

Clinical and immunologic reactivity of patients allergic to grass pollens and to multiple pollen species

I. Clinical and immunologic characteristics

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The heterogeneity of pollen-allergic individuals is well-known but poorly characterized. Twenty-six patients were studied to characterize their immunologic and clinical patterns. Thirteen patients were allergic only to grass pollens, and 13 other patients were allergic to grass-pollen and other pollen species, including Cupressaceae, plane tree, olive, and Parietaria. The IgE response was assessed by the titration of serum total IgE and orchard grass-specific IgE, as well as by IgE immunoblots to orchard-grass pollens. Clinical reactivity was assessed by nasal challenge with orchard-grass pollens before the pollen season and nasal and bronchial symptom-medication scores between April 1 and June 15. Pollen counts were obtained during this period of survey. Polysensitized patients had significantly increased levels of serum total and specific IgE and a greater heterogeneity of IgE immunoblots, suggesting an enhanced qualitative and quantitative IgE immune response. Polysensitized patients had nasal and bronchial symptoms occurring earlier than grass pollen-allergic individuals, confirming the priming effect caused by other plants flowering with an earlier season for both nasal and bronchial mucosa. Alternatively, the early symptoms may be attributable to the tree pollens or might reflect the higher grass-pollen IgE level in the polysensitized group. Bronchial symptoms appeared a few weeks after nasal symptoms. Nasal challenges were similar in both groups, and the severity of nasal symptoms during the season was not significantly different in both groups, suggesting that the intensity of symptoms is not related to the sensitization nor to the IgE immune response of the subjects. (J ALLERGY CLIN IMMUNOL 1991;87:737-46.)

The immune response to environmental allergens depends both on genetic and environmental factors.¹ Exposed to a common environment, only certain individuals develop an IgE-mediated immune response that differs from subject to subject. In the northern Mediterranean area, it has been observed that grass

Abbreviations used

BU:	Biological unit
IEF:	Isoelectric focusing
SPT:	Skin prick test
PGD ₂ :	Prostaglandin D ₂

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pollens represent the major pollen allergen and sensitize up to 85% of pollen-allergic individuals, one third of these individuals being allergic only to grass pollens.² However, it is not known if patients allergic only to grass pollens differ immunologically and clinically from patients allergic to many pollen species, although the heterogeneity of the IgE-mediated immune response to grass pollen has been observed.³⁻⁶

In 1968, Connell⁷ reported that the nasal mucosa becomes hyperresponsive to antigen challenge on consecutive days. This increased responsiveness was

called "the priming effect on the end organ" and appeared to be antigen nonspecific.⁸⁻¹² The nature and importance of the priming effect has only been reassessed within the past 10 years.¹⁰⁻¹⁴ It was confirmed that allergen, but not nonspecific irritants,¹⁵ could elicit the priming that was due, at least in part, to the cell influx after allergen challenge of the nasal mucosa^{9, 16-18} and possibly interactions between cytokines, inflammatory mediators, and effector cells of the allergic reaction.¹⁹ The changes in reactivity of the nasal mucosa during the pollen season highlight the priming effect observed after nasal challenge,^{18, 20, 21} and it appears that the tree-pollen season is an important trigger for the priming of the nasal mucosa.

A study was undertaken in 26 patients allergic either to grass (13 patients) or multiple pollen species (13 patients) to (1) characterize their clinical reactivity by nasal challenge and quantitative skin tests before the pollen season and symptom-medication scores during the pollen season, (2) determine if the priming effect of the nasal and bronchial mucosa was present in patients with multiple pollen species, and (3) assess their IgE-mediated immune response by total and specific IgE, as well as IgE immunoblots.

MATERIAL AND METHODS

Patients

Twenty-six patients (15 men, aged 14 to 36 years; mean $\pm$ SD, 22.4 ± 9.3 years) volunteered to enter the study after informed consent and approval of the Ethical Committee of the hospital. Patients 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, Uppsala, Sweden).
3. None of the patients had perennial allergic symptoms, although 18 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 immunotherapy 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.²

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 Stallergènes Laboratoires. A complete description of the preparation of the extracts was previously described.²² Briefly, extracts were prepared from pure pollens harvested either from cultivated (orchard grass, mugwort, and *Parietaria*) or wild plants during a period of 3 years. Undeveloped pollens were extracted by ammonium bicarbonate (0.125 mol/L) at pH 8.0, with continuous stirring at $+4^\circ\text{C}$ for 24 hours. The control of the potency of the extracts was done by RAST inhibition, IEF, and cross immunoelectrophoresis and compared with the internal standards. Extracts were labeled in BUs according to the proposals of the Nordic Countries Guidelines on Allergen Standardization.²³ The freeze-dried extracts were stored at $+4^\circ\text{C}$ and were used for skin tests.

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*), goldenrod (*Solidago virgaurea*), beech (*Fagus sylvatica*), alder (*Alnus glutinosa*), walnut (*Juglans regia*), mulberry (*Broussonetia papyrifera*), willow (*Salix nigra*), and common oak (*Quercus robur*). Extracts of ash and privet pollens were not tested because of cross-reactivity with olive pollens.²⁴

Skin prick tests. Modified SPTs were performed according to a method previously described in detail.²⁵ The modified SPT was performed by placing a small drop of each test extract and control solution on the volar surface of the forearm, and a hypodermic needle was passed through the drop and inserted into the epidermal surface at a low angle with the bevel facing up. The needle tip was then gently lifted upward to elevate a small portion of the epidermis. None of the patients had any factor that might have modified the performance of skin tests, and drugs that might have modified the performance of the tests were carefully avoided. All patients were tested by all the pollen extracts listed above and a 9% codeine phosphate solution 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.

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 one third in saline. Other pollen specificities titrated on undiluted sera included *Parietaria* and olive- and plane-tree pollen.

IgE immunoblots. IgE immunoblots were done after electrophoretic transfer to nitrocellulose of proteins separated by IEF according to a method previously described in detail.²⁶

IEF²⁷ of the orchard grass-pollen extract was performed on a 0.8% agarose thin-layer gel (Pharmacia Fine Chemicals, Uppsala, Sweden) containing 3% servylate 3 to 10-

and 1% servylate 4 to 6 (Serva, Heidelberg, Germany). The GelBond film (LKB, Broma, Sweden) was used as a support for the gel. The IEF procedure was performed with a current limited to 20 mA, a voltage of 2000 V, and a power of 35 W; the temperature of the coolant was set at 0° C for a total duration of 80 minutes.

The focused grass-pollen extract was transferred according to the method of Peltre et al.⁴ on nitrocellulose by passive diffusion. Free-protein binding sites of the nitrocellulose were blocked by TBS (0.1 mol/L of Tris HCl, 0.1 mol/L of NaCl, 2.5 mmol/L of MgCl₂, pH 7.4) containing 0.05% of Tween 20 (TBS-Tween) according to Batteiger et al.²⁸ India ink staining was used for detection of proteins on the nitrocellulose strips,²⁹ and 0.4 cm wide strips were incubated in 1/1000 India ink (Pelikan, Hannover, Germany) solution in TBS-Tween.

The nitrocellulose prints were washed for 5 minutes with distilled water at room temperature in multichannel plates (8-channel reservoir, Flow Laboratories, McLean, Va.) on a horizontal shaker. The prints were then incubated overnight with 4 ml of a 1/40 dilution of the respective sera. After strips were washed with TBS-Tween, they 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 mixture³⁰ prepared immediately before use by mixing the two following solutions: solution A was made by solubilizing 10 mg of nitro blue tetrazolium (Serva) in 30 ml of acid phosphatase, pH 9.5, buffer at 37° C; solution B consisted in 5 mg of 5-bromo-4-chloro-3-indolyl phosphate-toluidine salt (Serva) solubilized in 100 µl of *N,N*-dimethyl-formamide. The enzyme reaction was subsequently stopped by washing the strips in a stop solution containing 10 mmol/L of Tris HCl in 1 mmol/L of ethylenediaminetetraacetic acid at pH 7.5. The immunoblot patterns were classified according to the number of protein bands revealed from 1 to 10.

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

Total serum IgE. Total serum IgE was titrated by the Phadebas PRIST (Pharmacia Diagnostics AB) according to the package insert. When IgE levels were >500 KU/L, the serum was diluted by one third.

Evaluation of the clinical reactivity of the patients

Symptom scores. When the patients attended the clinic before the pollen season, they were taught the possible symptoms that could occur and the medication that they should take. Patients completed daily forms during the months of April, May, and June. The forms report nasal, bronchial, and ocular symptoms for 12 hours along with all the possible medications that could be used. The symptoms and medications were scored according to previous studies on immunotherapy.³¹⁻³³ Patients were considered as having

asthma when they presented at least two of the three following symptoms: shortness of breath, wheezing, or cough. Patients with asthma had a pulmonary function test at the peak of the pollen season to confirm the diagnosis of reversible airway disease.

Nasal provocation tests: Performance of the provocative challenge. Nasal provocation tests were performed as previously published³⁴ according to a technique modified from Naclerio et al.³⁵ All patients were tested between 8 AM and 12 AM to avoid possible circadian variations, by the same investigator, at a time when no pollen is found in the atmosphere, that is, in February. None of the patients were receiving treatment that might have affected the performance of nasal challenges. Capsules containing lactose or increasing concentrations of orchard-grass pollens from 50 to 156,250 grains (fivefold increment), prepared by the Stallergènes Laboratories, were insufflated into nostrils according to a technique modified from Rosenberg et al.³⁶ with a nasal Spinhaler (Fisons Laboratories, Loughborough, U.K.), and the patient refraining 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, score of 3; rhinorrhea, score of 1 to 3; nasal blockage, score of 1 to 3; itching of the nose, score of 1. This scoring system was defined according to the method of Østerballe,³⁷ and we had subsequently confirmed the validity of symptom scores in a previous study.³⁴ 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.^{33, 34} Moreover, we had already observed that the nasal challenge results were correlated to the intensity of symptoms during the pollen season.^{33, 38}

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.³⁵ Fifteen 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. It 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 radioimmunoassay with a monoclonal antibody against acylated histamine (Immunotech, Marseille, France).³⁹ PGD₂ was assayed by enzyme immunoassay (Stallergènes Laboratories) according to the method of Macclouf et al.,⁴⁰ and acetylcholinesterase from electric eel was the enzyme used in the assay.⁴¹ The techniques used and their validity were described in detail in a previous article.³⁴ Mediators are considered to be released when their level is at least twice as great as baseline.

TABLE I. Sensitization of the patients

Major sensitizing pollens	Plantain	Grass	Cupressaceae	Oak	Mulberry	Mugwort
Grass	13				1	
Grass	1	1		1		1
<i>Parietaria</i>						
Grass	1	1			1	
Olive						
Grass						
<i>Parietaria</i>	1	1	1	1		1
Olive						
Grass						
<i>Parietaria</i>	5	1	1		1	
Plane tree						
Grass						
<i>Parietaria</i>						
Plane tree	5	2	1	1	1	2
Olive						

Quantitative skin tests. SPTs were done as described above with six threefold dilutions (50 to 36 450 BU/ml) of a standardized orchard grass-pollen extract whose preparation was given above. Dose-response curves were constructed in a linear/log model for each group of patients with mean results of wheal diameters, and data were analyzed by means of parallel group bioassay.^{42,43} The significance between both groups was analyzed by Wilcoxon W test for the third allergen dilution.

Pollen counts. Pollen counts were obtained according to the method of Cour.⁴⁴ The sampler was placed 2 m above the ground.

Design of the study

Patients were tested by SPTs to all pollen species listed above, and the results were confirmed by Phadebas RAST. Patients were placed in the grass pollen-allergic group (when skin tests and RAST were positive only for grass-pollen extracts) and in the multiple pollen-allergic group (when skin tests and RAST were positive for many pollen species). All patients had the same investigations, including a titration of serum total IgE and orchard grass pollen-specific IgE, orchard grass-pollen IgE immunoblot, and clinical investigations. The nasal challenge with orchard grass-pollen grains and quantitative skin tests were performed before the pollen season, and symptom-medication scores were analyzed between April 1, 1987, and June 30, 1987.

Statistical analysis was performed by means of nonparametric tests.

RESULTS

Allergenic sensitivities of the patients

The allergenic sensitivities of the patients are presented in Table I.

The immunoblot analyses of orchard grass-pollen allergens are illustrated in Fig. 1. Patients allergic only

to orchard-grass pollens have IgE antibodies detecting fewer proteins than patients allergic to multiple pollen species and/or lower IgE antibody titers. Three polysensitized patients had IgE immunoblots similar to grass pollen-allergic patients. Moreover, there are some allergenic protein bands in immunoblotting that are only revealed in patients allergic to multiple pollen species (Fig. 1, A, arrows).

Total serum IgE was significantly ($p < 0.05$, Mann-Whitney U test) higher in patients allergic to multiple pollens (median $\pm$ SD, 689 $\pm$ 892 KU/L) than in those patients allergic only to grass pollens (median $\pm$ SD, 335 $\pm$ 456 KU/L).

Serum orchard grass pollen-specific IgE was significantly ($p < 0.05$, Mann-Whitney U test) higher in patients allergic to multiple pollens (median $\pm$ SD, 37.0 $\pm$ 14.3 PRU/ml) than in those patients allergic only to grass pollens (median $\pm$ SD, 24.3 $\pm$ 13.2 PRU/ml).

Skin test dose-response curves were similar in both groups (Fig. 2).

Nasal challenges

None of the subjects tested had significant symptoms with lactose (score always < 2). The results of nasal provocation tests are presented in Fig. 3. There was no major difference among both groups of patients in the threshold concentration of orchard-grass pollens required to elicit a positive challenge, that is, a symptom score of 5 (mean $\pm$ SD, 1419 $\pm$ 2205 grains for grass pollen-allergic subjects and 973 $\pm$ 1666 grains for polysensitized individuals).

A significant release of histamine was observed in six challenges in both groups, and the release of PGD₂ in 10 and 11 challenges. There was an overall agree-

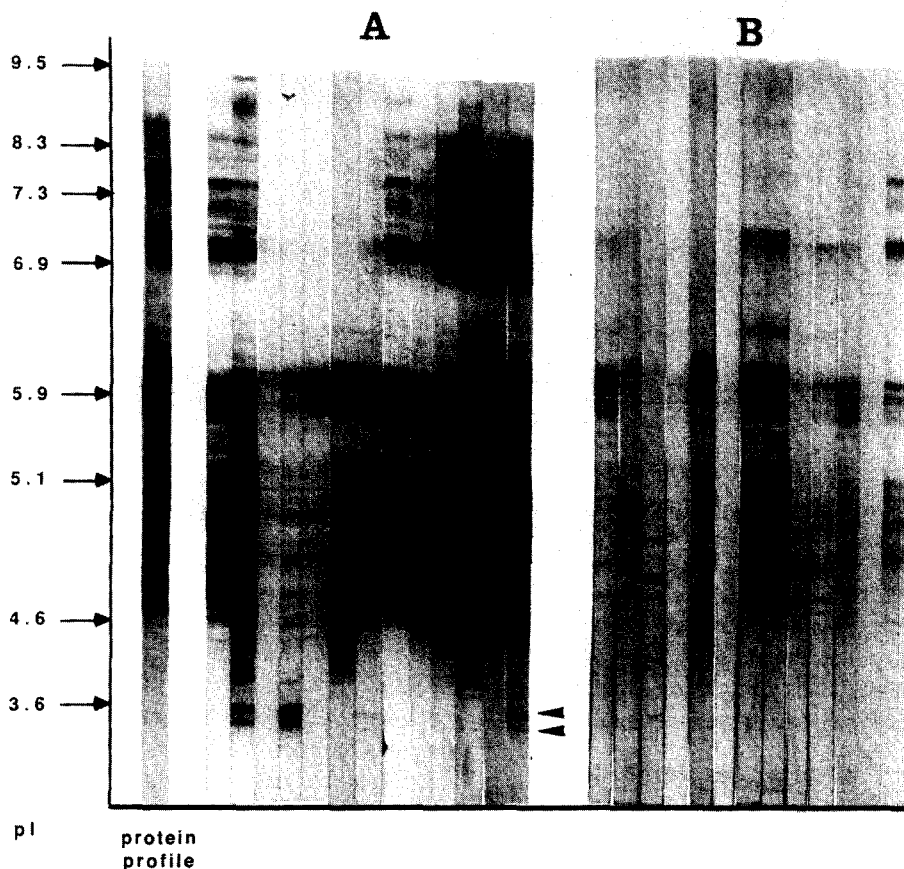


FIG. 1. IgE immunoblot of orchard grass-pollen extract. **A**, Patients allergic to multiple pollen species. **B**, Patients only allergic to grass-pollen extract.

ment between the release of PGD₂ and symptoms during challenges (Fig. 3).

There was no significant correlation between results of nasal challenge assessed by symptom score and either the immunoblot pattern or the serum-specific IgE titer (Spearman's rank-correlation test).

Pollen counts

Pollen counts for grass, Cupressaceae, and plane-tree pollens are presented in Fig. 4. Juniper-pollen counts (Cupressaceae) were very high during the first 5 weeks of the study (weeks 13 to 17). Plane-tree pollens were high between the third and sixth week of the study (weeks 15 to 18). Grass pollens started to increase slowly after week 15 and reached a significant amount at week 18. Pollens peaked between weeks 19 and 23. Oak pollens were extremely high between weeks 17 and 23, ranging from 150,200 to 923,800/m³. Urticaceae pollens (nettle and *Parietaria*) were very low throughout the study, ranging from 31 to 1298/m³ of air. Mulberry (*Broussonetia papyrifera*)-pollen counts were high between weeks 20 and 23, ranging from 6500 to 12,500 grains per

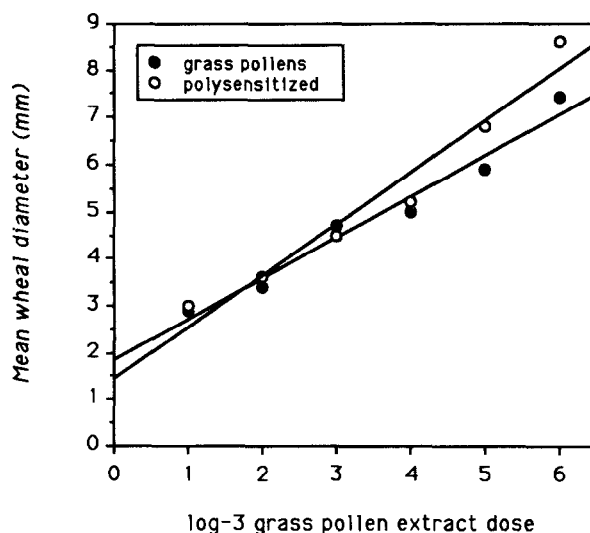


FIG. 2. Dose-response curves established for skin tests with serial concentrations of orchard grass-pollen extract in patients only allergic to grass pollens and in patients allergic to multiple pollen species; both dose-response curves were linear: $r = 0.98$ and 0.97 ; both dose-response curves were parallel: $F = 4.50$.

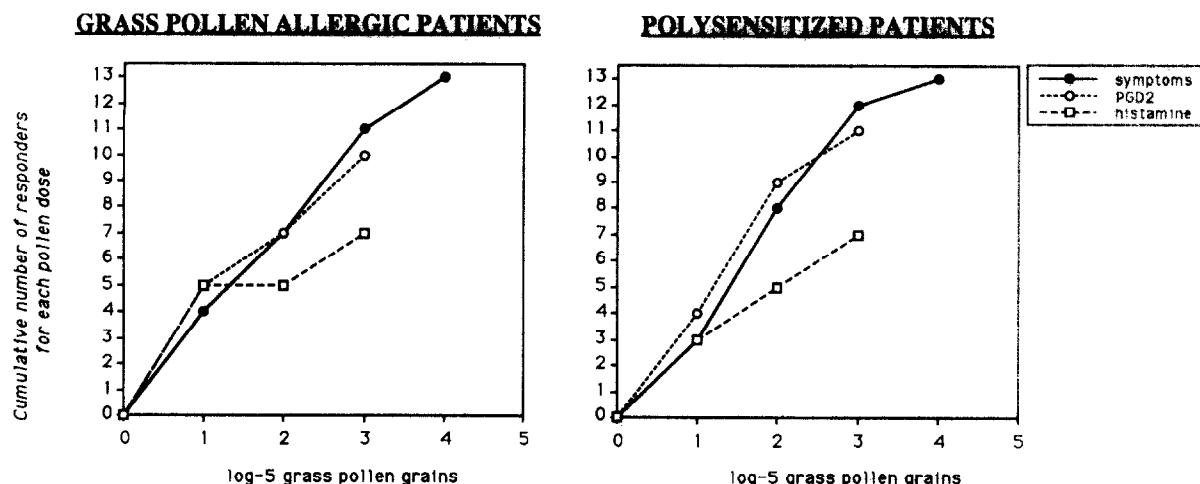


FIG. 3. Results of nasal challenges with orchard grass-pollen grains in patients only allergic to grass pollens and in patients allergic to multiple pollen species.

cubic meter of air. Plantain and olive pollens started to be present in pollen counts after week 20 (peak, 17,000/m³, week 23, and 18,000/m³, week 24, respectively). Mugwort pollinates in the autumn.

Symptom-medication scores during the pollen season

Symptom and medication scores were analyzed for asthma and rhinitis. All patients presented rhinitis, and 9/13 patients had asthma in the polysensitized group and 7/13 in the grass pollen-allergic group. Polysensitized individuals had an earlier onset of nasal and bronchial symptoms than patients allergic only to grass pollens (Fig. 4). The onset of symptoms of grass pollen-allergic patients coincided exactly with the onset of the grass-pollen season. Moreover, in both groups, bronchial symptoms appeared later than nasal symptoms. The mean symptom-medication scores were analyzed between April 1 and June 15 (Fig. 5), and although patients placed in the polysensitized group had slightly higher scores during the grass-pollen season, no significant difference was observed between both groups during this period (Mann-Whitney U test).

There was no significant difference between mean symptom-medication score and either the immunoblot pattern or the serum-specific IgE titer (Spearman's rank-correlation test).

DISCUSSION

The results presented in this study that indicate patients allergic only to grass pollens are immunologically different from patients allergic to multiple pollen species confirm the priming effect of the nasal mucosa

by tree pollens and demonstrate a similar priming effect on the bronchial mucosa. Bronchial symptoms appear a few weeks later than nasal symptoms. The intensity of symptoms is not related to the immunologic pattern nor to the sensitization of the patients as measured by nasal challenge with orchard grass-pollen grains and nasal symptom-medication scores during the season.

The allergenic sensitivity of the patients demonstrated that none of the grass pollen-allergic subjects was allergic to plants pollinating during the grass-pollen season. In contrast, all polysensitized individuals tested were allergic to plane tree (10/13) and/or Cupressaceae (6/13). The amounts of pollens inducing symptoms vary according to the pollen species. We had observed that Cupressaceae pollens induce symptoms when their number is >100,000/m³ of air (unpublished data), indicating that these pollens could affect patients during the first 4 weeks of the study. Plane-tree pollens and grass pollens require a lower number of grains in the air to cause allergic symptoms. Polysensitized patients had also positive skin tests to olive (7/13), *Parietaria* (11/13), oak (3/13), mulberry (3/13), plantain (4/13), and mugwort (3/13). *Parietaria* pollens were extremely low during the whole period of survey and started to be present in the atmosphere after grass pollens. Olive started to pollinate at the peak of the grass-pollen season. Mulberry pollinates at the same time as grass pollens. Some patients from the area have positive skin tests to plantain pollens, but it is not known whether they are eliciting symptoms. Their pollination occurs at the peak of grass pollens. Mugwort pollinates in the autumn. Thus, none of these five pollens interfered with

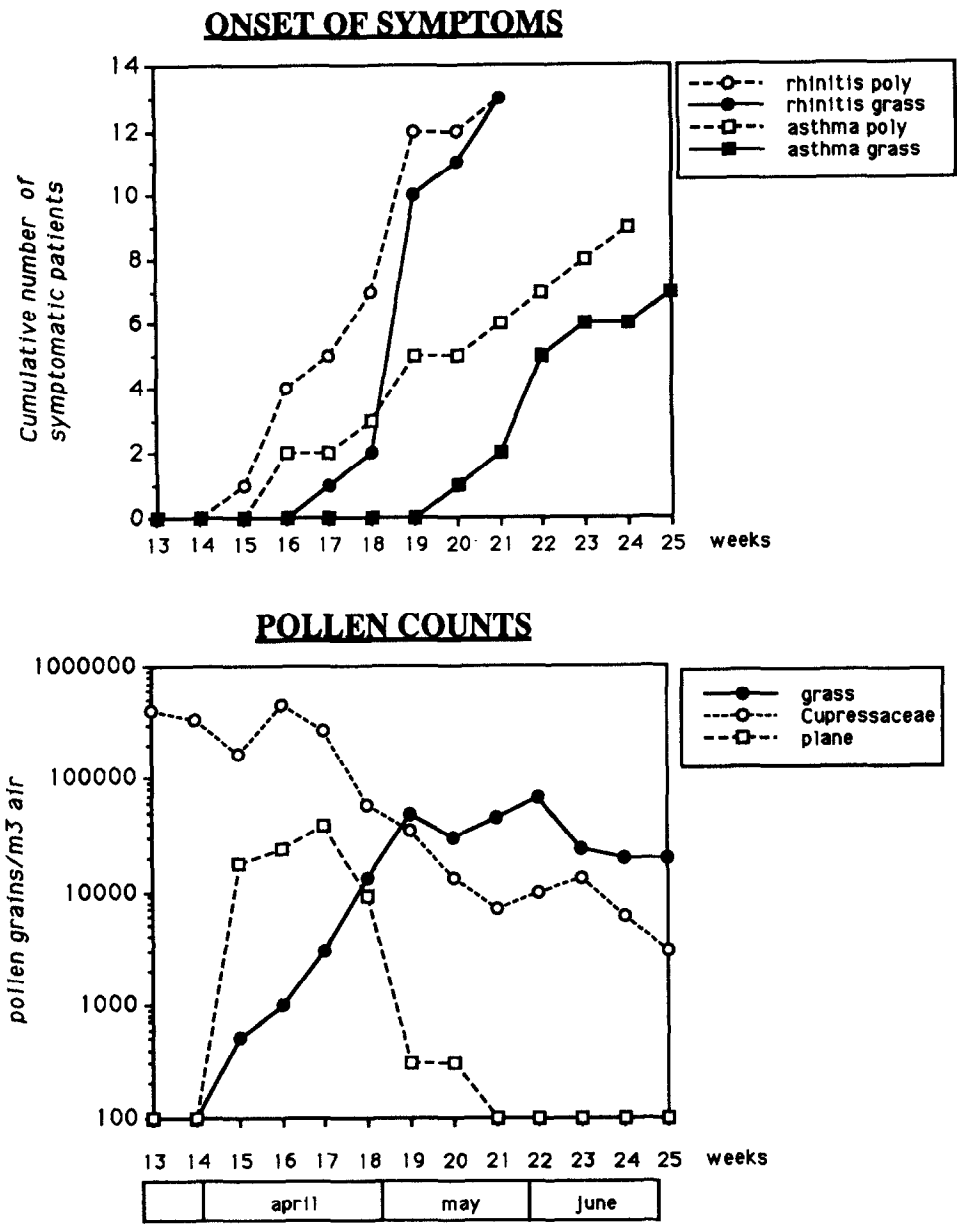


FIG. 4. Pollen counts for grass, Cupressaceae, and plane-tree pollens and onset of nasal and bronchial symptoms in patients allergic only to grass pollens and in patients allergic to multiple pollen species.

the study. Oak is a major pollinating plant of the area, but oak-pollen allergy is doubtful, since by nasal challenge with oak-pollen grains, we were unable to observe any positive test in patients with positive skin tests (unpublished observations); this pollen was considered to be of little clinical importance in our area.^{2,45} Moreover, since no grass pollen-allergic patient was sensitized to oak pollens, these pollens were unlikely to affect the results of the study.

The methods used in this study to assess the patients

clinically are well described and were used in previous studies from our group. Nasal challenges have been previously validated in immunotherapy or pharmacotherapy studies and were found to be reproducible.^{33, 34, 38} The results observed in the present study are very similar to results already published, and we confirmed a second time that the release of PGD₂ is better related to symptoms than that of histamine. Symptom-medication scores used in the present study had also been validated in previous studies,³¹⁻³³ and

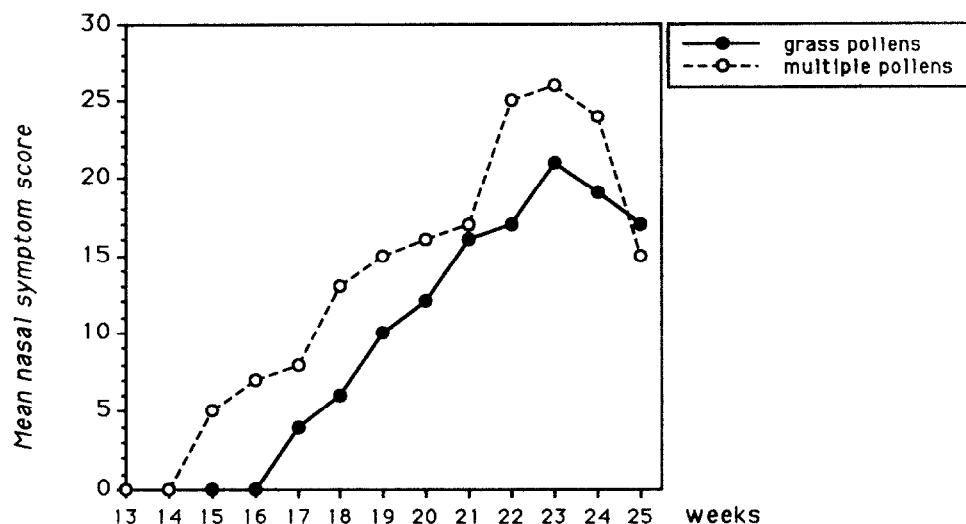


FIG. 5. Mean nasal symptom-medication scores in patients only allergic to grass-pollen extract and in patients allergic to multiple pollen species.

we observed a significant correlation between this parameter and skin tests³³ or nasal challenges.^{31, 36} For asthma, we did not analyze peak flows serially because they are not very informative in grass-pollen asthma, as demonstrated previously.⁴⁶⁻⁴⁸ Only 13 patients were studied in both groups, but we desired to study patients living in a restricted area and exposed to a similar environment. Therefore, we only followed up highly characterized patients. The onset of nasal symptoms occurred at the time of the onset of grass pollens in grass pollen-allergic individuals and a few weeks before in polysensitized patients, suggesting a priming effect on the nasal mucosa. This effect is likely to be due to plane-tree and juniper pollens, since these pollens were the only pollens to be in sufficient amounts in the air. A similar priming effect was observed for asthma. Bronchial symptoms occurred a few weeks after nasal symptoms in seven and nine patients placed in the grass pollen-allergic and multiple pollen-allergic groups. However, the earlier onset of symptoms in the polysensitized patient group may be attributable to tree pollens or might also reflect the higher level of grass IgE in this group. The intensity of symptoms during the pollen season was slightly, but not significantly, greater in the multiple pollen-allergic group than in the grass pollen-allergic group; thus, there is no synergistic effect that might have been expected with a priming effect. Nasal challenges and skin tests demonstrated that patients placed in both groups had a similar nasal and skin sensitivity.

The immunoblotting technique makes it possible to characterize the IgE immune response of patients.^{4, 5, 49} In the present study, we observed heterogeneous patterns as previously demonstrated in other

studies,^{4, 5, 49} but we could relate these findings to the patients' sensitivity. Since we wanted to determine the IgE immune response to grass pollens, we decided to study only the orchard grass-pollen IgE response because it is one of the most prevalent grass pollens in our area. Its immunoblotting characterization has been widely studied.⁴ Patients allergic only to grass pollens often present a lower number of IgE specificities against epitopes of orchard-grass pollen as well as lower mean levels of serum total IgE and orchard grass pollen-specific IgE. The lower number of IgE specificities detected by immunoblotting does not appear to be related to a higher concentration of grass-pollen IgE, since in a preliminary experiment, we observed similar patterns when sera with an identical IgE titer were run. In contrast, patients with multiple pollen sensitivities present IgE antibodies to most, if not all, epitopes and a greater IgE specific and non-specific response. These data emphasize the observation of Marsh and Bias^{3, 50} who noticed that both HLA type and basal total serum IgE level are important determinants in the expression of specific IgE responses in allergic subjects and that the association between specific IgE-mediated immune response and HLA type is masked in subjects with high IgE levels.

The enhanced IgE-mediated immune response of polysensitized patients is not related to the severity of allergic rhinitis, since nasal challenges with orchard grass-pollen grains and nasal symptom-medication scores during the pollen season are similar in both groups. Nor is it related to the intensity of skin tests to orchard-grass pollen. These latter observations are in accord with previous data on basophil degranulation⁵¹ and nasal challenge⁵² demonstrating

that the level of specific IgE antibodies was not directly related to the release of histamine from blood basophils in the first experiment and to the symptoms in the second. In contrast, differences in the IgE-mediated immune response may explain, at least partly, that with doses demonstrated to be effective in patients allergic only to grass pollen,^{31, 32} specific immunotherapy is less effective in polysensitized patients treated with the relevant allergens.⁵³

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