

Differences in clinical and immunologic reactivity of patients allergic to grass pollens and to multiple-pollen species

II. Efficacy of a double-blind, placebo-controlled, specific immunotherapy with standardized extracts

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The IgE response of patients only allergic to grass pollens differs from response of patients allergic to multiple-pollen species. The IgE immunoblots to orchard-grass pollens confirmed that polysensitized patients had more proteins revealed than patients only allergic to grass pollens. To determine if both groups of patients present a different response toward specific immunotherapy (IT), a double-blind, placebo-controlled study was performed in 70 patients. Patients receiving the active treatment had a rush IT with either a standardized orchard grass-pollen extract or with a standardized mixed-pollen extract prepared, depending on the sensitivity of the patients. The maintenance dose was defined as that dose effective in grass-pollen IT in previous experiments. The same equipotent maintenance dose was administered for all pollen species. Symptom-medication scores during the pollen season and nasal challenge with orchard grass-pollen grains demonstrated that grass pollen-allergic patients had a significantly improved efficacy by comparison to placebo treatment, whereas polysensitized patients had a nonsignificant improvement. Serum grass-pollen IgG was significantly increased after IT in both treated groups. This study demonstrate that the response toward specific IT differs in patients only allergic to grass pollens by comparison to polysensitized patients. (J ALLERGY CLIN IMMUNOL 1991;88:43-53.)

Key words: Immunotherapy, immunoblot, grass pollen, nasal challenge, skin tests, symptom scores

The IgE immune response to environmental allergens depends on genetic and environmental factors¹ and is heterogeneous.²⁻⁴ In the northern Mediterranean area, grass pollens are the major pollen allergen and sensitize up to 85% of pollen-allergic individuals, one third of these being only allergic to grass pollens.⁵ Patients only allergic to grass pollens differ immunologically from patients allergic to many pollen species, and it was suggested that specific IT is less ef-

Abbreviations used

BU:	Biological unit
IEF:	Isoelectric focusing
IT:	Immunotherapy
SPT:	Skin prick test
PGD ₁₂ :	Prostaglandin D ₁₂

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fective in patients sensitized to multiple-pollen species than in patients allergic only to grass pollens.⁶

Specific IT with grass-pollen extracts was found effective in double-blind, placebo-controlled studies, reducing symptoms of rhinitis, conjunctivitis, and asthma,⁷⁻¹⁹ as well as decreasing the nasal, bronchial, and/or ocular reactivity to allergens when the appropriate extract and IT schedules were used.^{8, 10, 13, 14, 17-19} However, the maintenance dose required to provide efficacy is still a matter of debate.

Low-dose regimens were consistently demonstrated to be ineffective in pollen allergy,²⁰ whereas the maximal tolerated dose²¹ often leads to systemic side reactions. The concept of an "optimal dose" was proposed, which consists of an allergen dose administered that decreased symptoms but induced a low rate of systemic reactions.²²⁻²⁴ This optimal dose has been defined only for patients allergic to grass²² or ragweed pollens,^{23, 24} and it is not known whether this dose is effective in patients allergic to multiple-pollen species.

A double-blind, placebo-controlled rush IT with standardized allergen extracts was undertaken in 70 patients allergic only to grass pollens or allergic to multiple-pollen species to compare (1) their IgE-mediated immune response by serum total and specific IgE as well as IgE immunoblots to orchard-grass pollen and (2) the responsiveness of both actively treated groups to optimal dose IT. The 36 patients allergic only to grass pollens received injections with an orchard grass-pollen extract or placebo, and the 34 patients allergic to multiple-pollen species received injections with the same orchard grass-pollen extract as well as with relevant allergens to which they were sensitized or placebo. The maintenance dose of each allergen injected was determined according to the optimal dose previously defined for grass pollens.^{9, 10, 12} The effectiveness of IT was determined by symptom-medication scores during the pollen season and objective parameters (nasal challenges, SPTs, and serum orchard grass-pollen IgG).

MATERIAL AND METHODS

Patients

Seventy patients (31 men, aged 14 to 44 years; mean, 25.4 ± 10.4 years) volunteered to enter in the study after informed consent. They were selected on the following criteria: (1) All patients had symptoms of rhinitis between April and July, and more than half also had symptoms of asthma and/or conjunctivitis. The duration of symptoms ranged from 2 to 30 years. (2) All patients had a positive prick test to a 1/100 (wt/vol) standardized orchard grass-pollen extract (Stallergènes Laboratories, Fresnes, France) and the presence of orchard grass pollen-specific IgE (Phadebas RAST, Pharmacia Diagnostics AB, Uppsala, Sweden). (3) None of the patients had a perennial allergic symptom, although a few patients presented positive skin tests to house dust mites, molds, and cat and dog dander. (4) None of the patients had received any form of specific IT to pollen extracts.

Characterization of allergenic sensitivities of the patients

Allergic sensitivities to pollen species were studied in all patients according to the pollen counts of the Montpellier area.⁵

Extracts. Standardized extracts from orchard grass (*Dactylis glomerata*), olive (*Olea europaea*), plane tree (*Platanus occidentalis*), mugwort (*Artemisia vulgaris*), and *Parietaria officinalis* pollens were prepared according to the proposals of the Allergen Subcommittee of the International Unions of Immunological Societies by the Laboratoires des Stallergènes. A complete description of the preparation of the extracts was previously described.²⁵ A control of the potency of an aliquot was done by RAST inhibition, IEF, and crossed immunoelectrophoresis and compared with the internal standards that were controlled by the same in vitro assays and skin test titration. According to the results of the control procedure, a given amount of the extracting solution was then poured into vials and subsequently lyophilized. A control of the final product by RAST inhibition and IEF was finally performed. Extracts were labeled in BUs according to the proposals of the Nordic countries guidelines on allergen standardization.²⁶ Standardization by means of skin tests makes it possible to label extracts of different allergen species in biologically equipotent units. The freeze-dried extracts were stored at $+4^{\circ}\text{C}$. These extracts used for skin tests were diluted in 50% glycerol, and extracts used for IT in human serum albumin diluent.

Nonstandardized extracts (1/20 wt/vol) were prepared with the same extraction methods for cypress (*Cupressus sempervirens*), juniper (*Juniperus communis*), nettle (*Urtica dioica*), English plantain (*Plantago lanceolata*), pigweed (*Amaranthus pratensis*), goosefoot (*Chenopodium album*), golden rod (*Solidago virgaurea*), beech (*Fagus sylvatica*), alder (*Alnus glutinosa*), walnut (*Juglans regia*), willow (*Salix nigra*), and common oak (*Quercus robur*). Extracts of ash and privet pollens were not tested because of cross-reactivity with olive pollens.²⁷

SPTs. Modified SPTs were performed according to a method previously described in detail²⁸ with a hypodermic needle on the volar surface of the forearm. None of the patients had any factor that might modify the performance of skin tests. All patients were tested by all pollen extracts listed above, and a 9% codeine phosphate solution was used as a positive control. Mean wheal diameters were measured 15 minutes after the performance of the test. Skin tests were considered to be positive when allergen-induced wheals were identical or larger than wheals induced by the codeine phosphate solution. The sensitivity of the patients to orchard-grass pollens was determined by skin test end point with threefold dilutions of the standardized extract, the end point being the threshold concentration of allergen eliciting a wheal size identical to wheal size elicited by the codeine phosphate positive control.

Serum-specific IgE. Serum-specific IgE was titrated with the Phadebas RAST according to the procedure of the package insert. All patients had a RAST to orchard grass. When the level of IgE was >17.5 PRU/ml, the serum was diluted 1/3 in saline. Other pollen specificities were sought according to the results of skin tests.

IgE immunoblots. IgE immunoblots were done after electrophoretic transfer to nitrocellulose of proteins separated by IEF according to a method previously described in detail.²⁹ IEF of the orchard grass-pollen extract was performed with a current limit to 20mA, a voltage of 2000 V, a power

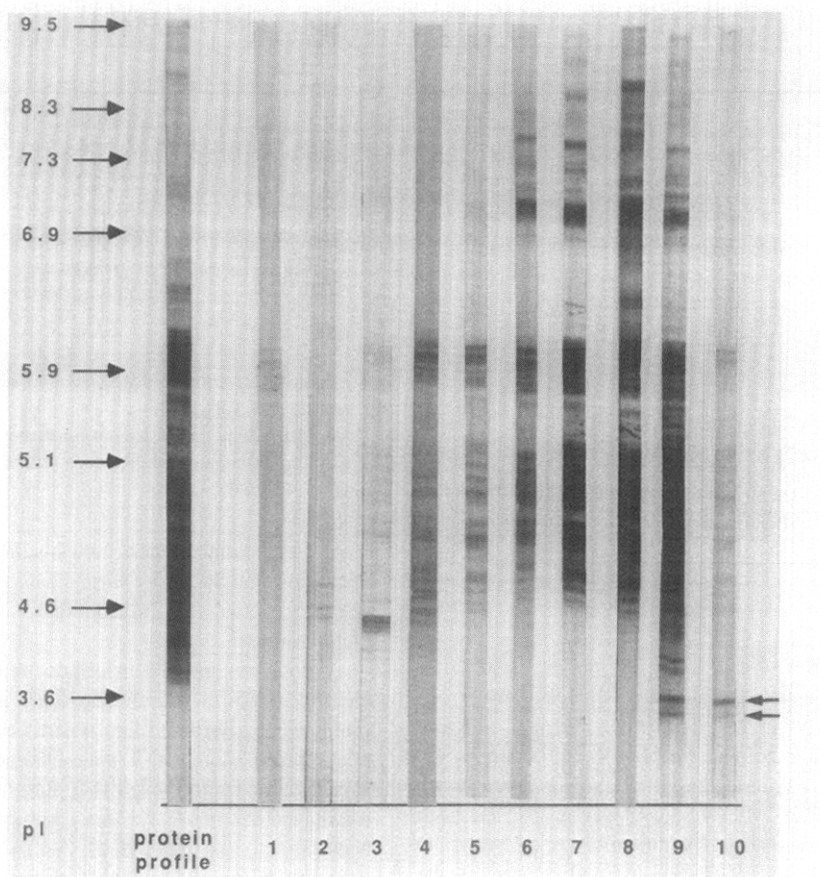


FIG. 1. IgE immunoblotting patterns of orchard grass-pollen allergens.

of 35 W, and the temperature of the coolant set at 0° C for a total duration of 80 minutes. The focused grass-pollen extract was passively transferred according to the method of Peltre et al.³ on nitrocellulose. Free-protein binding sites of the nitrocellulose were blocked by TBS (0.1 mol/L of Tris HCl, 0.1 mol/L of NaCl, and 2.5 mmol/L of MgCl₂, pH 7.4) containing 0.05% of Tween 20 (TBS-Tween) according to Batteiger et al.³⁰ India ink staining was used for detection of proteins on the nitrocellulose strips.³¹ (After the nitrocellulose prints were washed, they were incubated overnight with 4 ml of a 1/40 dilution of the respective sera. After a wash with TBS-Tween, the strips were incubated for 2 hours with 4 ml of 1/1000 alkaline phosphatase-conjugated goat antihuman IgE (Tago Inc., Burlingame, Calif.). The strips were finally developed with 1 ml per strip of a substrate chromogen mixture³² prepared immediately before use by mixing the two following solutions of nitro blue tetrazolium (Serva, Heidelberg, Germany) and 5-bromo-4-chloro-3-indolyl phosphate toluidine salt (Serva) solubilized in 100 µl of N,N-dimethylformamide. The enzyme reaction of the strips was subsequently stopped by a wash in a stop-solution containing 10 mmol/L of Tris HCl in 1 mmol/L of ethylenediaminetetraacetic acid, pH 7.5. According to the number of protein bands of orchard-grass pollen recognized and the intensity of the allergen pattern, immunoblots have been classified from 0 to 10 (Fig. 1).

Sera from nonatopic and nonpollen-sensitive individuals were used as controls. Patients' sera from both groups were mixed in the same gel plate to avoid technical pitfalls. The investigator that performed immunoblots was not aware of the sensitivity of the patients.

Immunotherapy

Extracts used. Only standardized extracts of orchard grass, olive, plane tree, and *Parietaria* pollens were used, although some patients were also sensitive to other pollen species. The clinical importance of these other pollen species is insignificant (other tree pollens tested) or they pollinate in the winter and early spring (cypress and juniper pollens) or in the autumn (mugwort) (Table I). These extracts were prepared as indicated above. A histamine placebo was prepared as previously indicated.¹¹

IT protocol. A double-blind, placebo controlled rush IT was performed according to a protocol already published^{9, 11, 33} and indicated in Table II. The same biologically equipotent maintenance dose of 2000 BU was administered for each pollen species administered. This dose was defined as being "optimal" in the treatment of a group of grass pollen-allergic patients since (1) it decreases significantly the skin test reactivity to the specific allergen after 7 days,^{9, 34} (2) it increases significantly serum orchard-grass, pollen-specific IgE,^{10, 12} and (3) it decreases significantly

TABLE I. Extracts used during IT

	Active treatment	Placebo
Orchard grass	16	17
Orchard grass	4	3
<i>Parietaria</i>		
Orchard grass	3	5
Olive		
Orchard grass	2	4
<i>Parietaria</i>		
Olive		
Orchard grass	4	3
<i>Parietaria</i>		
Plane tree		
Orchard grass	3	2
<i>Parietaria</i>		
Plane tree		
Olive		

symptom-medication scores during the pollen season after a short treatment course.^{9, 10, 12} Extracts of different pollen species were mixed in the same vial. When a systemic reaction or a large local reaction of >10 cm occurred, the dosage increment was reduced, but all patients could reach the maintenance dose. After the maintenance dose had been reached, it was administered five times at weekly intervals. Between April 1 (after investigations) and October 1, the maintenance dose was reduced by one half and administered every 2 weeks.

Investigations

Symptom scores. When the patients attended the clinic in April, they were taught the possible symptoms that could occur and the medication that they should take. Patients completed daily forms during the months of May and June. Nasal and ocular symptoms for 12 hours were recorded on the forms, along with all the possible medications that could be used. The symptoms and medications were scored according to previous studies,^{9, 10-12} and the average symptom and medication scores were calculated for each patient.

Nasal provocation tests: Performance of the provocative challenge. Nasal provocation tests were performed by the same investigator as previously published³⁵ according to a technique modified from Naclerio et al.³⁶ Patients were tested within 3 weeks at the end of IT (early March), at a time when no offending pollen is found in the atmosphere. None of the patients was receiving treatment that could affect the performance of nasal challenges. None of the patients had been suffering from any form of allergic or infectious rhinitis for the past 2 weeks before the challenge.

Capsules containing lactose or increasing concentrations of orchard-grass pollens from 50 to 156,250 grains (fivefold increment) prepared by the Laboratoires des Stallergènes were insufflated into nostrils according to a technique modified from Rosenberg et al.³⁷ with a nasal Spinhaler (Fisons Laboratories, Loughborough, England), the patient refrain-

TABLE II. Protocol of IT

Day		Bu
1	9 AM	20
	9:30 AM	100
	10 AM	200
	11 AM	400
	2 PM	600
2	9 AM	900
	11 AM	1500
3	9 AM	2000

The maintenance dose of 2000 BU was administered five times at weekly intervals.

During the pollen season, patients received 1000 BU every 2 weeks. (The dose was identical for each pollen species.)

ing from breathing during the insufflation. For each concentration, the Spinhaler was actuated five times. Each capsule was opened after insufflation to assess that it was completely empty.

Lactose was initially insufflated and eventual symptoms were recorded for 15 minutes. Then, increasing numbers of grains were insufflated into nostrils every 15 minutes until a symptom score of 5 was obtained: five consecutive sneezes, 3; a rhinorrhea, 1 to 3; nasal blockage, 1 to 3; and itching of the nose, 1. This scoring system was defined according to the method of Østerballe,¹⁸ and we subsequently confirmed the validity of symptom scores in a previous study.³⁵ It was observed that the release of PGD₂ in nasal secretions was significantly ($p < 0.005$) associated with a symptom score of 5. The reproducibility of this test was examined, and it was found to be high.^{11, 35} Moreover, we have already observed that the nasal challenge results were correlated to the intensity of symptoms during the pollen season.^{10, 12, 22}

Nasal washes. Before any challenge, a wash with 5 ml of saline in each nostril was performed three times to decrease the level of mediators. Then, 100 µl of oxymethazoline was sprayed in each nostril, according to the technique of Naclerio et al.³⁶; 15 minutes after the insufflation of lactose or each allergen dose, a nasal wash was done. The wash fluid was stored on ice until the conclusion of the experiment. The fluid was then centrifuged at +4° C for 15 minutes at 15,000 g. The sol phase was separated from the gel phase by pipetting and stored at -20° C until it was assayed.

Titration of mediators in nasal secretions. Histamine was titrated by RIA with a monoclonal antibody against acylated histamine (Immunotech, Marseille, France).³⁸ PGD₂ was assayed by enzyme immunoassay according to the method of Maclouf et al.,³⁹ and acetylcholinesterase from electric eel was the enzyme used in the assay.⁴⁰ The techniques used were described in detail in a previous article.³⁵ Mediators are considered to be released when their level is at least twice that of baseline.

Quantitative SPTs. All allergic patients had quantitative SPTs with the standardized orchard grass-pollen extract.

TABLE III. Comparison of the patient groups

	Grass-pollen treated		Multiple-pollen treated	
	Placebo	Active	Placebo	Active
No. of patients	17	16	17	16
Age (yr)	21.6 ± 11.6	20.8 ± 9.7	26.5 ± 12.6	24.3 ± 8.5
SPT end point	6.9 ± 1.6	7.2 ± 0.9	8.1 ± 1.6	7.5 ± 1.3
Specific IgE (PRU/ml)	35.5 ± 12.1	38.1 ± 20.1	22.8 ± 14.6	25.2 ± 11.2
Rhinitis symptoms				
Duration (yr)	10.2 ± 5.3	9.6 ± 6.2	9.2 ± 5.9	8.5 ± 6.1
Severity (score*)	3.5 ± 0.6	3.2 ± 0.9	3.5 ± 0.3	3.1 ± 0.6
Asthma (%)	41.2	31.2	29.4	35.2
Conjunctivitis (%)	58.8	56.25	64.8	50.0

*The severity was scored according to previous studies from 0 to 4.^{8,11}

SPTs were done with six threefold dilutions from 50 to 36,450 BU/ml. Dose-response curves were constructed in a linear/log model for each patient's group with mean results of wheal diameters and data analyzed by means of parallel-line bioassay.^{41, 42} The significance between placebo-treated and active-treated groups was analyzed by Wilcoxon W test for the third allergen dilution.

Serum-specific IgE. Serum-specific IgG was titrated by means according to the method of Puttonen and Maasch.⁴³ Briefly, RAST disks were prepared according to the method of Ceska et al.⁴⁴ with the orchard-pollen extract. IgG coupled on the disk was revealed by *Staphylococcus aureus* protein A radiolabeled with ¹²⁵I. The results are presented in percent binding of total added counts.

Pollen counts. Pollen counts for all pollen tested were obtained according to the method of Cour.⁴⁵

Design of the study

Patients were tested by SPTs to all pollen species listed above, and the results were confirmed by Phadebas RAST. We did not perform RAST to other pollen species when the SPT was negative, since it has been observed that skin tests are more sensitive than IgE determinations. Only patients with concordant results between skin test and RAST for olive-, plane tree-, *Parietaria*-, and/or mugwort-pollen extracts were included in the study. Patients were placed in grass pollen-treated groups (when skin tests and RAST were only positive for orchard grass-pollen extracts) and in multiple pollen-treated groups (when skin tests and RAST were positive for many pollen species) and randomly assigned to one of the four treatment groups: placebo-grass pollens (group 1), placebo-multiple pollens (group 2), grass-pollen extract (group 3), and multiple-pollen extract (group 4). A different investigator performed the IT protocol and the assessment of the patients.

Before IT and before the pollen season (between January 1 and 31), patients had a quantitative skin test and the titration of serum grass-pollen IgG. Moreover, between March 1 and 21, they had a nasal challenge, a quantitative skin test, and the titration of serum grass-pollen IgG. During the season, all patients had follow-up by a symptom-

medication score they recorded every day from May 15 to July 1.

Statistical analysis

Statistical analysis was performed with nonparametric tests. The Spearman's rank test was used for regression analysis, the Wilcoxon W test for paired data, the Mann-Whitney U test for unpaired comparisons, and contingency table analysis for other data.

RESULTS

Homogeneity between groups

Among the 70 patients included, 66 completed the whole study. The characteristics of patients placed in the placebo-treated groups and patients who received allergens are presented in Table III. All parameters studied were comparable in the four treated groups.

Allergenic sensitivities of the patients

The allergenic sensitivities of the patients for grass, olive, plane tree, and *Parietaria* pollens are presented in Table I.

The immunoblot analysis of orchard-grass pollens demonstrates that patients only allergic to grass pollens have a pattern ranging from 1 to 5 ($m \pm SD$, 2.03 ± 1.38) (Fig. 2). In contrast, patients allergic to multiple-pollen species have a broader range of immunoblot patterns ranging from 1 to 9 ($m \pm SD$, 5.64 ± 2.53 ; $p < 0.0001$, Mann-Whitney U test). Moreover, there are some allergenic protein bands in immunoblotting that are revealed only in patients allergic to multiple-pollen species (Fig. 1, arrows, immunoblot pattern 10).

Serum orchard grass-specific IgE was significantly ($p < 0.05$, Mann-Whitney U test) higher in patients allergic to multiple pollens (median $\pm SD$, 34.5 ± 19.3 PRU/ml) than in patients only allergic to grass pollens (median $\pm SD$, 22.3 ± 14.1 PRU/ml).

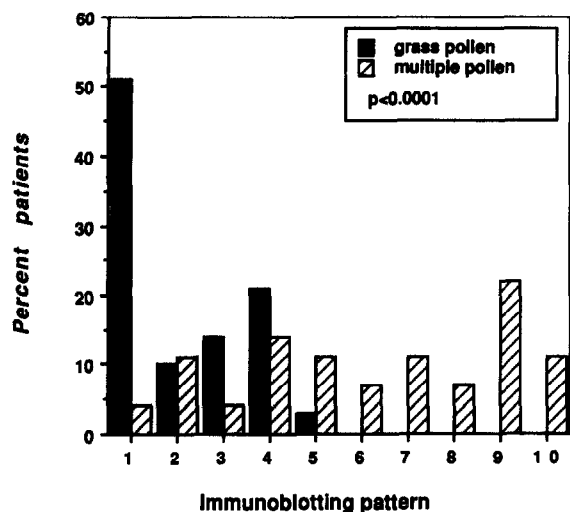


FIG. 2. Distribution of orchard grass-pollen IgE immunoblotting patterns in patients allergic only to grass pollens and in patients allergic to multiple-pollen species. Statistical analysis by Mann-Whitney U test.

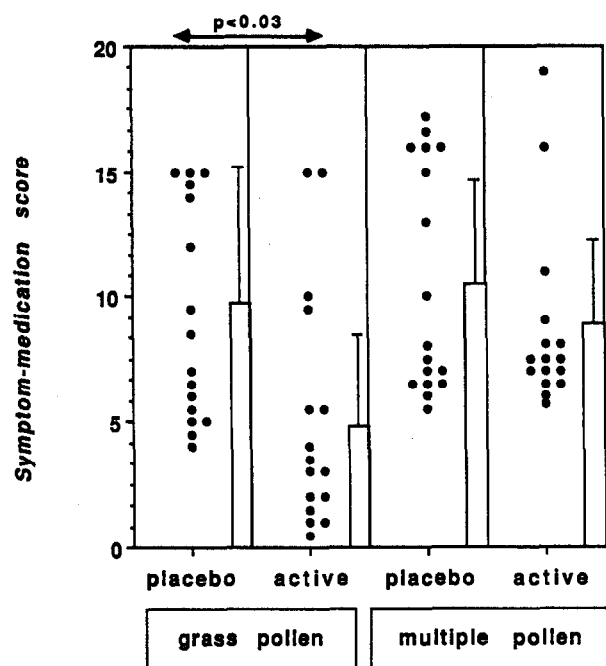


FIG. 3. Symptom and medication scores were analyzed for rhinitis and conjunctivitis.

Protocol of IT and side reactions

All patients could reach the maintenance dose, but because of some local and/or systemic reactions, some patients reached the maintenance dose after a longer course of IT. Three patients in the grass pollen-treated group and four in the multiple pollen-treated group experienced a systemic reaction. None of the

SYMPTOM SCORES NASAL CHALLENGE

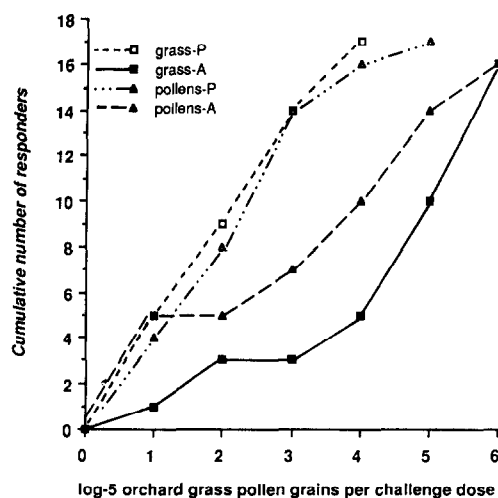


FIG. 4. Results of nasal challenge with orchard grass-pollen grains in the four treated groups of patients. *P*, placebo; *A*, active treatment; *grass*, patients allergic to grass pollens; *pollens*, patients allergic to multiple-pollen species. $p_{\text{active/placebo}} < 0.01$ in the grass pollen-treated group (Mann-Whitney U test).

patients treated by placebo had a systemic reaction. Before their investigation, patients placed in the grass pollen-treated group received a mean cumulative dose of 15897 ± 312 BU of orchard-grass pollens, and patients who were in the multiple pollen-treated group received a mean cumulative dose of 16371 ± 940 BU of each pollen species.

Symptom-medication scores

Symptom and medication scores were analyzed for rhinitis and conjunctivitis (Fig. 3). It was found that patients in both placebo-treated groups had a similar mean symptom-medication score and that those patients placed in both treated groups had a lower mean symptom-medication score. However, only patients placed in the grass pollen-treated groups had a significant clinical improvement by comparison to placebo treatment ($p < 0.04$, Mann-Whitney U test). Some polysensitized patients placed in the active-treatment group were largely improved. There was no significant correlation between the immunoblotting pattern and the efficacy of IT.

Pollen counts were lower during the pollen season than in many other years, but the peak of the season for each tested species corresponded to the period of survey.

Nasal challenges

None of the subjects tested had significant symptoms with lactose (score always < 2). The results of nasal provocation tests are presented in Figs. 4 and

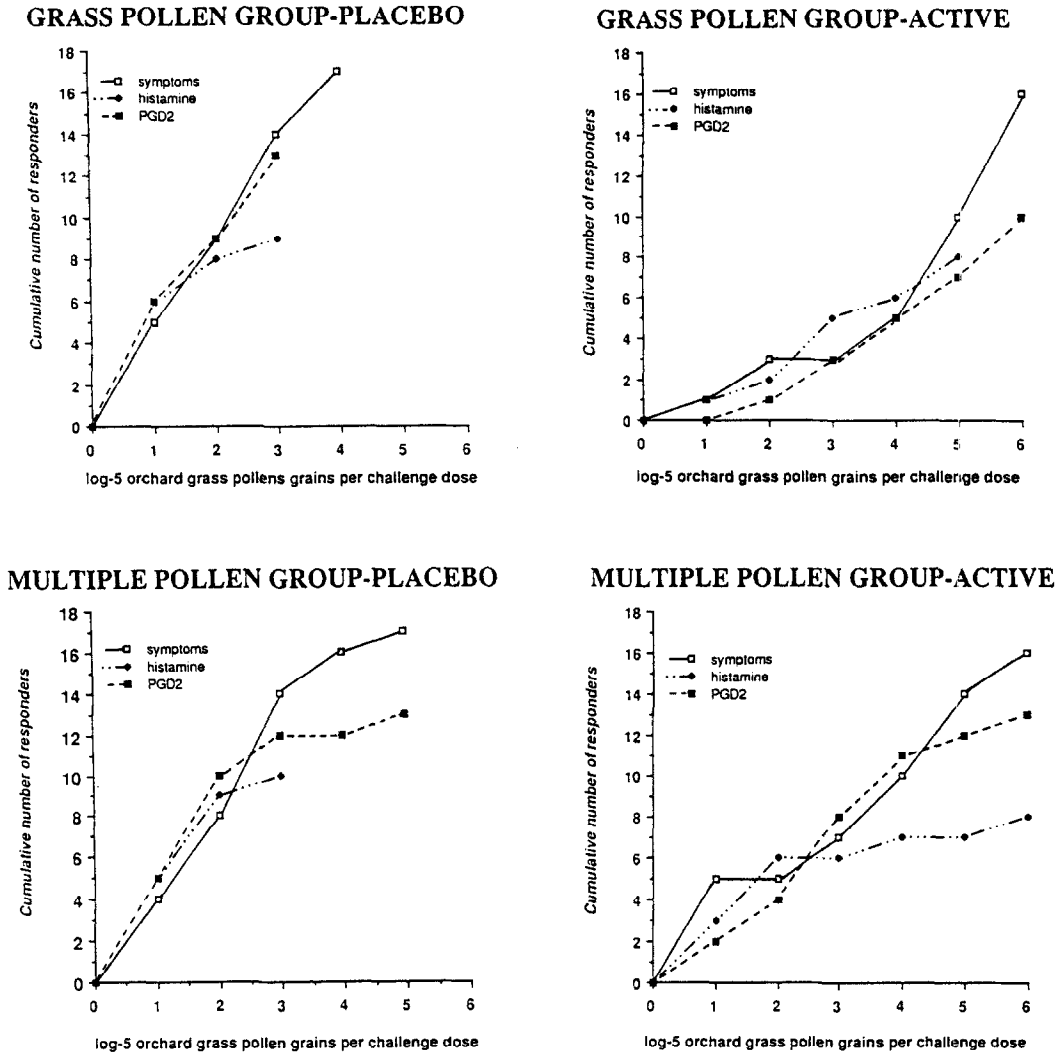


FIG. 5. Symptom scores and release of mediators during nasal challenge with orchard grass-pollen grains.

5. Patients in both placebo-treated groups had a similar nasal response to pollen grains ($m \pm SD$; 1544 ± 558 grains for group 1 and 3086 ± 7510 grains for group 2). Patients treated by allergen had a different response to nasal challenge according to their sensitivity. Patients in the grass pollen-treated group (group 3) reacted for a significantly ($p < 0.01$, Mann Whitney U test) greater mean number of grains ($m \pm SD$; 69175 ± 70655 grains) than patients in the corresponding placebo-treated group (44-fold increment). In contrast, patients in the multiple pollen-treated group (group 4) reacted for a nonsignificantly greater mean number of grains ($m \pm SD$; 28687 ± 51437 grains) by comparison to the corresponding placebo treatment (9.3-fold increment). There was no significant difference between active-treated groups.

A significant release of histamine was observed in serum to 12 challenges and a release of PGD₂ in 10

to 12 challenges. There was an overall agreement between the release of mediators and symptoms during challenges (Fig. 5).

There was no significant correlation between the results of nasal challenge and the immunoblots, although many patients with a pattern grade 1 to 3 reacted for a high number of pollen grains and patients with a pattern grade 7 to 9 reacted for a low number.

Quantitative skin tests

Results of quantitative skin tests are presented in Fig. 6. All dose-response curves were linear (linear correlation coefficient), and those between placebo-treated and active-treated groups were parallel (F test). There was only a significant ($p < 0.01$, Wilcoxon W test) difference between skin tests performed before and after IT in patients treated by grass pollens (group 3).

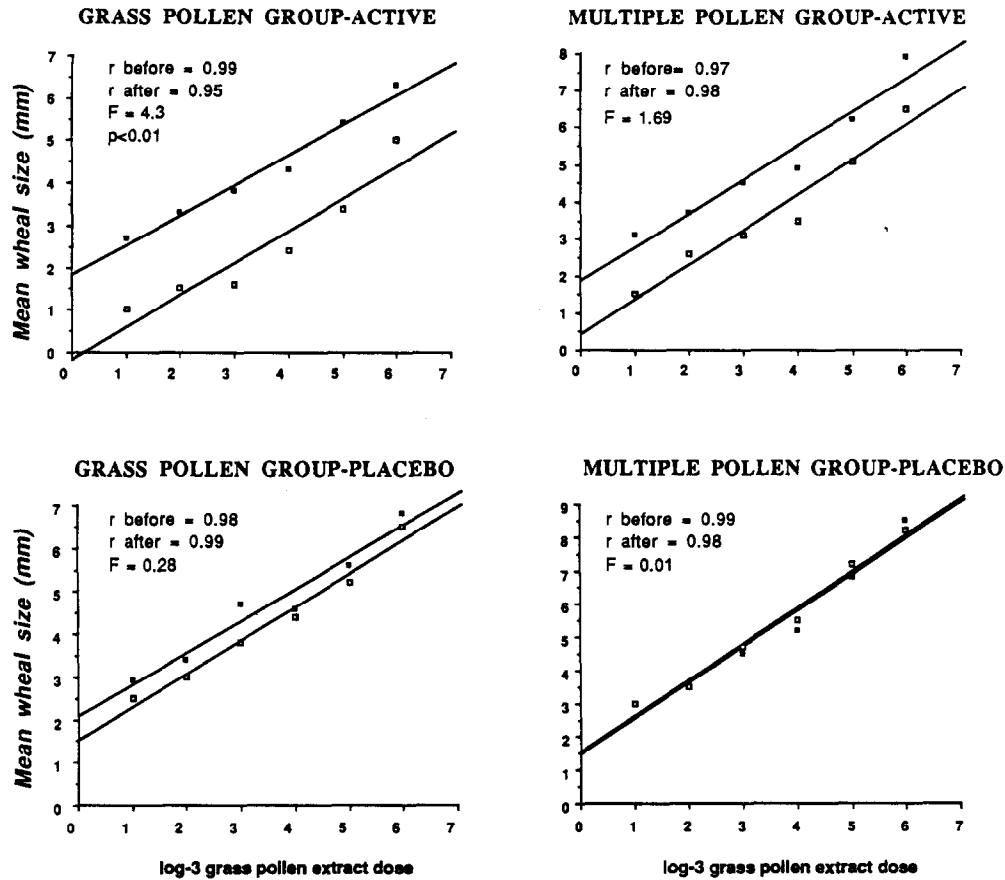


FIG. 6. Evolution of skin tests before (■) and after (□) IT. Statistical analysis by parallel-line bioassay. Dose-response curves are linear, $r > 0.75$, and parallel, $F < 5.05$. Statistical difference for skin tests performed before and after IT by Wilcoxon W test for the results of the third allergen dilution.

TABLE IV. Results of in vitro assays

	Grass-pollen treated		Multiple-pollen treated	
	Active	Placebo	Active	Placebo
IgG (% binding)				
Before IT	6.88 ± 3.17	6.88 ± 3.17	4.78 ± 1.34	5.39 ± 2.38
After IT	12.59 ± 9.46	6.90 ± 2.91	9.15 ± 3.59	5.23 ± 2.17
<i>p</i> Value	<0.01	NS	<0.02	NS

NS, Not significant.
Statistical analysis between first and second values by Wilcoxon W test.

Serum-specific IgG

Mean serum-specific IgG level did not change after IT in the placebo-treated group, whereas it was significantly ($p < 0.02$ and $p < 0.04$, Wilcoxon W test) increased in both active-treated groups (Table IV). There was no significant correlation between nasal symptom-medication scores and IgG or IgE levels.

DISCUSSION

The results presented in this study indicate that patients only allergic to grass pollens are immunologically different from patients allergic to multiple pollen species and confirm that specific IT with an equipotent biologic dose of standardized extract effective in patients allergic to grass pollens is ineffective in patients

allergic to multiple-pollen species receiving a mixture of standardized pollen extracts.

The immunoblotting technique makes it possible to characterize the IgE immune response of patients.^{3, 4, 29, 46} Patients who are allergic to grass pollens only often present a low number of IgE specificities against separated proteins of orchard-grass pollen as well as a mean lower level of serum orchard grass pollen-specific IgE. In contrast, patients with multiple-pollen sensitivities present all possible immunoblotting patterns. This heterogeneity in the IgE-mediated immune response is not related to the severity of allergic rhinitis because nasal challenges and symptom-medication scores during the pollen season are similar in both placebo-treated groups.

This study confirms the value of specific IT with appropriate extracts and dosage schedules in patients only sensitized to grass pollens.^{8-18, 33, 34} Symptom-medication scores were significantly reduced in the active-treated group, and these results were confirmed by nasal challenges, SPTs, and serum-specific IgG. Nasal challenges were done with grass-pollen grains as previously published,^{9, 11, 22, 47} and in this study, we also examined the release of histamine and PGD₂.³⁵ As noticed by Creticos et al.,^{24, 48} it was observed that both symptoms and release of mediators are significantly reduced by specific IT. The lack of efficacy of IT in the polysensitized pollen-treated group might be related to the low level of pollens in the atmosphere during the trial. However, this hypothesis does not appear relevant since nasal challenge and skin tests to orchard grass were not improved in the polysensitized group and since the low level of pollens in the atmosphere did not prevent the grass pollen-treated group from being significantly protected.

Some polysensitized patients presented a clinical benefit, as demonstrated by nasal challenge and symptom-medication scores during the pollen season, but mean results of nasal challenge and symptom-medication scores were not significantly different between patients treated by placebo or standardized allergens. Only mean orchard grass-specific IgG antibody levels were significantly increased in the active-treated group. These findings confirm a preliminary study of our group⁶ and do not differ from two studies of Grammer et al.⁴⁹ who observed that the IgG immune response toward grass, tree, and ragweed allergens is increased in polysensitized patients after IT. We used a similar biologic dose for each pollen extract. This dose was previously demonstrated to be effective in patients allergic only to grass pollens, and the present study confirms these data. It is less effective in polysensitized patients. The lack of efficacy of

specific IT was demonstrated both by nasal challenge and when symptom-medication scores were analyzed during the pollen season, although all relevant pollen species were administered. We used a single grass-pollen extract. Some polysensitized patients might have had IgE antibodies against epitopes absent in orchard grass, but nasal challenges and skin tests, purposely done with orchard-grass pollens, were not significantly different in the placebo-treated and the active-treated groups. Another explanation is related to the immune response of patients. Many polysensitized patients had an increased IgE response toward orchard-grass pollen, as found by immunoblots and serum-specific IgE, and some of these patients may not respond favorably to IT done with the optimal allergen dose required to treat patients only sensitized to grass pollens but may be improved by higher doses. This hypothesis suggests that the optimal dose might be different in both active-treated patients' groups, but it is difficult to increase the allergen doses with standardized extracts because of increased side effects. Another explanation is that allergen mixtures are less stable than single species. This has been demonstrated for cat dander or ragweed pollen mixed in house dust mite extracts.⁵⁰ An enzymatic degradation of pollen extracts has already been observed,⁵¹ but this usually occurs during storage either at temperatures higher than temperatures used in our study or during a longer period of time.

With safer extracts, such as polymerized extracts,^{14, 22, 33, 52} it may be possible to increase allergen doses administered to polysensitized patients and to find that specific IT is effective in such subjects.

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