Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids

I. Rush immunotherapy with allergoids and standardized orchard grass–pollen extract

J. Bousquet, M.D.,* A. Hejjaoui, M.D.,* W. Skassa-Brociek, M.D.,* B. Guérin, Ph.D., H. J. Maasch, Ph.D.,*** H. Dhivert, M.D.,* and F. B. Michel, M.D.***

Montpellier and Joinville le Pont, France, and Reinbeck, Germany

Forty-five grass pollen–allergic patients were randomly assigned to three groups according to their skin test and RAST sensitivities and the severity of seasonal rhinitis. Eleven patients were treated with placebo (group 1), 19 patients (group 2) were treated with a six-mixed grass-pollen allergoid prepared by mild formalinization with a two-step procedure, and 15 other patients were treated with a standardized orchard grass–pollen extract (group 3). Because of a different immunotherapy schedule, only patients placed in groups 1 and 2 received the extracts in a double-blind fashion. Rush immunotherapy was performed in 3 to 6 days, and the maintenance dose was subsequently administered weekly for 4 weeks and every 2 weeks until the end of the grass-pollen season. During the season, a coseasonal treatment was administered. Systemic reactions occurred during the rush protocol in 36.8% of patients treated with allergoid and 20% of patients who received the standardized extract. Only patients treated with allergoid had systemic reactions during maintenance dose. The reactions observed with the standardized extract were more severe. Total doses of allergoid ranged from 2350 to 13,500 protein nitrogen units. Symptom and medication scores during the peak of the season were analyzed. Patients treated with the standardized allergen had a significant reduction of the number of days of symptoms during the month of June (9.5 ± 6.7 days; $p < 0.005$) and of medication scores (1.3 ± 1.4; $p < 0.01$) compared to patients receiving placebo (19.4 ± 8.1 days; medication score, 2.8 ± 2.1). Patients treated with allergoid also had a significant reduction of symptomatic days (11.3 ± 7.6; $p < 0.01$) and medication score (1.7 ± 1.5; $p < 0.05$) compared to patients receiving placebo. There was no significant difference between the two treated groups. The mean skin prick test end point was significantly reduced in both treated groups after immunotherapy and unchanged in the placebo-treated group. Serum grass-pollen IgG measured by solid-phase radioimmunoassay with Staphylococcus aureus *protein A was significantly increased after immunotherapy in both treated groups and remained unchanged in the placebo-treated group. This study demonstrates that both a standardized allergen extract and a formalinized allergoid are effective in the treatment of grass-pollen rhinitis. (J ALLERGY CLIN IMMUNOL 1987;80:591-8.)*

In the northern Mediterranean area, grass pollens represent the major pollen allergen.[1] Immunotherapy with standardized grass-pollen extracts was demonstrated to be effective in children or adults.[2-8] Within the past 15 years, attempts have been made to reduce the allergenicity and to retain the antigenicity of pollen extracts. Mild formalinization was used with grass-[9]

Abbreviations used
BU: Biological unit
IR: Index of reactivity
RI: Rush immunotherapy
PNU: Protein nitrogen unit

From the *Clinique des Maladies Respiratoires, Centre Hospitalier Universitaire, Montpellier, France, **Laboratoires des Stallergenes, Joinville le Pont, France, and ***Allergopharma Joachim Ganzer KG, Reinbeck, Germany.

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Reprint requests: J. Bousquet, M.D., Clinique des Maladies Respiratoires, Centre Hospitalier Universitaire, Avenue du Major Flandre, 34059 Montpellier Cedex, France.

TABLE I. Protocol of rush immunotherapy with the standardized orchard grass–pollen extract*

	Wt/vol	IR†
Day 1		
9 A.M.	0.1 ml 10^{-5}	0.01
9:30 A.M.	0.5 ml 10^{-5}	0.05
10 A.M.	0.1 ml 10^{-4}	0.1
10:30 A.M.	0.2 ml 10^{-4}	0.2
2 P.M.	0.4 ml 10^{-4}	0.4
Day 2		
9 A.M.	0.6 ml 10^{-4}	0.6
2 P.M.	0.8 ml 10^{-4}	0.8
Day 3		
9 A.M.	0.1 ml 10^{-3}	1
Day 7		
	0.2 ml 10^{-3}	2

*Alyostal ST (Stallergènes Laboratories)

†A strength of 100 IR corresponds to the strength of an allergen extract that induces a wheal size identical to that induced by a 9% codeine phosphate solution used as a positive control.

TABLE II. Rush immunotherapy with the mixed grass-pollen allergoid

	PNU
Day 1	
9 A.M.	20
9:30 A.M.	40
10 A.M.	80
11 A.M.	120
2 P.M.	200
Day 2	
9 A.M.	300
11 A.M.	400
2 P.M.	600
Day 3	
9 A.M.	800
Day 7	
	1000

and ragweed-pollen extracts.[10] The "allergoids" prepared were demonstrated to have such properties,[11] and double-blind studies in humans have demonstrated that they are effective in ragweed-pollen allergy.[12, 13] In grass-pollen allergy, allergoids were demonstrated to be more effective than a semidepot extract based on pyridine extraction and alum precipitation,[14] but no placebo-controlled study has been carried out with grass pollens, and the reference extract used in the latter study may not compare favorably with standardized extracts.[3]

A double-blind, placebo-controlled study was undertaken in the form of a rush protocol in order to compare the safety and efficacy of a mixed grass-pollen allergoid and a standardized grass-pollen extract that was found to be effective in a previous study.[3]

MATERIAL AND METHODS

Patients

Forty-five patients ranging in age from 12 to 43 years (Mean ± SD: 24.1; ± 10.1 years) were included in the study after informed consent. They were selected on the basis of the following criteria: (1) All patients had rhinitis during the grass-pollen season, and more than half also had symptoms of asthma and/or conjunctivitis. The duration of symptoms during the pollen season ranged from 3 to 19 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 at a RAST class 3 to 4 in the Pharmacia (Pharmacia Diagnostics AB, Uppsala, Sweden) scoring system. (3) None of the patients had a multiple pollen allergy assessed by prick tests to the pollens found in the Montpellier area[1] and RAST to the most common pollen species: *Parietaria*, olive, cypress, and English plantain pollens, (4) None of the patients had received any form of specific immunotherapy to grass pollens. (5) None of the patients was under systemic corticosteroids at the time of immunotherapy.

Extracts

Standardized orchard grass–pollen extract. Standardized and lyophylized orchard grass–pollen extracts were obtained from Stallergènes Laboratories. These extracts were prepared according to the proposals of the allergen subcommittee on Standardization of the International Unions of the Immunological Societies. The details of pollen extraction and control has been previously described in detail.[15] Briefly, pollens of the same species collected during a period of 3 years were microscopically analyzed and found to be free from contamination with either other pollen species or plant matter. Pollens were extracted in ammonium bicarbonate (pH 8.4) overnight at +4° C. The extracts were compared with an internal reference preparation by means of RAST inhibition and isoelectric focusing. The strength of the extracts was expressed in IR equivalent to BU defined according to Aas and Belin,[16] but the positive control solution was a 9% codeine phosphate solution instead of a 1% histamine hydrochloride solution. The same extract was used for immunotherapy and in vivo tests.

Six-mixed grass-pollen allergoid. The six grass-pollen allergoid was prepared by the new two-step procedure described by Marsh et al.[10] Briefly, equal weights of pollens from six different grass species (*Dactylis glomerata, Festuca elatior, Holcus lanatus, Lolium perenne, Phleum pratense,* and *Poa pratensis*) were defatted at room temperature and extracted with 0.125 mol/L of ammonium bicarbonate at +4° C for 16 hours. The extract was then lyophilized and used to prepare the allergoid and for in vitro tests.

TABLE III. Scoring system for medications

No medication	0
Nasal sprays of disodium cromoglycate	1
Nasal sprays of beclomethasone	2
Terfenadine	3
Prednisolone (oral)	4

TABLE IV. Diary charts provided for the patients

Rhinitis	No.
No symptom	0
Episodes of sneezing (>5)	1
Nasal blockade	1 or 1
Rhinorrhea	1 or 2
Pruritus of the nose	1

Daily symptom scores were recorded.

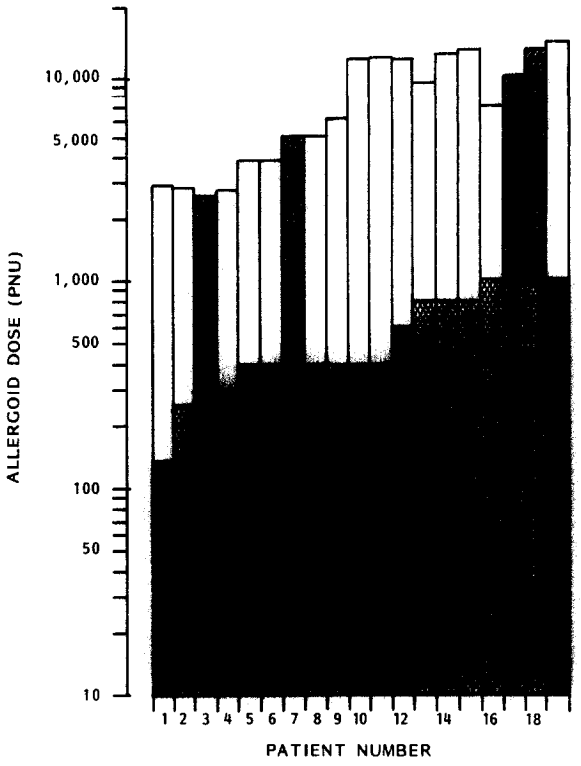

FIG. 1. Doses of mixed grass-pollen allergoid injected into the patients. Maximal dose reached without systemic reactions (▩); maximal dose reached with a systemic reaction (■); cumulative dose reached without systemic reactions (□); and cumulative dose reached with a systemic reaction (▨).

The lyophilized extract was subsequently dissolved in a 0.1 mol/L of phosphate buffer, and 0.2 mol/L of formaldehyde was added to the allergen solution to elicit a 10 mg/ml pollen extract. This solution was incubated for 14 days at a controlled pH of 7.5. For the second incubation step, the solution was diluted fourfold with a 0.1 mol/L of phosphate buffer (pH 7.5) and was incubated at 32° C for 21 days. The resulting allergoid solution was dialyzed at +4° C to remove formaldehyde and buffer salts and subsequently lyophilized. For immunotherapy, the allergoid was dissolved in 0.9% saline containing 0.4% phenol and was standardized in PNUs.

Placebo. A placebo preparation containing 0.9% NaCl, 0.4% phenol as a preservative, and 0.5 to 0.005 mg/ml of histamine hydrochloride was used.

Rush immunotherapy protocol

Immunotherapy with standardized orchard-pollen extract. The RI consisted in a rapid increase of allergen dose that made it possible to reach the maintenance dose in 4 days (Table I). After having reached the maintenance dose of 2 IR, it was administered every week for 4 weeks and subsequently every 2 weeks until April 1. The RI started in December or January. A coseasonal immunotherapy was administered every 2 weeks until October 1 with the dose reduced by one half.

Immunotherapy with the six-mixed grass-pollen allergoid. The RI consisted of a rapid increase of allergoid doses during 3 days, and the doses were subsequently increased weekly to reach a maintenance dose of 1000 PNU (Table II). When a systemic reaction or a large local reaction of a diameter >10 cm occurred, the dosage increment was stopped, and the maintenance dose was the dose reached before this reaction. The maintenance dose was subsequently administered every week for 4 weeks and every 2 weeks thereafter until April 1. The RI started in January or February. A coseasonal immunotherapy was administered every 2 weeks after April 1 with the dose reduced by one half.

Evaluation of the patients

Symptom scores. Patients attending the clinic in April were taught the possible symptoms that could appear and the type of medication needed (Table III). A chart presenting all the treatments that might be required was available to the patients and their doctors. Patients completed daily forms during the months of May and June, since the predicted onset of the season ranges from May 1 to May 15; however, because of a rainy and cold month of May, the pollen season started in June, and only the month of June was studied. The forms report nasal symptoms for 12 hours along with all the possible medication that could be used. The symptoms and medications were scored, and the average medication score and the number of days of hay fever for each patient were calculated (Table IV).

Pollen counts. The pollen counts were performed according to the technique of Cour.[17]

Skin test titration. Prick tests were performed by the Stallerpointe (Stallergènes Laboratories) according to a technique previously described in detail[3, 18] with a 9% co-

TABLE V. Comparison of the three groups of patients

	Placebo (group 1)	Allergoid (group 2)	Allergen (group 3)
No. of subjects	11	19	15
Age (yr)	25.3 ± 9.6	26.2 ± 14.8	20.7 ± 7.7
RAST (percent binding)	18.0 ± 11.6	18.2 ± 14.2	17.2 ± 13.5
Skin tests			
End point	6.1 ± 1.8	6.4 ± 1.3	6.9 ± 1.5
Mean size of greatest wheal (mm)	6.6 ± 1.9	7.3 ± 2.6	7.3 ± 3.2
Rhinitis			
Severity (grade)*	3.1 ± 1.2	3.0 ± 1.1	3.3 ± 0.6
Duration (yr)	8.5 ± 5.7	9.2 ± 6.9	7.9 ± 8.1
Asthma (percent)	64	74	73
Conjunctivitis (percent)	45	53	73

There was no significant difference between groups by Mann-Whitney U test.
*Rhinitis was graded from 0 to 4 according to the severity of symptoms and the medications required by the patients the year before immunotherapy.

TABLE VI. Systemic reactions observed during immunotherapy

	Allergoid (group 2)	Allergen (group 3)
Rush protocol (percent)		
Patients	36.8	20.0
Injections	6.0	2.7
Reactions treated	30.0	100.0
Adrenaline (sc)	11.1	75.0
Terbutaline (sc)	11.1	75.0
Maintenance immunotherapy (percent)		
Patients	21.0	0
Injections	<2	
Reactions treated	50.0	
Adrenaline (sc)	0	
Terbutaline (sc)	0	

sc = Subcutaneous. All treated patients received antihistamines and corticosteroids.

deine phosphate positive control solution and threefold dilutions of orchard grass–pollen extract from 50,000 to 8 BU/ml. The end point titer was recorded.

Serum orchard grass–pollen IgE. Serum grass–pollen IgE was measured with the classic Phadebas RAST (Pharmacia Diagnostics AB). RAST discs were prepared according to the technique of Ceska et al.,[19] and results are presented as percent binding of total added counts.

Serum orchard grass–pollen IgG. Six grass pollen–specific IgG was measured according to the method described by Puttonen and Maasch[20] with RAST discs prepared according to Ceska et al.[19] IgG coupled on the disc was revealed by *Staphylococcus* protein A radiolabeled with ^{125}I. The results are presented (1) as percent binding of total added counts and (2) in percent of IgG values from initial values (baseline). The value of the IgG determination before immunotherapy is taken as 100%.

Design of the study

Randomization of patients. Patients were randomly assigned to one of the three treatment groups. Thirty patients were enrolled in a double-blind study and received either a placebo (group 1) or a mixed grass-pollen allergoid (group 2). Fifteen patients were treated with a standardized orchard grass–pollen extract (group 3).

Parameters studied. Before RI and between April 1 and April 15 (i.e., before the pollen season), patients had a skin test titration and the titration of serum grass-pollen IgE and IgG.

During the pollen season, all patients had follow-up by a symptom score diary that they filled in every day from June 1 to June 30.

Statistical analysis of the data

Data were analyzed by use of nonparametric statistical analysis for group comparison. The Wilcoxon W test for paired data and the Mann-Whitney U test were used.

RESULTS

Homogeneity among groups of patients

The homogeneity between groups is presented in Table V and was analyzed by means of the Mann-Whitney U test. There is no major difference among the three groups.

Protocol of immunotherapy and side reactions

None of the patients receiving placebo presented any systemic reaction, whereas 36.8% treated by allergoids had a systemic reaction during the RI (Ta-

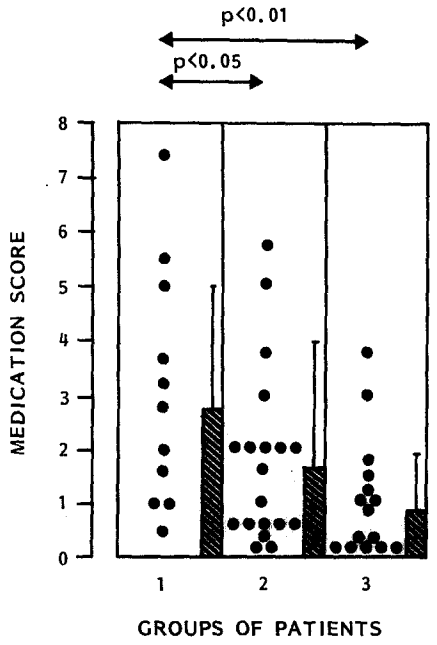

FIG. 2. Medication scores in patients treated with placebo *(1)*, the mixed-pollen allergoid *(2)*, or the standardized orchard grass-pollen extract *(3)*. Statistical analysis by Mann-Whitney U test.

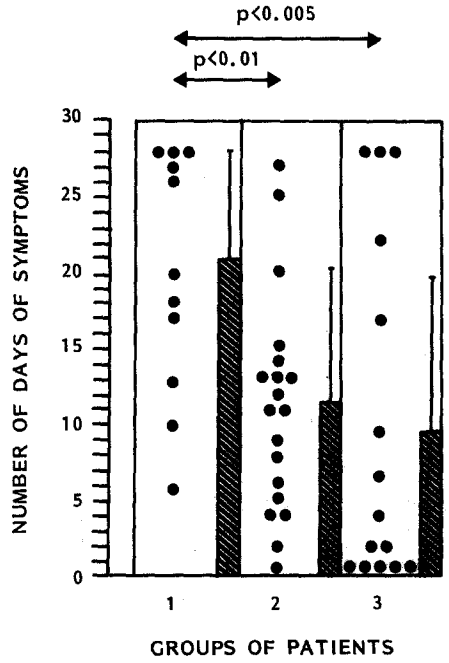

FIG. 3. Symptom scores expressed in number of days of rhinitis in patients treated with placebo *(1)*, the mixed grass-pollen allergoid *(2)*, or the standardized orchard grass-pollen extract *(3)*. Statistical analysis by Mann-Whitney U test.

ble VI). Among the nine injections that elicited a systemic reaction, six reactions were characterized by mild urticaria and were not treated. One reaction was slightly more severe and cleared with antihistamines and oral corticosteroids. One injection had induced asthma that required subcutaneous terbutaline sulfate, and the seventh reaction appeared to be more severe and was treated with subcutaneous adrenalin. Twenty-one percent of these patients had a mild systemic reaction during maintenance immunotherapy that was eventually treated by antihistamines and oral corticosteroids. Three of the patients having reactions during maintenance treatment did not present any systemic reaction during the RI (Fig. 1). None of these patients presented more than one systemic reaction (Table VII).

Twenty percent of patients treated with the standardized orchard grass–pollen extract had a systemic reaction that was usually more severe than that induced by the allergoids. None of the latter patients presented any untoward systemic reaction during the maintenance treatment.

All patients treated with the standardized orchard grass–pollen extract could reach the maintenance dose in 3 to 6 days. Most patients placed in the allergoid group could not reach the theoretical maintenance dose because of either systemic and/or large local reactions (Fig. 1). The cumulative allergoid doses ranged from 2900 to 13,500 PNU.

Clinical efficacy

Arithmetic means of daily medication scores and the number of days of rhinitis are presented in Figs. 2 and 3. It can be observed that there is a significant reduction in symptoms and medications in the two treated groups ($p < 0.05$ to $p < 0.005$). Patients treated with the standardized orchard grass–pollen extract had lesser symptoms and lower medication scores, but the differences did not reach significance. Five patients in group 3 (orchard grass–pollen extract) and only one patient in group 2 (mixed grass–pollen allergoid) did not present any symptom during the month of June.

Pollen counts

By comparison to previous years, grass-pollen counts were relatively low during the month of survey, but they were present in the atmosphere throughout the month of June.

Skin test titration

The evolution of skin tests after treatment demonstrates that patients treated with placebo did not present any significant change in their end point titer, whereas patients treated either with allergoid or the standardized orchard grass–pollen extract had a sig-

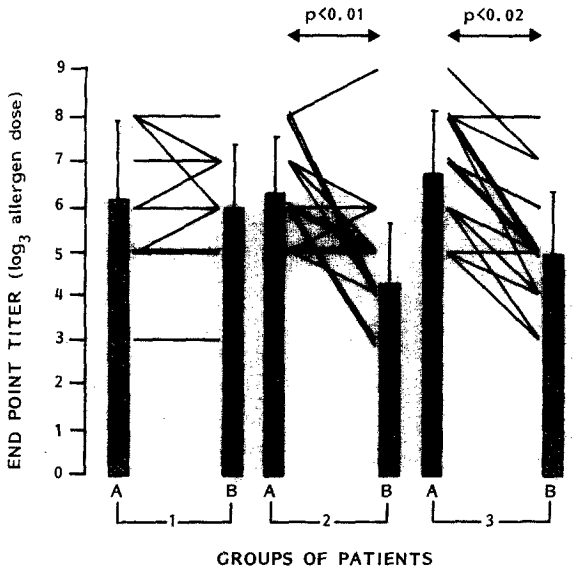

FIG. 4. Evolution of skin prick test end point before *(B)* and after *(A)* immunotherapy with placebo *(1)*, the mixed grass-pollen allergoid *(2)*, or the standardized orchard grass-pollen extract *(3)*. Statistical analysis by Wilcoxon W test.

nificant ($p < 0.01$ and $p < 0.02$) decrease in their skin test end point (Fig. 4).

Grass pollen–specific IgE

Grass-pollen IgE was very similar before and after immunotherapy in the placebo-treated group and was nonsignificantly increased in the other two treated groups (Table VIII).

Grass pollen–specific IgG

Patients who received the placebo had similar mean IgG levels before and after immunotherapy (Table VIII). By contrast, patients who were treated with either the six-mixed grass-pollen allergoid or with the standardized allergen extract had a significant increase in IgG. When the increase of IgG after immunotherapy was considered, it can be observed in Fig. 5 that in the placebo-treated group, the percentage was significantly ($p < 0.01$ and $p < 0.005$) lower than in the other two treated groups.

DISCUSSION

RI with a standardized grass-pollen extract or a mixed-pollen allergoid is effective in lessening symptoms of grass-pollen rhinitis in patients only allergic to grass pollens. However, both treatments elicit a rather high number of systemic reactions. In the treated groups, skin prick tests were significantly decreased, and serum-specific IgG significantly increased.

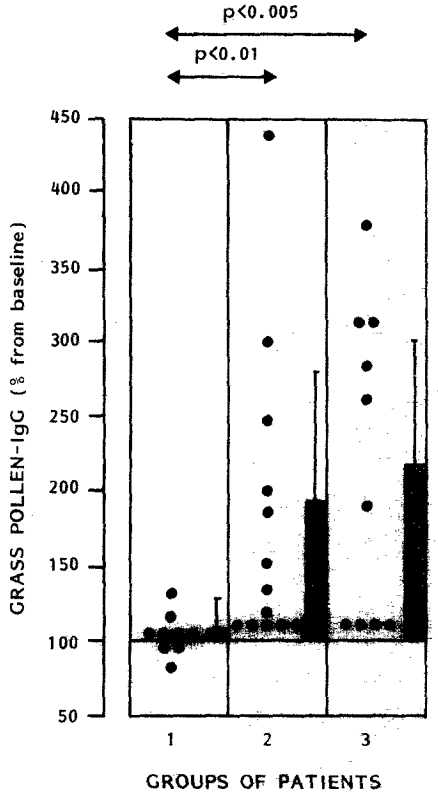

FIG. 5. Evolution of serum grass pollen IgG after immunotherapy with placebo *(1)*, mixed grass-pollen allergoid *(2)*, or standardized orchard grass-pollen extract *(3)*. Statistical analysis by Mann-Whitney U test.

The frequency of systemic reactions with the standardized allergen extract was similar to that previously reported in either grass-pollen[2-8] or ragweed-pollen[21] immunotherapy. The reactions observed with the mixed grass-pollen allergoid are also similar to those reported in the literature for grass-pollen[14] or ragweed-pollen[12] immunotherapy. However, it appears that the doses eliciting systemic reactions are much lower in Europe with grass pollens than in the United States with either ragweed pollens or grass pollens, since some patients react with a dose as low as 120 PNU. These differences may be explained by the fact that grass-pollen counts of the East Coast of the United States are at least 10 times lower than counts found in Germany or in southern France* where the three studies with grass-pollen allergoids have been performed. In the present study, an alternative possibility for the high frequency of systemic reactions may be that allergoids should not be administered through RI because the protective immune response cannot be established. This study does not favor the initial con-

*Cour P. Unpublished data.

TABLE VII. Characteristics of the systemic reactions experienced by the patients

Extract	Patient No.	Reaction			
		Schedule	No.	Type	Treatment
Allergoid	1	RI	1	Urticaria	None
	3	Maintenance	1	Urticaria	None
	4	RI	2	Urticaria	None
	5	RI	2	Urticaria	None
	7	RI	1	Urticaria	None
		Maintenance	1	Urticaria	Anti-H_1 histamine
	10	RI	1	Urticaria	Anti-H_1 histamine, prednisone
	12	RI	1	Asthma	Terbutaline (IM)
	17	Maintenance	1	Urticaria	None
	18	Maintenance	1	Urticaria	Anti-H_1 histamine, prednisone
	19	RI	1	Urticaria, angio-edema	Adrenaline
Allergen	3	RI	1	Urticaria, asthma	Adrenaline, terbutaline (IM)
	7	RI	1	Urticaria, angio-edema	Adrenaline
	9	RI	1	Urticaria, asthma	Terbutaline (IM), anti-H_1 histamine

IM = intramuscular.

TABLE VIII. Evolution of serum grass pollen–specific IgE and IgG during immunotherapy

	Placebo (group 1)		Allergoid (group 2)		Allergen (group 3)	
IgE (percent binding)						
Before immunotherapy	18.0 ± 11.6		18.2 ± 14.2		17.2 ± 13.5	
After immunotherapy	17.2 ± 11.9	NS	20.3 ± 15.6	NS	19.7 ± 12.6	NS
IgG (percent binding)						
Before immunotherapy	6.9 ± 2.1		8.8 ± 2.0		7.9 ± 1.9	
After immunotherapy	6.3 ± 2.5	NS	15.6 ± 4.3	$p < 0.01$	16.9 ± 5.1	$p < 0.01$

NS = not significant. Statistical analysis by Wilcoxon W test.

cept that allergoids will retain immunogenicity and loose allergenicity, since systemic reactions occurred. However, as demonstrated in Tables VI and VII, the reactions were milder than reactions induced by a standardized extract, and the RI may not be adequate.

We used a single grass-pollen species in the standardized extract and a mixed grass-pollen allergoid. This difference was due to the fact that in Europe grass-pollen allergoids are only available as a mixture. Moreover, it is much easier and safer to standardize a single grass-pollen species than a mixture; therefore, we preferred to use an orchard grass–pollen extract. This grass-pollen species is prevalent in the south of France and shares many common allergens with other grass pollens. In a previous study we have demonstrated (unpublished data) that an orchard grass–pollen extract elicited comparable results to a five-mixed grass-pollen extract when extracts were injected into patients.

The patients investigated in this study presented a homogeneous sensitization characterized by their symptoms, skin tests, and orchard grass–pollen IgE. These homogeneous patterns are not uncommon in this area in patients only allergic to grass pollens.

Clinically, both the standardized orchard grass–pollen extract and the mixed grass-pollen allergoid were demonstrated to be effective in alleviating symptoms and reducing medication. It was not possible to establish daily symptom-medication scores because the pollen season was not totally identical in all parts of the area. However, there were no main differences in the mean results, and the patients placed in the

three groups were evenly distributed. The total pollen counts were lower than in most other years, but the peaks were high. The results observed with the standardized orchard grass–pollen extract were very similar to results observed in previous years with the same extract and protocol.[3] The results obtained with this extract are also comparable with results of other groups with standardized grass-pollen extracts.[2-8] The symptom and medication scores of the patients treated with the mixed grass-pollen allergoid were significantly better than scores of the placebo-treated group but slightly and nonsignificantly worse than scores of patients treated with the standardized extract. However, standardized extracts are more difficult to use in practice than allergoids, and since classic immunotherapy with the allergoids may be better tolerated than RIs, treatments with allergoids can be used as an alternative to standardized extracts.

Skin tests were significantly reduced in patients treated either with allergen or allergoid. This finding has already been noticed in grass-pollen[2-6, 8] or mite[17] immunotherapy with nonmodified extracts, but it appears to be the first demonstration with allergoids. Serum IgG was increased in both the treated groups as is usually reported.[2-8, 12, 21] These two objective parameters confirm that standardized extracts and allergoids are immunologically effective in the treatment of patients allergic to grass pollens.

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