

Immunotherapy with a calcium phosphate-adsorbed five-grass-pollen extract in seasonal rhinoconjunctivitis: a double-blind, placebo-controlled study

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Summary

Background Calcium phosphate-adsorbed allergen extracts are used for subcutaneous immunotherapy to avoid the use of aluminium adjuvants.

Objectives A double-blind, placebo-controlled study was performed in order to confirm the safety and assess the efficacy of a standardized five-grass-pollen extract adsorbed onto calcium phosphate for specific immunotherapy (IT).

Methods Twenty-nine patients with seasonal rhinoconjunctivitis were randomized to receive either the active preparation (16 patients) or placebo (13 patients), in a 1-year study. During the increasing dose phase, an extract ranging from 0.1 IR per ml to 50 IR per ml was administered at a rate of one subcutaneous injection per week until a maintenance dose was reached. The patients were assessed by symptom diary and rescue medications during seasonal exposure and specific nasal and skin reactivity before and after IT. Immunological parameters (specific IgE and IgG₄ antibodies) were assessed before, during and after IT.

Results The overall symptoms score (mean AUC) was not significantly different between the IT group and the placebo group during grass-pollen exposure (49.6 vs. 56, respectively). The total medication score (mean AUC) was significantly lower in the IT group than in the placebo group (11 vs. 41, $P < 0.01$, Mann–Whitney U -test). The cumulative symptom/medication score was significantly lower in the IT group than in the placebo group (64.5 vs. 102.3, $P < 0.05$, U -test). A significant increase in nasal reactivity threshold was observed after IT in the IT group (21.4 IR/mL before IT vs. 63.4 IR/mL after IT, $P < 0.01$, Wilcoxon), whereas no significant changes were observed in the placebo group (31.0 IR/mL before IT vs. 37.7 IR/mL after IT). IT induced a significant reduction in grass pollen cutaneous reactivity in the actively treated group ($P < 0.001$). A significant increase in serum-specific IgG₄ antibody response was observed in the IT group (3.1% before IT vs. 10.1% after IT, $P < 0.001$). Nine patients in the IT group developed moderate immediate systemic reactions vs. two patients in the placebo group.

Conclusion Specific immunotherapy with calcium phosphate-adsorbed standardized grass pollen extract was safe and effective for the treatment of patients with seasonal allergic rhinoconjunctivitis.

Keywords: allergic rhinitis, grass pollen, specific immunotherapy, skin prick test, nasal provocation test, specific IgE, specific IgG₄.

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Introduction

Specific immunotherapy (IT) of human allergic disorders has been used for several decades and involves injections of gradually increasing doses of allergen extracts in sensitized patients. IT has been widely used in pollen allergy rhinitis and its clinical efficacy has been demonstrated in several controlled trials [1–4]. Various approaches have been undertaken to improve IT, by either modifying the allergen, the route of administration or the dosages and administration schedules. Modified allergen extracts have been developed in an attempt to make immunotherapy more effective and to reduce side-effects. A widely used physical modification consists of adsorption onto aluminium hydroxide suspension in order to confer the benefits of a depot preparation with a low incidence of side-effects, while providing sufficient immunogenicity to elicit a significant clinical effect. Although less widely used than aluminium hydroxide, calcium phosphate was initially used as an adjuvant with diphtheria-tetanus-pertussis (DTP) vaccines for many years and was found to be safe and effective [5]. Calcium phosphate has therefore been used to induce physical modification of allergens [6]. Unlike aluminium adjuvants, calcium phosphate does not induce local reactions such as subcutaneous nodules [7] or IgE production [8]. Immunotherapy with calcium phosphate-adsorbed allergens is largely prescribed in France and in some European countries, but its efficacy has only been reported in an open study [9] and has not been confirmed by a controlled study. A previous study showed that calcium phosphate-adsorbed allergens induced low systemic side-effects [10]. In a retrospective survey, calcium phosphate-adsorbed allergens were shown to be well tolerated in comparison with an aqueous allergen extract

[11]. The present double-blind placebo-controlled study was therefore designed to assess the efficacy and safety of immunotherapy with a calcium phosphate-adsorbed five-grass-pollen extract in patients suffering from seasonal rhinoconjunctivitis.

Methods

Patients

Twenty-nine patients (age range 18–44 years) allergic to grass pollen were enrolled in the study. Patients reported typical symptoms of rhinoconjunctivitis during the grass pollen season from May to July, confirmed by a positive skin prick test (SPT) to five grass pollen extracts (wheal > 5 mm), serum grass pollen specific IgE antibody levels > class 2, as determined by RAST, and positive grass pollen nasal provocation test (NPT). Four patients had previously documented seasonal grass pollen asthma. Patients with perennial rhinitis or severe seasonal asthma, patients receiving systemic corticosteroids and patients treated by specific immunotherapy during the past 5 years were excluded. Other exclusion criteria were the known contraindications of immunotherapy according to the European Academy of Allergology and Clinical Immunology guidelines [12]. Two patients were withdrawn from the study before the pollen season (one patient in the IT group because of repeated large local reactions and one in the placebo group because of poor compliance), so that 15 patients in the IT group and 12 patients in the placebo group completed the study. The patients' clinical data on inclusion are shown in Table 1.

Table 1. Patients' clinical data

	Treatment	
	IT	Placebo
Number of patients	16	13
Gender	9	6
Female		
Male	7	7
Mean age (range) (yr)	29 (18–44)	31 (20–42)
Mean duration of rhinitis (range) (yr)	8 (2–16)	11 (2–28)
Skin prick test (mean wheal mm) (SD)	9.69 (1.8)	9.0 (3.7)
Specific IgE (kIU/L)		
Mean (range)	38.7 (2.4–100)	35.5 (0.8–99.1)

The study was approved by the Ethics Committee of the Necker-Enfants Malades Hospital, Paris, France.

Allergen extracts

A purified standardized allergen extract, composed of equal parts of five grass pollens (orchard, meadow, rye, sweet vernal and timothy), was manufactured by Stallergènes SA (Antony, France). The same batch was used throughout the study for IT, NPT and SPT. The allergen extracts were standardized with reference to an internal standard by RAST inhibition, isoelectrofocusing and immunoblotting for determination of allergenic potency. The biological activity of the extracts was determined by SPT and the potency was expressed as the arbitrary index of reactivity (IR). A potency of 100 IR was designated as the strength of grass pollen extract that elicited a geometric mean wheal diameter of 7 mm on skin prick test in 30 subjects sensitive to the corresponding allergen. The batch-to-batch consistency of the allergen extracts was accepted within a limit of variation less than 50% of the potency. Quantification of the major allergen showed that the extract used for this study contained 7 µg of *Phl p 5* per 100 IR (determined by O. Cromwell at Allergopharma, Reinberg, Germany). For SPT, the grass pollen extract was dissolved in 50% glycerol. A depot extract adsorbed onto calcium phosphate (Phostal, Stallergènes SA) was used for IT. Placebo consisted of diluent including calcium phosphate without adding histamine. Active IT treatment and placebo had an identical appearance. All extracts were stored at 4 °C until use and the stability assigned by the manufacturer was much longer than the total duration of the study.

Immunotherapy protocol

Patients received 16 injections at weekly intervals during the induction phase, with increasing concentrations of IT treatment or placebo equivalent. The dose schedules are shown in Table 2. The maintenance dose was given once a fortnight before the beginning of the grass pollen season, then once a month during the pollen season (with a 50% reduction of the volume of solution injected). Dose modifications were allowed for medical indications according to the routine specific immunotherapy procedure.

Study design

This was a double-blind, placebo-controlled, parallel-group study. Patients were enrolled in the trial between September 1997 and October 1997 and randomly assigned to either IT or placebo with a block size of four (two SIT and two placebo preparations). The study ended in September 1998. SPT and NPT were performed before

Table 2. Induction phase immunotherapy schedule (weekly injections)

Injection No.	Allergen unit IR/mL	Volume (ml)	Dose (IR)	Dose (µg <i>Phl p 5</i>)*
1	0.1	0.1	0.01	0.0007
2	0.1	0.2	0.02	0.0014
3	0.1	0.4	0.04	0.0028
4	0.1	0.8	0.08	0.0056
5	1	0.1	0.1	0.007
6	1	0.2	0.2	0.014
7	1	0.4	0.4	0.028
8	1	0.8	0.8	0.056
9	10	0.1	1	0.07
10	10	0.2	2	0.14
11	10	0.4	4	0.28
12	10	0.6	6	0.42
13	50	0.1	5	0.35
14	50	0.2	10	0.7
15	50	0.4	20	1.4
16	50	0.6	30†	2.1

*100 IR/mL = 7 µg *Phl p 5*. †Maintenance dose.

treatment (September 1997) and after the pollen season, at the end of the trial (September 1998). Blood samples were obtained for antibody analysis before treatment, during the pollen season (June 1998) and after the pollen season.

Symptom and medication scores

All patients recorded their daily symptom scores and drug requirements from mid-April to mid-July. The severity of individual symptoms was assessed on a scale of 0–3 for the nose (sneezing, blocking, running and itching), eyes (redness, itching and tearing) and chest (coughing, wheezing and breathlessness). Associated adverse events and all medical events were also recorded. Patients were allowed to regularly use anti-allergic drugs in the form of antihistamines (levocabastine eye drops and cetirizine 10 mg tablets) and inhaled β-agonist (salbutamol) to control symptoms. In the case of rhinitis symptoms not controlled by H1 histamine receptor antagonist, the investigator was allowed to prescribe a maximum of two short courses (less than 5 days) of oral betamethasone (0.5 mg) during the pollen season. In the case of severe asthma, the patient was allowed to use inhaled beclomethasone (250 µg). Long-acting antihistamines and nasal corticosteroids were not allowed. Drugs were scored as follows: each eye drop, each puff of salbutamol or beclomethasone scored 1, each cetirizine tablet scored 2, and each betamethasone tablet scored 3. Cetirizine was scored as 2 as a first-line rescue treatment, while salbutamol

was expected to be used less frequently, as this study addressed rhinitis and not asthma. The mean weekly symptom and medication scores were calculated for each individual patient.

Nasal provocation test (NPT)

The patients' nasal sensitivity was analysed by NPT responses. None of the patients was receiving treatment likely to affect the performance of the nasal provocation test. None of the patients had suffered from allergic or infectious rhinitis during the 2 weeks preceding the test. Freeze-dried allergen extracts were dissolved in normal saline immediately before use and transferred to plastic hand-held nebulizers. Saline solution was initially insufflated and any symptoms were recorded after 15 min. One dose (0.1 mL) of allergen solution was instilled into each nostril (close to the inferior nasal turbinate) during apnoea or expiration. A nose-clip was applied after each insufflation and kept in place for 10 min, allowing the patient to breathe through the mouth to prevent allergen inhalation during nasal inspiration. Increasing doses (from 0.1 IR/mL to 100 IR/mL in 10-fold increments) were then insufflated into the nostrils every 15 min until a total symptom score of 6 was obtained. The scoring system, previously described by Bousquet et al. [13], was as follows: more than five consecutive sneezes = 3; rhinorrhea = 1–3; nasal blocking = 1–3; itching of the nose = 1.

Skin prick tests (SPT)

The patients' skin sensitivity was analysed by quantitative

SPT with five grass pollen extracts. SPTs were performed in duplicate on both forearms with five threefold dilutions from 2 to 166 IR/mL. A 9% codeine phosphate solution was used as the positive control and glycerinated saline solution as the negative control. Allergen solutions were coded and used blindly by the investigators. The skin prick test reactions were evaluated by measuring the wheal diameter according to the following formula: $d = d1 + d2/2$. Dose–response curves were constructed according to a linear/log model and mean wheal diameter results were analysed by means of a parallel-line bioassay [14].

Determination of IgE and IgG₄ levels

Serum samples were analysed for specific IgE using the EIA CAP System (Pharmacia Diagnostics, Uppsala, Sweden), and specific IgG₄ were measured by a radio-immunoassay (Pharmacia Diagnostics) according to the manufacturer's instructions. Specific IgE levels are expressed as kU/L and specific IgG₄ levels are expressed as a percentage of a reference standard serum.

Pollen counts

Daily pollen counts between March and July 1998 in the area of Paris and Rouen (located 120 km to the west of Paris) were obtained from the French National Network for Aerobiological Observation (RNSA).

Statistics

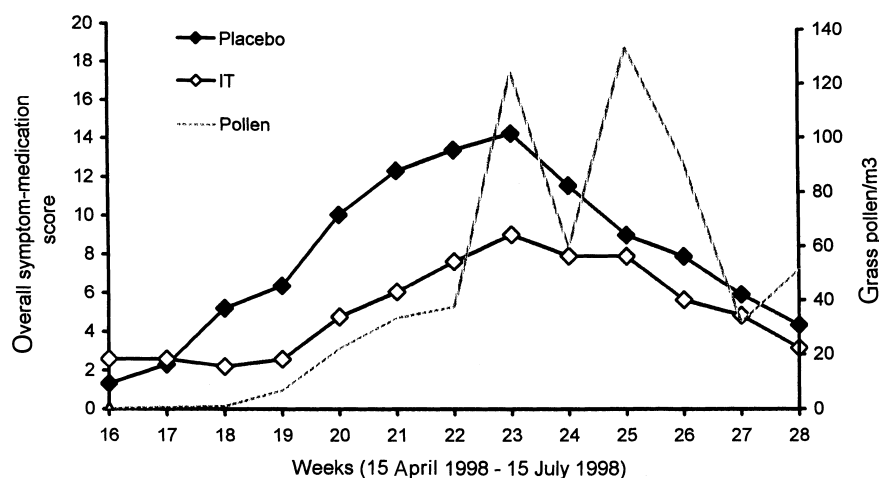
The baseline characteristics of the IT and placebo groups

Table 3. Symptoms and drug scores (mean values) for IT ($n = 15$) and placebo ($n = 12$) groups during the pollen season

	Mean scores* (AUC)				
	IT	Placebo	Mean difference	95% confidence interval†	P value
<i>Symptoms</i>					
Overall total (rhino-conjunct.)	49.5	56.0	6.4	− 18.6–31.5	NS
Nose (total)	33.5	38.6	5.1	− 12.7–23.1	NS
Eyes (total)	16.0	17.3	1.2	− 8.6–11.2	NS
<i>Drugs</i>					
Overall total	11.1	40.8	29.6	6.5–52.7	0.005
Cetirizine tab.	2.9	6.1	3.2	0.7–5.6	0.007
Levocabastine eye drops	3	6.9	3.9	− 2.0–9.8	0.2
Betamethasone tab.	0.05	2.5	2.4	− 0.2–5.1	0.004

*For each patient, the total symptom score during the pollen season (15 April to 15 July) was calculated for the active and placebo groups using the AUC method and compared using the Mann–Whitney *U*-test. †For differences between means (placebo minus IT).

Fig. 1. Mean overall cumulative symptom-medication scores and mean grass pollen counts (pollen grains per cubic meter of air) during the grass pollen season (weeks 16–28). The differences between the two groups were significant at weeks 19–24 ($P < 0.05$) as analysed each week by Mann-Whitney *U*-test.



were compared using the Mann-Whitney *U*-test for continuous variables and χ^2 test for categorical variables. Data were statistically analysed by minimum, maximum and mean values, 95% confidence interval of the mean, arithmetic and geometric means, standard deviation and standard error of the mean. Results for placebo and active treatment groups were compared using a non-parametric test (Mann-Whitney *U*-test). In some cases a non-parametric Wilcoxon signed ranks test for paired series was used for intragroup analysis.

Results

Patients

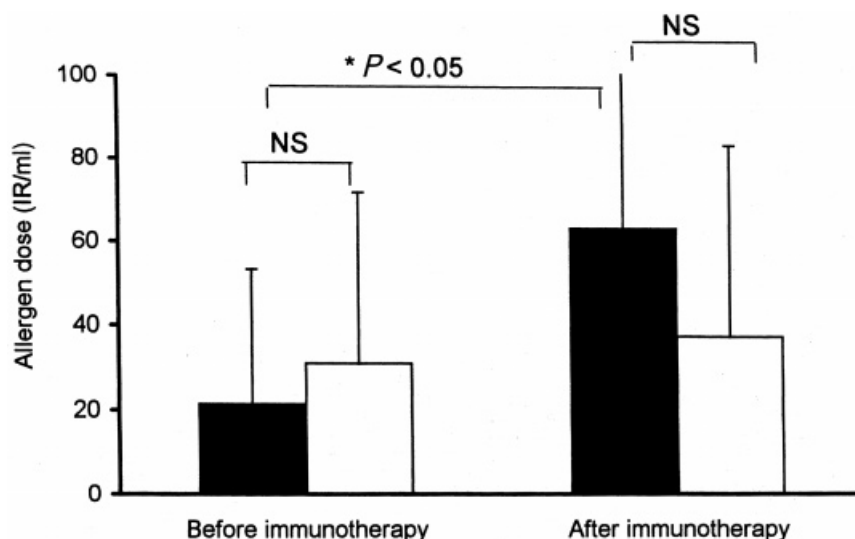
Patients in the IT group received a mean cumulative dose of 220.4 ± 90 IR of five grass pollens (range: 46.6–304.6

IR) corresponding to a mean dose of $15.4 \mu\text{g}$ of *Phl* p5 major allergen. Five patients (33%) in the IT group did not reach the maximum maintenance dose of 30 IR due to local and/or systemic reactions and received a maximum maintenance dose between 10 IR and 20 IR.

Symptom and medication scores

The effects of IT and placebo on symptom and medication scores with 95% confidence intervals for mean differences for all comparisons are shown in Table 3. The overall symptoms score (mean AUC) did not differ significantly between the IT group and the placebo group during grass pollen exposure (49.6 vs. 56, respectively). Mean nasal and conjunctival symptoms were not significantly different between the two groups when tested as single endpoints. The overall medication score (AUC) was significantly

Fig. 2. Results of nasal provocation tests with grass pollen extract. The positive nasal provocation doses were not statistically different between the IT group (■) and the placebo group (□) (Mann-Whitney *U*-test), but the difference was significant before and after IT in the IT group (Wilcoxon *W*-test).



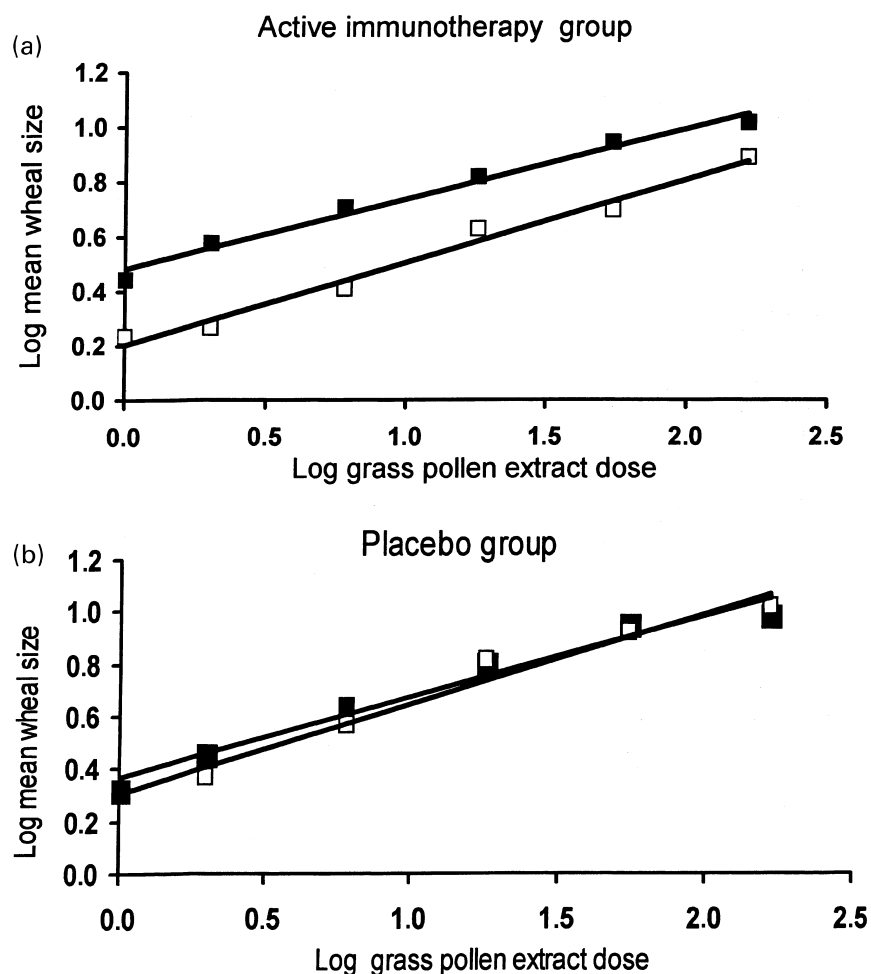


Fig. 3. Changes in skin tests before (■) and after (□) IT in the IT group (a) and in the placebo group (b). Statistical analysis by parallel-line bioassay.

lower ($P = 0.005$, Mann–Whitney) in the IT group than in the placebo group. Drug use was lower in the IT group for cetirizine ($P = 0.007$), levocabastine eye drops ($P > 0.05$) and oral betamethasone ($P = 0.004$). The mean overall symptom–medication score for the entire pollen season was significantly lower in the IT group than in the placebo group (64.5 vs. 102.3 (1 to 74.5), $P = 0.03$, Mann–Whitney). Between weeks 18 and 24 of the pollen season, the symptom–medication scores were lower with IT than with placebo (Fig. 1). In both groups, the symptom–medication scores peaked at week 23, which coincided with the first peak pollen count.

Interestingly, the second peak pollen count, which occurred at week 25, was not associated with a rebound of the symptom–medication score.

Nasal provocation test responses

Changes in nasal allergen sensitivity are shown in Fig. 2. No difference in nasal threshold grass pollen dose was

observed between the active and placebo groups at the pre-treatment visit before the pollen season (21.4 IR vs. 31 IR, respectively). A threefold higher concentration of allergen extract (63.4 IR) was necessary in the IT group to elicit a positive response on NPT after IT ($P < 0.05$, Wilcoxon), but no such change was observed in the placebo group (31 IR before IT vs. 37.7 after IT, $P > 0.05$, Wilcoxon). After IT, the threshold dose in the IT group was higher than in the placebo group (63.4 IR vs. 37.7 IR, respectively), but these differences were not statistically significant ($P > 0.05$, Mann–Whitney).

Skin prick test responses

Quantitative skin prick test results are shown in Fig. 3. A significant difference ($P < 0.001$, Wilcoxon) was observed for each allergen concentration tested between the skin tests performed before and after IT in patients treated by grass pollens. In contrast, no significant change in skin test responses was observed in the

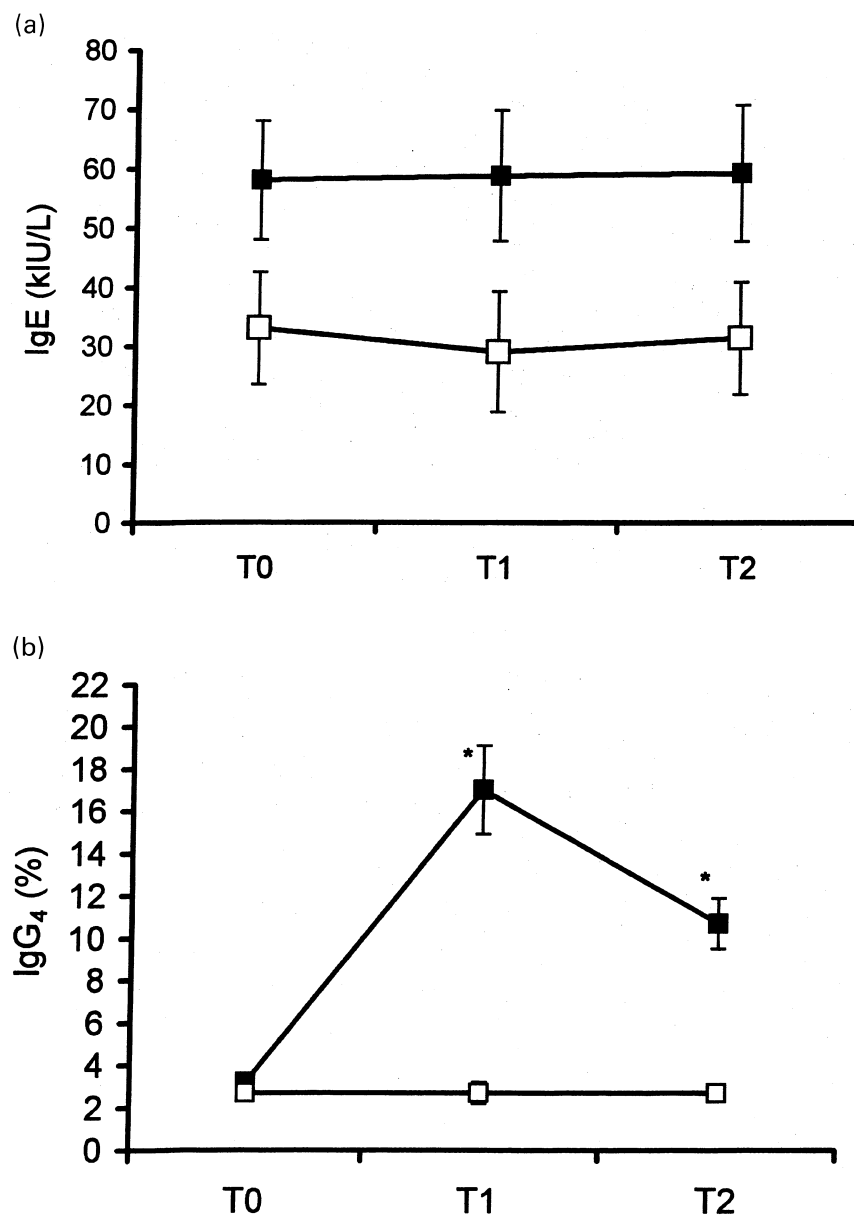


Fig. 4. Means $\pm$ SEM of serum specific IgE (a) and specific IgG₄ (b) levels (SIT, $n = 15$ (■); placebo, $n = 12$, □) before treatment (T0), during the pollen season (T1), and after the pollen season (T2). Values for IT and placebo were adjusted to T0 levels. * $P < 0.001$.

placebo group. For the 100 IR/mL concentration, the mean wheal diameter decreased from 9.6 mm before IT to 6.7 mm after IT in the IT group vs. a slight increase from 9 mm to 9.3 mm in the placebo group. Comparison of the two groups showed a highly significant increase in allergic skin threshold for the IT group (95% CI for the difference between the means, $P = 0.001$, Mann–Whitney).

Serum IgE and IgG₄ levels

Before treatment, mean serum-specific IgE levels were

higher in the IT group than in the placebo group, but this difference was not statistically significant. During and after the pollen season, mean serum-specific IgE levels remained unchanged in both the IT group and the placebo group (Fig. 4a).

Mean serum-specific IgG₄ levels (Fig. 4b) in the IT group increased more than fivefold (from 3.2% to 17%, $P < 0.001$, Wilcoxon) during the pollen season, then decreased slightly after the season (from 17% to 10.7%). In the placebo group, specific IgG₄ levels remained unchanged during and after the pollen season (2.7% at T0, then 2.7% at T1 and 2.7% at T2).

Safety

Potentially allergic reactions were carefully recorded after every injection in order to assess safety. Local reactions at the injection site, with swelling and erythema greater than 5 cm in diameter, were observed in six of the 16 patients in the IT group, but not in any of the patients in the placebo group. Systemic reactions, such as mild exacerbations of rhinoconjunctivitis and urticaria, were documented for seven patients in the IT group and two patients in the placebo group. Mild reversible acute asthma was reported in two patients in the IT group. All systemic reactions occurred during the 30 min after injection. No delayed reactions were observed. No severe systemic reactions were observed in either group.

Discussion

This study demonstrates that immunotherapy with a standardized calcium phosphate-adsorbed five-grass-pollen extract is effective and safe. To our knowledge, this is the first double-blind placebo-controlled study using a calcium phosphate-adsorbed allergen extract. Symptom-medication scores in the active treatment group were significantly decreased during pollen exposure compared with the placebo group. Moreover, the efficacy of immunotherapy in this study was also confirmed by decreased target organ (nasal) and skin sensitivity. Despite the fact that calcium phosphate allergen extracts are not comparable with other modified allergenic extracts, these results are in line with those of other controlled studies performed with aqueous or aluminium hydroxide-modified grass pollen allergens [15–17]. The observed discrepancy between the non-significant reduction in symptom scores and the significant reduction in the use of symptomatic drugs, when the two groups were compared, highlights the question of the outcome of immunotherapy, taking into account the potential subjective self-assessment of symptoms by the patient.

It should be stressed that the patients included in this study were highly sensitized to grass pollens and faced a significant allergen exposure, and that the clinical improvement would probably have been better after a longer study duration, as described by other authors [18,19]. However, if we use the criteria recently described by Malling [1] to evaluate the efficacy of IT, this study can be considered to demonstrate a moderate efficacy (between 45% and 59% improvement). We have shown that grass pollen immunotherapy resulted in an increased threshold for nasal reactivity. As demonstrated in several other studies, the reduction in nasal reactivity, which was correlated with improvement in clinical symptoms, is now widely accepted to be an objective criterion of the efficacy of immunotherapy [13,20,21]. These changes in target organ sensitivity were correlated with a reduction in the release of

inflammatory mediators and local cellular recruitment [20,22,23]. Skin prick tests have been extensively studied during specific IT, and indicate that IT induces suppression of skin reactivity [4,18,23], which could be related to a decreased number of cutaneous mast cells [24].

Pollen immunotherapy has been reported to blunt the usual rise in specific IgE that occurs during the pollen season [4,20]. However, we did not observe any variations in the level of serum IgE antibodies to orchard grass pollen in the IT group or placebo group, as shown in other studies [3,18]. Our results agree with previous data showing that calcium phosphate-adsorbed allergen extracts do not induce an increase in serum IgE levels as usually observed during IT with aluminium-depot allergen extracts [8]. The marked increase of serum-specific IgG4 antibody level observed in the IT group, a well-known phenomenon [25], indicates that IT with calcium phosphate-adsorbed grass pollen extracts had the same immunomodulatory effects as those induced by administration of aluminium hydroxide-modified allergens [16–18,20].

The potential benefit of IT must be weighed against its known risks [26,27]. The IT schedule used in this study was designed to evaluate the tolerance to a maintenance dose of 30 IR previously used with aqueous extracts only. The adverse effects observed in the IT group showed the same patterns as those previously described in other studies using lower doses of calcium phosphate-adsorbed allergens IT [9,10].

It should be stressed that these local and systemic allergic reactions occurred just as rapidly with calcium phosphate-adsorbed allergens as with aqueous extracts. However no severe anaphylactic reactions were observed.

In conclusion, this first controlled study of IT with calcium phosphate-adsorbed five-grass-pollen extract demonstrated that this extract was effective and well tolerated in the treatment of seasonal allergic rhinoconjunctivitis.

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