollen allergy.

Regular visits for clinical evaluation and safety assessment were scheduled. Quantitative skin tests and conjunctival provocation tests were performed, and blood samples were taken before starting treatment, after 1 year, and at the end of the trial.

The main efficacy assessment criteria were symptoms and rescue medication scores. Secondary assessment criteria were changes in skin and conjunctival specific reactivity as well as blood parameters, analyzed after 1 and 2 years of immunotherapy. The safety of this treatment modality was assessed by the frequency and severity of adverse effects.

The study was conducted according to Good Clinical Practices rules after approval by the ethics committee and Italian health authorities. The patients' parents gave informed consent before inclusion.

### Treatment

Active treatment consisted of a standardized *P judaica* extract (Stallergènes, Antony, France) in drops to be taken sublingually and held under the tongue for 2 minutes before being swallowed.

The extract was standardized in index of reactivity (IR) units. The in-house reference extract (called 100 IR) is defined as the concentration eliciting a wheal with a mean diameter of 7 mm in 30 skin-tested patients with allergies. The level of the Par J 1 major allergen in the 100 IR extract was 70  $\mu$ g/mL.

Four allergen concentrations were used: 1, 10, 100, and 300 IR per milliliter.

Placebo consisted of a glycerinated phenolated saline solution with an appearance and taste similar to those of the active treatment.

The build-up treatment phase started with 2 drops of the first concentration (1 IR/mL), increasing by 2 drops per day up to 10 drops per day, followed by the next concentration (10 IR/mL) increasing from 2 to 10 drops per day and then continued with a similar progression from 2 to 20 drops of the next concentration (100 IR/mL) and 2 to 20 drops of the highest concentration (300 IR/mL). After administration of 20 drops of 300 IR per milliliter daily for 1 month, the maintenance dose was 20 drops of 300 IR per milliliter 3 times a week until the end of the study.

The cumulative dose of allergen extract received by each patient was 75,000 IR per year (375 times higher than with parenteral specific immunotherapy). The cumulative dose of Par J 1 major allergen was 52.5 mg over 2 years.

### Patient monitoring

**Symptom-medication score.** Patients were instructed to keep a diary for daily evaluation of nasal symptoms (sneezing, rhinorrhea, or nasal blockage), ocular symptoms (itching, redness, or weeping) and lung symptoms (cough, dyspnea, or wheezing) according to a 0-3 grading scale (0 = no symptoms, 1 = mild, 2 = moderate, 3 = severe symptoms). When necessary, the patients were allowed to use antiallergic drugs (antihistamines, ocular and nasal cromoglycate, nasal corticosteroids and, if necessary, oral corticosteroids as well as antiasthmatic drugs), but they had to record drug intake for symptomatic treatment on the diary card.

Arbitrary scores were attributed to the class of drugs used to treat rhinitis symptoms (0.25 point for each dose of topical cromoglycate, 0.5 point for each dose of nasal corticosteroid, 2 points for each dose of antihistamine, and 3 points for each oral corticosteroid tablet).

Daily mean symptom and medication scores, calculated each week during the pollen season, were plotted against time for each treatment group.

**Skin tests.** Quantitative skin tests with 5 dilutions (0.2 IR, 6.6 IR, 20 IR, 60 IR, 100 IR) of *P judaica* extract were performed in duplicate on the patient's forearm on inclusion and 1 year and 2 years after starting treatment. A positive control (9% codeine phosphate solution) and a negative control (glycerinated saline solution) were used.

Skin reactions were recorded after 20 minutes. Each wheal was outlined with a fine-tip roller pen and transferred by means of transparent tape to the corresponding sheet of the case report form.

The geometric means of the diameters of wheals induced by each concentration, measured with a scanner, were used to plot the dose-response curve for each patient with use of the least squares method:  $\log A = a + b \times \log C$  (where A = wheal diameter, a = intercept, b = slope, and C = concentration).

**Conjunctival provocation test.** Allergen-specific conjunctival challenges were performed on inclusion and 1 year and 2 years after treatment was started.

Drops of increasing allergen concentrations (0.4 IR, 1.2 IR, 3.7

IR, 11 IR, 33 IR, 66 IR, 100 IR, lyophilized *P judaica* extract reconstituted at 3-fold dilutions with nonphenolated saline solution), were placed into the lower conjunctival fornix of the right eye (the left eye was used as the control) until a clinical reaction was observed. Only the early-phase reaction occurring within 15 to 30 minutes was investigated. Clinical reactions, such as conjunctival itching and burning, conjunctival hyperemia, weeping, nasal blockage or posterior rhinorrhea, were assessed according to an arbitrary scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe) described by Abelson et al.<sup>8</sup>

The dose eliciting a score of at least 7 was considered to be the threshold dose, and its variation after 1 and 2 years of treatment was compared in the active treatment and placebo groups.

**In vitro parameters.** *Parietaria*-specific IgE and specific IgG<sub>4</sub> were measured with commercial kits according to the supplier's instructions (CAP System-Pharmacia, Uppsala, Sweden) on serum samples taken before starting treatment, after 1 year of treatment, and at the end of the trial.

IgE values were expressed in kallikrein units per liter and IgG<sub>4</sub> values were expressed as a binding percentage compared with a reference standard serum.

### Safety assessment

Patients and investigators were asked to record all adverse effects in detail, regardless of whether they were related to treatment, according to Good Clinical Practices rules.

### Pollen counts

The *Parietaria* pollen season in Italy starts in February in southern Italy and in March/April in northern Italy and lasts until the beginning of November. Pollen grain counts were recorded each year of the study in each center and pollen curves were plotted.

### Statistical analysis

Nonparametric data were analyzed by the Mann-Whitney *U* test for intergroup comparison and Wilcoxon's rank sum test for intragroup comparison. Statistical tests comparing 2 entities were 2 tailed and *P* < .05 was considered to be significant.

## RESULTS

### Patients

The 2 groups did not differ in terms of demographic variables, history of rhinitis, conjunctival provocation tests, skin reactivity, and immunologic parameters.

Of the 41 randomized patients, 3 from the active group and 3 from the placebo group left the study before the first pollen season and another 2 patients (1 active and 1 placebo) dropped out of the trial during the second year. Thirty-three patients (16 in the active treatment group and 17 in the placebo group) were therefore evaluable at the end of the trial. One patient in the active group stopped the treatment before the end of the second season but was eligible for efficacy assessment. All patients were included in the safety analysis.

During the study 5 patients dropped out of the active group (4 because of adverse events and 1 for noncompliance with study procedures) and 4 patients dropped out of the placebo group (1 because of adverse events, 1 for personal reasons, and 2 because of deterioration of the allergic disease). Few patients were cosensitized to other allergens. Demographic data are shown in Table I.

### Clinical efficacy assessment parameters

Rhinitis symptom scores during the first and second pollen season are shown in Fig 1, A and B. On inclusion, patients in the active treatment group appeared to have more severe symptoms than those in the placebo group. Analysis of the course of symptoms showed a reduction of symptom scores during the second season in patients receiving active immunotherapy. Malling<sup>9</sup> emphasized that only a reduction in symptom scores greater than or equal to 30% can be considered clinically relevant.

Significantly more patients had a  $\geq 30\%$  reduction in rhinitis symptoms in the active treatment group than in the placebo group (*P* = .02) (Fig 2).

Medication scores for rhinitis were not significantly different between the 2 groups throughout the study, as shown in Fig 3, A and B. Actively treated patients took significantly fewer antiallergic drugs only during 1 week of the first pollen season (*P* = .05).

### Skin tests

The diameter of the wheal obtained with the 100 IR concentration on the dose-response curve estimated by regression analysis before treatment and after 1 year and 2 years of treatment was compared in the 2 groups by the nonparametric Mann-Whitney *U* test. After 1 year of treatment no change was observed in the active group, whereas an increase in skin reactivity was observed in the placebo group, resulting in a significant difference (*P* = .02) between the 2 groups (Fig 4). After 2 years of treatment, skin reactivity decreased in the active group and the difference between the 2 groups became highly significant (*P* = .002).

### Conjunctival provocation test

An increase in the threshold dose indicates that the patient has become more tolerant to the allergen.

The threshold dose did not differ between the 2 groups before treatment was started. After 1 year of treatment the threshold dose increased in both groups, but to a significantly greater extent in the active group (*P* = .02), as shown in Fig 5.

During the second year, the threshold dose decreased in both groups, but to a lesser extent in the active group, although the significant difference (*P* = .02) between the 2 groups on the nonparametric Mann-Whitney *U* test was maintained.

### In vitro parameters

**Specific IgE level.** Specific IgE levels were variable and no significant difference was detected between groups.

**Specific IgG<sub>4</sub> level.** Before treatment was started, the percentage of specific IgG<sub>4</sub> was higher in the placebo group than in the active group, but the difference was not statistically significant. As shown in Fig 6, no change was observed in the placebo group throughout the study, whereas specific IgG<sub>4</sub> levels increased during the treatment period in the active group.

By analyzing the variation of specific IgG<sub>4</sub> levels in

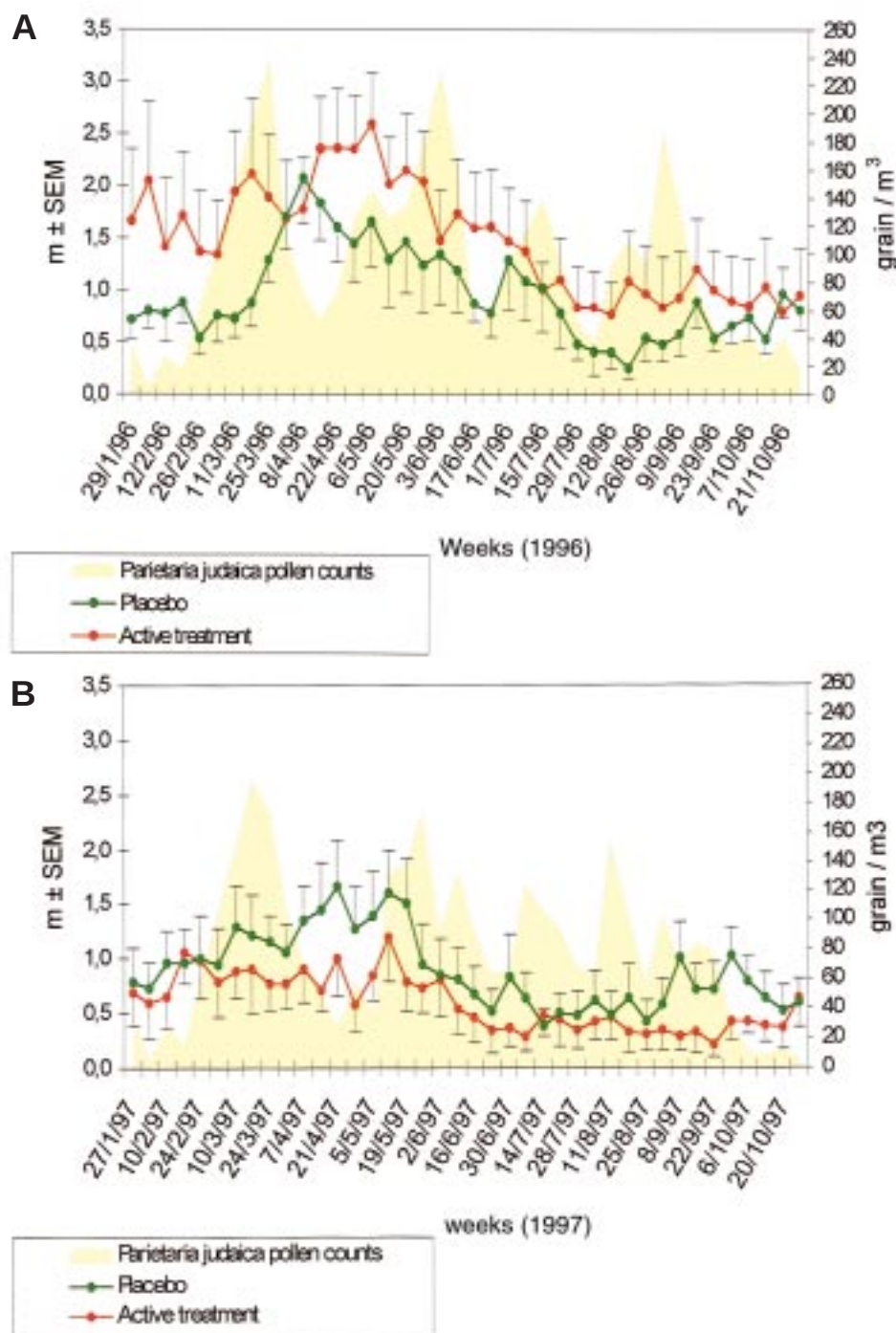


**FIG 1.** Rhinitis symptom scores. Daily means  $\pm$  SEM of rhinitis symptoms (sneezing, rhinorrhea, and nasal blockage; each symptom scored from 0 = absent to 3 = severe) were plotted against time during first pollen season (1996) (A) and second pollen season (1997) (B). Shaded area, *Parietaria* pollen counts. Active treatment group was clearly less symptomatic during second year.

the active and placebo groups, a significant difference was observed ( $P = .02$ ).

### Adverse events

The adverse events observed during the trial are listed in Table II. Nineteen patients (12 in the active group and 7 in

the placebo group) had, sometimes repeatedly, one of the listed adverse events. The most common adverse events were gastrointestinal complaints. These complaints led to withdrawal from the trial in 4 cases in the active group and in 1 case in the placebo group. The adverse events were effectively treated by dose reduction and antihistamines.

TABLE II. Adverse events

| Adverse event                         | Active group | Placebo group |
|---------------------------------------|--------------|---------------|
| Anaphylactic reaction                 | 0            | 0             |
| Itching in mouth                      | 3            | 2             |
| Labial swelling                       | 2            | 2             |
| Gastrointestinal complaints           | 19           | 1             |
| Conjunctivitis                        | 1            | 1             |
| Rhinitis                              | 1            | 1             |
| Mild asthma attack                    | 0            | 1             |
| Severe asthma attack                  | 0            | 1             |
| Others (generalized pruritus)         | 1            | 1             |
| Total No. of adverse events           | 26           | 10            |
| No. of patients having adverse events | 12           | 7             |

Only one patient receiving placebo had to be hospitalized for a severe asthma attack and 1 patient receiving active treatment was hospitalized for a wrist fracture.

Safety was considered to be good because no major adverse event was reported.

## DISCUSSION

This study compared the efficacy of sublingual immunotherapy with standardized *P. judaica* extract versus placebo in children with *Parietaria* pollinosis. The major outcome, indicating efficacy of treatment, was reduction in rhinitis symptom scores. Because no baseline assessment was performed, the active group was more severely symptomatic at the start of the trial, and this difference persisted during the first pollen season. As classically reported, no effect of immunotherapy was observed shortly after treatment was started. A marked reduction of the symptom score was observed during the second year, with no difference in antiallergic drug intake. The magnitude of the reduction is an important issue, as stressed by Malling.<sup>9</sup> For example, a 10% reduction may be statistically significant but clinically irrelevant. No "gold standard" has been defined for the magnitude of reduction of symptoms or drugs considered to be effective in everyday clinical practice, but a reduction of at least 30% could justify treatment involving a potential risk of adverse effects. The results of this study showed a clinical benefit of sublingual immunotherapy with high doses of allergen in terms of this parameter. However, a baseline assessment should be performed in future studies to ensure equal groups to be compared in terms of symptoms and medication scores.

Second, the decrease in skin reactivity and the increase in the threshold dose for conjunctival challenge suggest that this treatment modulates the immune response to the allergen. The higher percentage of specific IgG<sub>4</sub> in the placebo group from the start of the trial is in line with the less-severe symptoms observed in this group, whereas this parameter increased as the symptom scores decreased in the active group.

The cumulative dose of allergen was 375-fold greater than that usually administered in subcutaneous

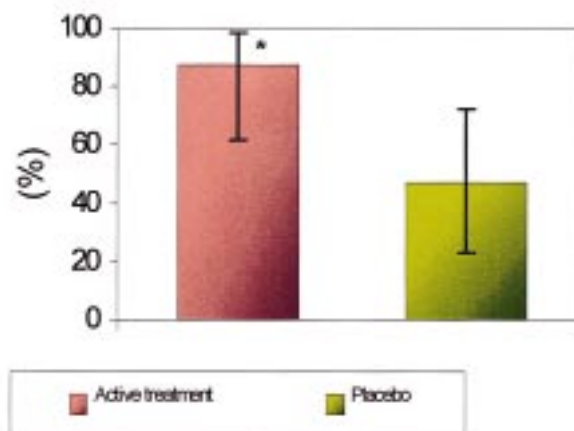


FIG 2. Proportion of patients with reduction of at least 30% of rhinitis symptom scores. A total of 87.5% of patients receiving active treatment reduced their symptom scores by at least 30%, which can be considered to be clinically relevant.<sup>9</sup> Significant difference was observed between groups (asterisk,  $P = .02$ ). Error bars indicate exact 0.95 confidence interval (binomial).

immunotherapy. Despite this large dose of allergen, the adverse effect rate was not significantly higher in the active treatment group, except for gastrointestinal complaints, which constituted the most frequent adverse events. These data confirm the safety of this modality of allergen administration because no severe systemic reaction was observed. A number of controlled studies<sup>6,7,10-21</sup> have reported data on the efficacy and safety of treatment of allergic disorders by sublingual immunotherapy. It is difficult to compare the results of these studies because of the great variability of duration of treatment, doses of allergen extract administered, and number and type of patients and allergens used.

For methodologic reasons, only 4 of these studies<sup>6,12,14,15</sup> were included in the efficacy and safety analysis of the WHO Position Paper concerning sublingual immunotherapy.<sup>4</sup> These studies demonstrated the clinical effectiveness of sublingual-swallow immunotherapy with grass, *Parietaria*, and mite extracts. The safety profile was excellent in adults. However, systemic adverse effects (urticaria or asthma) were reported in one study<sup>12</sup> performed with mite extract in children. These adverse effects may be due to the type of allergen and the allergic nature of perennial asthma.

Other studies have been published since completion of the WHO Position Paper. A long-term study<sup>19</sup> that used mite monomeric allergoid tablets demonstrated a clinical benefit and reduction of allergic inflammation after specific conjunctival challenge. Another study,<sup>20</sup> in a large population with hay fever, demonstrated a significant steroid-sparing effect during the grass pollen season, and in another trial<sup>21</sup> the dyspnea score of children with mild asthma caused by olive tree pollen sensitization was reduced after sublingual immunotherapy.

In a carefully designed (double-blind, double-dummy) study Quirino et al<sup>22</sup> compared the efficacy of these 2 treatment modalities with grass pollen extracts. The

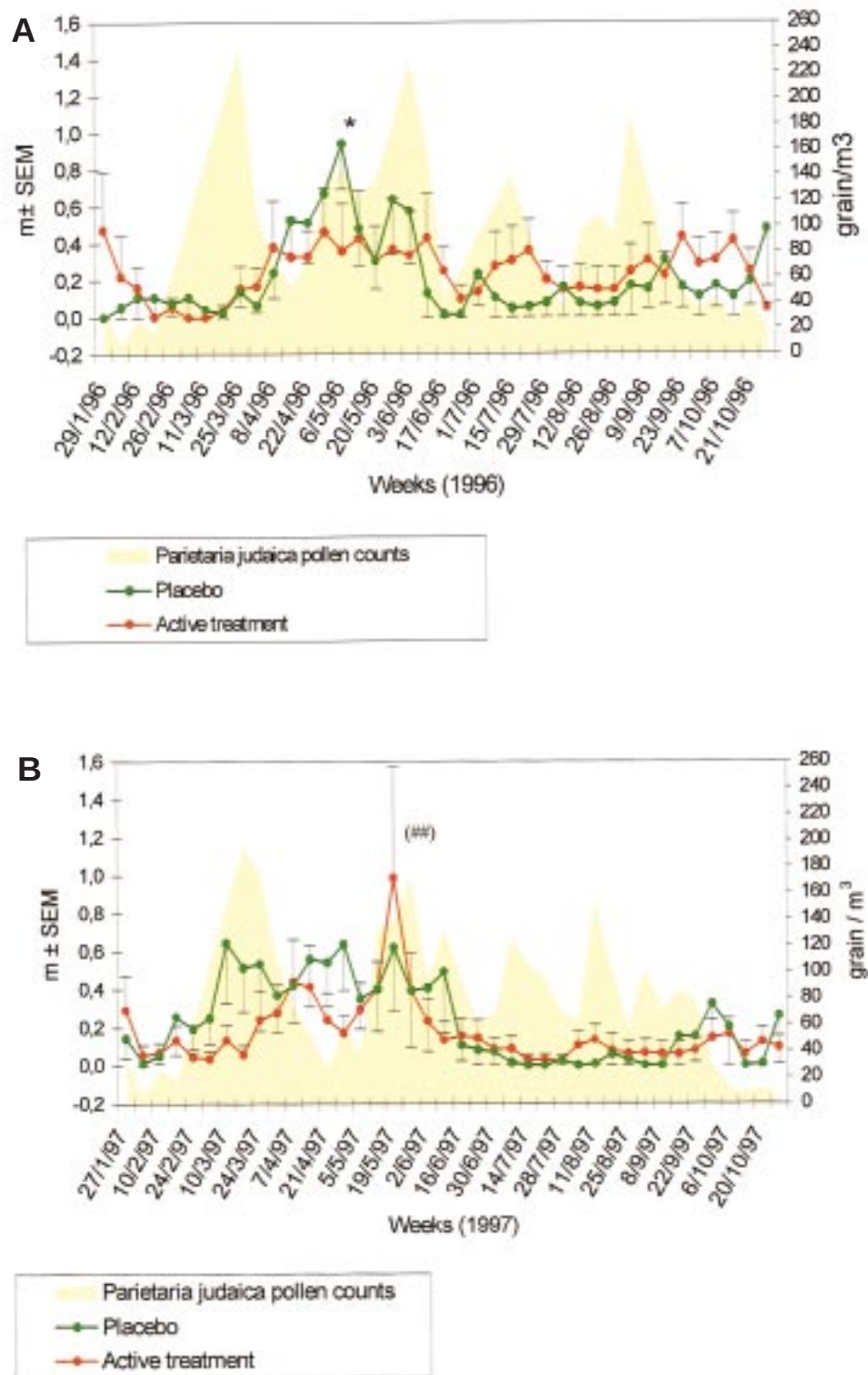


**FIG 3.** Antirhinitis medication scores. Daily means  $\pm$  SEM of antiallergic drugs (arbitrarily scored: 0.25 point for each dose of topical cromoglycate, 0.5 point for each dose of nasal corticosteroids, 2 points for each dose of antihistamine, and 3 points for each oral corticosteroid tablet) were plotted against time during first pollen season (1996) (A) and during second pollen season (1997) (B). Shaded area, *Parietaria* pollen counts. Medication scores did not differ significantly between groups throughout study. Asterisk, Patients in active treatment group took significantly fewer antiallergic treatment only during 1 week of first pollen season ( $P = .05$ ); two pound signs, 1 patient took 3 oral corticosteroid tablets.

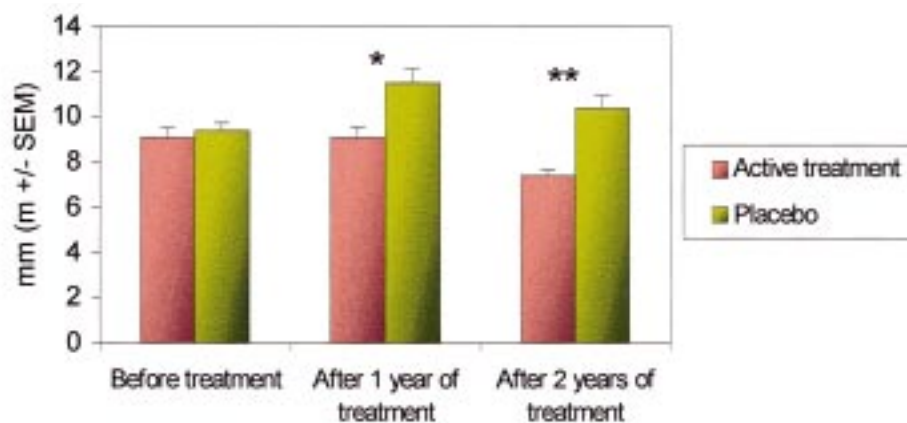


**FIG 4.** Quantitative skin tests. Groups were compared in terms of diameter of wheal obtained with 100-IR concentration on dose-response curve estimated by regression analysis. After 1 year of treatment, increase in skin reactivity was observed in placebo group (*asterisk*,  $P = .02$ ) and, after 2 years of treatment, skin reactivity decreased in active treatment group and difference between groups became highly significant (*two asterisks*,  $P = .002$ ).

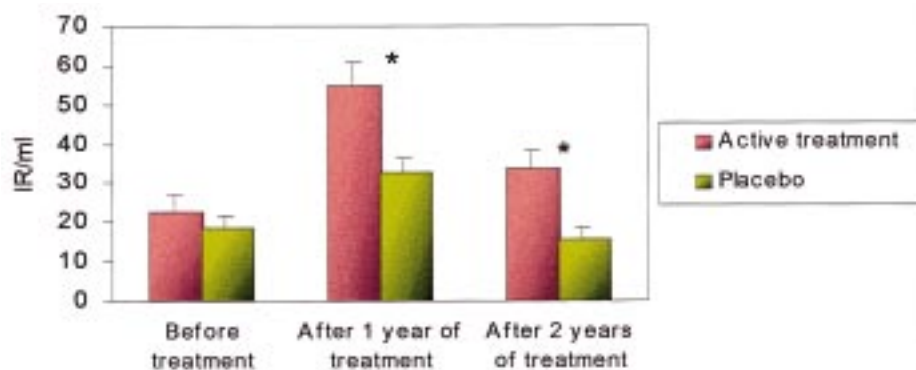


**FIG 5.** Threshold dose for conjunctival provocation tests (mean  $\pm$  SEM). After 1 year of treatment threshold dose increased in both groups, but to significantly greater extent in active treatment group (*asterisk*,  $P = .02$ ). During second year threshold dose decreased in both groups, but to lesser extent in active treatment group, maintaining significant difference (*asterisk*,  $P = .02$ ) between groups.

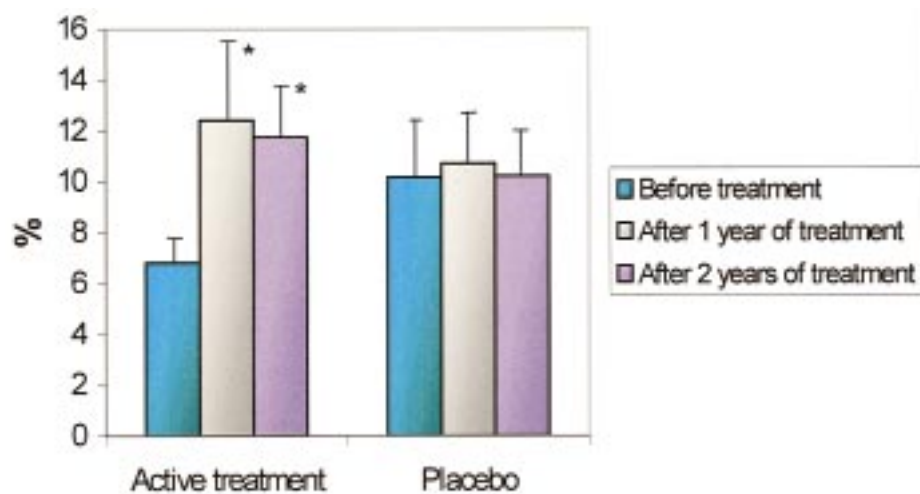


**FIG 6.** Anti-*Parietaria*-specific IgG<sub>4</sub> as percent binding (mean  $\pm$  SEM). There was no change in placebo group throughout the study, whereas specific IgG<sub>4</sub> increased during treatment period in active treatment group. Analysis of variation of specific IgG<sub>4</sub> in active treatment group and placebo group revealed significant difference (*asterisk*,  $P = .02$ ).

results showed that sublingual and injection therapy were equally effective in terms of clinical parameters. Objective parameters were only changed for patients treated by injection. These results suggest differences in the mechanisms involved in these 2 treatment modalities.

Passalacqua et al,<sup>23</sup> studying the pharmacokinetics of sublingual allergen administration, showed that the allergen was not degraded by saliva and that no direct absorption by sublingual mucosa could be detected. A significant amount of radiolabeled allergen (Par j 1) persisted in the sublingual region for up to 48 hours after administration. The oral mucosa is particularly rich in Langerhans' cells, which have been shown to act as effective antigen-presenting cells and to produce IL-12. Because this cytokine is capable of preferentially promoting the development of Th1 cells, a hypothetical mechanism of the "tolerogenicity" of the sublingual route might involve Th1/Th2 differentiation and regulation of IgE production.<sup>24</sup>

Another important aspect is the economic issue. Although the allergens used for sublingual immunotherapy are more expensive than those used for injection immunotherapy, particular attention must be paid to the "social cost" of injection immunotherapy (in terms of time off work for scheduled visits for injections and related medical costs), which offsets the cost differences between these 2 treatment modalities.<sup>25</sup>

Larger studies with a dose-ranging design to determine the optimal relationship between clinical efficacy, safety profile, and cost, together with more data concerning the mechanisms of action, are necessary.

Data from the literature and our own results suggest that sublingual immunotherapy may be used in young children to improve the control of allergy. The impact of this treatment modality on the natural history of the disease is another question that needs to be addressed.

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