

IgE antibodies to recombinant pollen allergens (Phl p 1, Phl p 2, Phl p 5, and Bet v 2) account for a high percentage of grass pollen-specific IgE

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Background: Pollen from different grass species are some of the most potent elicitors of Type I allergy worldwide. The characterization of antigenic structures and IgE epitopes common to different grass species is relevant to define reagents for diagnosis and specific therapy of grass pollen allergy.

Objective: The purpose of this study was to estimate the percentage of IgE directed to common, cross-reactive, or both types of epitopes shared by recombinant pollen allergens (Phl p 1, Phl p 2, Phl p 5, and Bet v 2) and natural pollen extracts from nine different monocots (*Anthoxanthum odoratum*, *Avena sativa*, *Cynodon dactylon*, *Lolium perenne*, *Phragmites australis*, *Poa pratensis*, *Secale cereale*, *Triticum sativum*, *Zea mays*) by using sera from different populations.

Methods: Natural pollen extracts from nine different monocot species were characterized regarding their allergen contents by using specific antibodies and by IgE immunoblot inhibition with recombinant allergens. The percentage of grass pollen-specific IgE that was preabsorbed with a combination of recombinant timothy grass pollen allergens (Phl p 1, Phl p 2, and Phl p 5) and recombinant birch profilin (Bet v 2) was determined by ELISA in sera from 193 European, American, and Asian subjects.

Results: IgE to recombinant pollen allergens accounted for a mean 59% of grass pollen-specific IgE. A lower inhibition of IgE binding to certain natural extracts (*C. dactylon* and *Z. mays*) could be attributed to the absence of immunologically detectable group 5 and group 2 allergens in these species.

Conclusion: We define four recombinant pollen allergens that account for a substantial proportion of grass pollen-specific IgE. The recombinant pollen allergens characterized may represent candidates not only for diagnosis but also for patient-tailored immunotherapy of grass pollen allergy.

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Key words: Recombinant pollen allergens, Phl p 1, Phl p 2, Phl p 5, profilin, Bet v 2, IgE cross-reactivity, immunoabsorption, grass pollen allergy

Abbreviations used

BSA: Bovine serum albumin
TBS: Tris-buffered saline

Grass pollen allergens belong to the most frequent and potent elicitors of allergic symptoms. At least 40% of type I allergic patients are sensitized against grass pollen allergens.¹ Immunoabsorption studies have indicated that IgE to grass pollen allergens account for a high percentage of total IgE in sensitized individuals.² Characterization and standardization of grass pollen allergens by immunochemical, protein-chemical, and, in part, molecular cloning techniques resulted in the definition of allergen groups that occur in different grass species.^{3,4} Group 1 allergens with a molecular weight of approximately 30 kd,⁵ (e.g., Phl p 1,⁶ the major timothy grass [*Phleum pratense*] pollen allergen) represent targets for IgE antibodies in more than 95% of patients allergic to grass pollens. Group 2/3 allergens of 10 to 12 kd molecular weight are recognized by IgE antibodies of approximately 60% of patients allergic to grass pollen⁷⁻⁹; 70% of patients allergic to grass pollen react with group 4 allergens at 50 to 60 kd,¹⁰ 70% to 80% of patients react with group 5 allergens,^{11,12} and 20% display IgE reactivity to profilins at 14 kd.¹³⁻¹⁶ Group 6 allergens with a molecular weight of 10 kd share IgE epitopes with group 5 allergens and are expressed in certain grass species only.^{17,18} Additional grass pollen allergens with homologies to tree pollen allergens, among them Lol p 11¹⁹ and Cyn d 7,²⁰ were described.

We have recently isolated complementary DNA (cDNA) coding for three major timothy grass allergens (Phl p 1,⁶ Phl p 2,⁹ and Phl p 5¹²) and timothy grass pollen profilin,¹⁵ which shares IgE epitopes with birch pollen and other profilins.¹⁶ Recombinant timothy grass pollen allergens were expressed in *Escherichia coli* and have been shown to bind to IgE antibodies from individuals allergic to grass pollen.²¹ Moreover, it could be demonstrated that a panel consisting of a few recombinant allergens, Phl p 1, Phl p 2, Phl p 5, and profilin was sufficient to diagnose grass pollen allergy in a representative panel of patients allergic to grass pollen from

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TABLE I. Demographic and serologic characterization of individuals allergic to grass pollen tested in Fig. 1

Patient No.	Demographic data					Total IgE kU/L	Timothy kUA/L	rPhl p 1 kUA/L	rPhl p 2 kUA/L	rPhl p 5 kUA/L	rBet v 2 kUA/L
	Sex	Age (yr)	Allergies	Symptoms	Treatment						
1	F	31	g,t,w,mi,a	r,c,as	—	356	8.9	<0.35	0.6	<0.35	13.9
2	M	34	—	—	—	11	<0.35	<0.35	<0.35	<0.35	<0.35
3	F	44	g,t	r,c	—	180	21.0	11.5	8.3	6.6	<0.35
4	F	34	g,t,w	r,c	ah	200	61.0	25.1	15.2	60.1	<0.35
5	M	33	g	r,c	crom	40	7.0	1.6	1.6	3.1	<0.35
6	F	35	g,t,a	r,c	ah,hy	110	12.9	5.7	3.0	5.6	<0.35
7	M	44	g,t,w,mi,a,mo	r,c	cor	>2000	83.1	43.4	8.4	29.4	<0.35
8	M	34	g	r,c	ah	180	18.8	11.9	<0.35	8.9	<0.35
9	F	29	g,t,w,mi,a	r,c	ah	72	6.1	3.4	<0.35	3.5	<0.35
10	M	36	g,t,w	r,c	—	96	17.2	4.2	1.6	7.6	2.9
11	M	28	g,t,w,mi	r,c	ah	287	69.3	13.2	8.4	49.7	<0.35
12	M	38	g,t,w,mi,mo	r,c,as	hy	573	80.4	18.4	4.8	20.6	3.4
13	M	38	g,t,mi,a,mo	r,c,as	hy	943	34.4	13.6	21.0	10.9	<0.35

g, Grass pollen; t, tree pollen; w, weed pollen; mi, mites; a, animals; mo, molds; r, rhinitis; c, conjunctivitis; as, asthma; ah, antihistamines; crom, cromoglycate; hy, grass pollen-specific hyposensitization; cor, corticosteroids.

Austria,²² as well as in populations from different parts of the world.²³ Purified recombinant timothy grass pollen allergens were found to induce specific and dose-dependent histamine release from basophils of individuals allergic to grass pollen.²⁴ IgE immunoblot inhibition experiments provided evidence that recombinant Phl p 1 shares IgE epitopes with natural group 1 isoallergens from other grass species.²⁵

If the above-defined panel of recombinant timothy grass pollen allergens is considered not only for diagnostic purposes but eventually for specific immunotherapy of grass pollen allergy, it is necessary to determine whether the recombinant molecules account for a high percentage of grass pollen-specific IgE. In this study we investigated the degree of IgE epitopes shared by a combination of four recombinant pollen allergens (timothy grass pollen allergens Phl p 1, Phl p 2, Phl p 5 and recombinant birch profilin, Bet v 2) and natural grass pollen extracts from nine different grass species as determined by ELISA competition experiments by using sera from geographically different populations.

METHODS

Patients' sera

Sera from 193 patients allergic to grass pollen were obtained from different parts of Europe (Austria: $n = 81$; Denmark: $n = 26$; France: $n = 22$; Sweden: $n = 22$; and Switzerland: $n = 16$), North America (Canada: $n = 12$), and Asia (Japan: $n = 14$). Patients were selected according to a positive case history (presence of at least one of the symptoms [rhinitis, conjunctivitis, or asthma] during the grass pollen season) and positive skin prick test reactions. The serologic characterization of all but the additional 52 Austrian sera has been reported previously.²³ The 52 additional Austrian sera contained timothy grass pollen-specific IgE as determined by RAST (CAP, Pharmacia, Uppsala, Sweden). Twelve representative sera of the 52 Austrian sera were characterized in detail by determination of IgE antibodies to timothy grass pollen extract and to recombinant pollen allergens (rPhl p 1, rPhl p 2, rPhl p 5 and rBet v 2) by

using the CAP system (Pharmacia). The demographic and serologic characterization of these 12 representative patients and a nonallergic control person is displayed in Table I.

Preparation and analysis of natural grass pollen extracts and recombinant allergens

Pollen from nine grass species with different taxonomic relationships to *Phleum pratense* (timothy grass) and different geographic distributions were selected for the experiments. Pure pollen from *Anthoxanthum odoratum* (sweet vernal grass), *Avena sativa* (cultivated oat), *Cynodon dactylon* (Bermuda grass), *Lolium perenne* (rye grass), *Phragmites australis* (Australian reed), *Poa pratensis* (Kentucky bluegrass), *Secale cereale* (cultivated rye), *Triticum sativum* (cultivated wheat), and *Zea mays* (maize) was purchased from Allergon (Välinge, Sweden). Protein extracts were prepared by homogenization of 0.5 gm pollen in 50 ml distilled water containing 5 mmol/L phenylmethylsulfonyl fluoride with an ultraturax (Ika, Heidelberg, Germany). Proteins were further extracted by shaking at 4°C for 3 hours. Extracts were then centrifuged at 16,000 g for 15 minutes to remove insoluble particles. The supernatants were placed in aliquots in 1 ml portions, frozen in liquid nitrogen, lyophilized, and stored at -20°C until use. Lyophilized extracts were analyzed by sodium dodecylsulfate–polyacrylamide gel electrophoresis,²⁶ and Coomassie Brilliant Blue staining²⁷ (Bio-Rad, Richmond, Calif.) was performed to estimate the quality and quantity of proteins. Recombinant timothy grass pollen allergens (rPhl p 1, rPhl p 2, rPhl p 5) and recombinant birch profilin (rBet v 2) that had been expressed in *Escherichia coli* were purified as described.^{24, 28}

Immunologic characterization of natural grass pollen extracts

Approximately 200 $\mu\text{g/cm}$ gel of each grass pollen extract was separated by preparative sodium dodecylsulfate–polyacrylamide gel electrophoresis²⁶ and blotted onto nitrocellulose.²⁹ Nitrocellulose-blotted allergen extracts were characterized according to the presence of allergens by using sera from representative patients allergic to grass pollen with a well-characterized allergen profile ($n = 12$; Table I) and, for control purposes,

with serum from a nonallergic control individual (patient no. 2, Table I) by IgE immunoblotting.²²

Nitrocellulose-blotted natural grass pollen extracts were also probed with a rabbit anti-group 1 antiserum R4,^{12,30} a rabbit anti-recombinant Phl p 2 antiserum (Dolecek and Valenta, unpublished data), a rabbit anti-group 5 antiserum R5,^{12,30} a rabbit anti-recombinant Phl p 6 antiserum (Vrtala S and Valenta R, unpublished data, 1996) and a rabbit anti-celery root profilin antiserum RP1.^{14,31} A normal rabbit serum was used for control purposes. Bound rabbit antibodies were detected with a ¹²⁵I-labeled donkey anti-rabbit antiserum (Amersham, Buckinghamshire, U.K.) and visualized by autoradiography. The presence of group 4 allergens was investigated by probing nitrocellulose-blotted natural grass pollen extracts with mouse monoclonal antibodies 3C4 and 2D8 raised against Phl p 4.^{10,32} Bound mouse antibodies were detected with a ¹²⁵I-labeled sheep anti-mouse antiserum (Amersham) and visualized by autoradiography.

The presence of allergens that share IgE epitopes with recombinant Phl p 1, Phl p 2, Phl p 5, and profilin in the natural grass pollen extracts was also studied by IgE immunoblot inhibition experiments. Sera from the 12 representative patients (Table I) were preabsorbed with a mixture of the four recombinant pollen allergens (each at a concentration of 10 µg/ml) or, for control purposes, with 40 µg/ml bovine serum albumin (BSA) before detection of nitrocellulose-blotted grass pollen extracts. Bound IgE was detected as described.¹⁴

ELISA competition experiments

For ELISA inhibition experiments, sera from 193 patients were diluted 1:10 in Tris-buffered saline (TBS) and preabsorbed with a mixture of four recombinant allergens (rPhl p 1, rPhl p 2, rPhl p 5, and rBet v 2; each at 10 µg/ml) and, for control purposes, with 40 µg/ml BSA at 4° C overnight. The amount of recombinant allergen was selected, according to pilot experiments, to be in excess of specific IgE antibodies. ELISA plates (Nunc Maxisorb, Roskilde, Denmark) were coated with natural grass pollen extract (200 µg/ml TBS) at 4° C overnight. Plates were washed three times with buffer A (TBS, 0.05% vol/vol Tween 20) and blocked with TBS, 0.05% vol/vol Tween 20, and 1% wt/vol BSA at 37° C for 2 hours. Plates were incubated with 100 µl/well of the preabsorbed sera at 4° C overnight and washed five times with 200 µl/well of buffer A. An alkaline phosphatase-coupled mouse monoclonal anti-human IgE antibody (PharMingen, San Diego, Calif.) diluted 1:1000 in TBS, 0.05% vol/vol Tween 20, and 0.5% wt/vol BSA was used to detect bound IgE antibodies. After 2 hours of incubation at 4° C, plates were washed three times with buffer A and the color reaction was started with alkaline phosphatase substrate (Sigma Diagnostics, St. Louis, Mo.). The optical density was determined with an ELISA reader (Dynatech, Denkendorf, Germany). All determinations were carried out as duplicates.

RESULTS

Demographic and serologic characterization of representative patients allergic to grass pollen

Twelve representative individuals allergic to grass pollen are described in Table I. The group consisted of 5 women and 7 men with a mean age of 35 years. All patients had at least allergic rhinitis and conjunctivitis during the grass pollen season. Although three patients had received hyposensitization treatment, most of the other patients took only symptomatic medication. The

analysis of sera by RAST (CAP, Pharmacia) revealed that in all but one serum (from patient no. 12) the levels of IgE directed against the sum of recombinant timothy grass pollen allergens and recombinant Bet v 2 were comparable or even higher than the timothy grass pollen-specific IgE levels. Recombinant Phl p 1 and rPhl p 5 were recognized by all but one serum (from patient no. 1). Serum 1 contained high levels of profilin-specific IgE and very low levels of Phl p 2-specific IgE, and according to immunoblot experiments (Fig. 1) it bound to group 4 allergens at approximately 50 to 60 kd. Ten of the 12 sera that displayed IgE reactivity against rPhl p 2 and 3 sera reacted with profilin.

Characterization of natural grass pollen extracts with specific antibodies, antisera, and IgE-immunoblot inhibition experiments

In a first step we examined the presence of certain allergen groups in pollen extracts from nine monocots by using specific antisera and monoclonal antibodies. Nitrocellulose-blotted pollen extracts were probed with rabbit antisera raised against group 1, 2, 5, and 6 allergens, as well as against profilin. Monoclonal antibodies raised against group 4 allergens were included in the analysis. Table II summarizes the results of the immunologic screening. Although group 1 and group 4 allergens, as well as profilins, were detected in all extracts tested, almost no reactivity of the rabbit anti-group 5 antiserum with *C. dactylon* and no reactivity with *Z. mays* was observed. The rabbit anti-Phl p 2 antiserum failed to react with extracts from *C. dactylon*, *Ph. australis*, and *Z. mays*. No group 6 related proteins were detected in *Av. sativa*, *L. perenne*, *Ph. australis*, *S. cereale*, *T. sativum*, and *Z. mays*.

In a next step natural grass pollen extracts were characterized by using serum IgE from patients with a well-characterized allergen profile (Table I) and by IgE inhibitions with recombinant pollen allergens (Phl p 1, Phl p 2, Phl p 5, and Bet v 2) (Fig. 1). All sera with group 1 and group 5 reactivity bound to bands of approximately 25 to 35 kd in all nine species tested (Fig. 1, panels 3 to 13, lane, -). IgE reactivity to this band was inhibited best in extracts (*C. dactylon*, *Ph. australis*, and *Z. mays*), which, according to the antibody testing, contained little or no detectable group 5 allergens (Fig. 1, panels 3 to 13, lane, +). Most sera with high IgE levels to rPhl p 2 (patient nos. 3, 4, 7, 11, and 13) reacted weakly or failed to bind in the group 2 molecular weight range (10 to 12 kd) in *C. dactylon*, *Z. mays*, and *Ph. australis* indicating the absence of immunologically active group 2 allergens in these species. The inhibition of IgE binding to group 2 allergens from different extracts also varied in different sera. Although IgE binding of serum 13 to group 2 allergens in *L. perenne*, *An. odoratum*, *Av. sativa*, and *Po. pratensis* was significantly inhibited by preincubation with rPhl p 2, the binding of serum 3 was not reduced. As exemplified by serum 1, profilins (14 kd) and group 4 allergens (in the high molecular

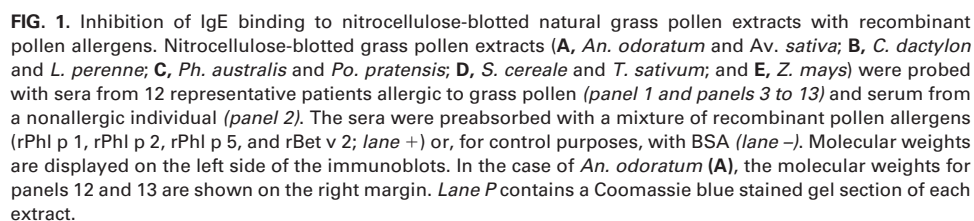


TABLE II. Presence of group 1, 2/3, 4, 5, or 6 allergens and profilin in pollen of different grass species as determined by specific antisera/antibodies and by the IgE binding/inhibition experiments

Allergen	MW	Natural grass pollen extracts								
		Ant o	Ave s	Cyn d	Lol p	Phr a	Poa p	Sec c	Tri s	Zea m
Group 1	25-35 kd	+	+	+	+	+	+	+	+	+
Group 2/3	10-12 kd	+	+	—	+	—	+	+	+	—
Group 4	40-60 kd	+	+	+	+	+	+	+	+	+
Group 5	25-35 kd	+	+	+/-	+	+	+	+	+	—
Group 6	~10 kd	+	—	+	—	—	+	—	—	—
Profilin	~14 kd	+	+	+	+	+	+	+	+	+

MW, Molecular weight; +, strong immunologic reaction at expected molecular weight; +/-, weak immunologic reaction; —, lack of binding.

weight range at approximately 50 to 60 kd) were present in all grass species tested.

The results obtained by the IgE inhibition experiments were in agreement with the antibody data and are summarized in Table II. Group 1 and group 4 related allergens, as well as profilins, are present in all monocot species tested. No substantial amounts of immunologically reactive group 2, 5, and 6 related allergens could be detected in *C. dactylon* and *Z. mays*.

Recombinant pollen allergens (Phl p 1, Phl p 2, Phl p 5, and Bet v 2) account for a high percentage of grass pollen-specific IgE

The sharing of common IgE epitopes between recombinant pollen allergens (rPhl p 1, rPhl p 2, rPhl p 5, and rBet v 2) and natural pollen extracts from nine grass species was determined in 193 sera from patients of different origin (Europe, America, and Asia) who were allergic to grass pollen by ELISA competition experiments. Table III displays in detail the average inhibition of IgE binding to each extract obtained in each population. The average inhibition of IgE binding to the nine grass pollen extracts determined in 193 sera from all populations was 59%. In 67.6% of the tested sera, a 50% or greater inhibition of IgE binding to natural extracts was observed after preabsorption with recombinant pollen allergens. In 18.1% of the sera, inhibition of IgE binding ranged between 25% and 50%, and a less than 25% inhibition was measured in only 14.3% of the sera. A lack of inhibition of IgE binding to natural grass pollen extracts after preabsorption with recombinant allergens was found in only five sera (2.5%). Three of these sera were from Sweden, and two were from Japan. The grass pollen-specific IgE levels determined in these sera by RAST were very low (<2 kUA/L), and no ELISA-detectable IgE to any of the four recombinant pollen allergens (rPhl p 1, rPhl p 2, rPhl p 5, and rBet v 2) could be measured.

Variations in the degree of IgE inhibition depending on patient populations and botanical relationships among the grass species

Preabsorption of sera from Austria, Canada, Denmark, France, and Switzerland with recombinant aller-

gens led to an inhibition of IgE binding that ranged between 68% and 58%. In sera from Japanese and Swedish patients, the extent of inhibition was smaller (55% and 42%, respectively).

Differences in the degree of inhibition were also observed on the basis of the taxonomic relationships between grass species. The extent of average inhibition of IgE binding to pollen extracts prepared from grasses of the same group as timothy grass were as follows: *An. odoratum*: 65%, *Ae. sativa*: 66%, *L. perenne*: 64%, *Po. pratensis* 66%, *S. cereale*: 63% and *T. sativum*: 63%. A significantly lower average inhibition of IgE binding to pollen extracts of botanically more distantly related grasses was observed (*C. dactylon*: 47%, *Ph. australis*: 55%, *Z. mays*: 45%).

DISCUSSION

We have previously defined a panel of recombinant pollen allergens (recombinant timothy grass pollen allergens rPhl p 1,⁶ rPhl p 2,⁹ and rPhl p 5,¹² and recombinant birch profilin rBet v 2¹³⁻¹⁶) that allowed serologic diagnosis of grass pollen allergy not only in an Austrian population of individuals allergic to grass pollen²² but also in sera obtained from different parts of the world (Europe, America, and Asia).²³ Although these data indicated that the above panel of recombinant allergens contained sufficient IgE epitopes to permit a component-based serologic testing of grass pollen allergy, nothing was known as to the extent of common IgE epitopes shared between this panel of recombinant allergens and natural pollen extracts from other grass species.

Patients are exposed to a large variety of different grasses. If the use of recombinant allergens is considered not only for diagnosis but also for specific immunotherapy, the degree of IgE epitopes shared by recombinant allergens and different natural allergen extracts needs to be assessed in a representative panel of sera. In an immunoabsorption approach similar to that used by Gleich and Jacob,² who determined the percentage of pollen-specific IgE antibodies in total IgE, we measured the percentage of grass pollen-specific IgE directed against a mixture of 4 recombinant pollen allergens (rPhl p 1, rPhl p 2, rPhl p 5, and rBet v 2). One hundred

TABLE III. Inhibition of IgE binding to nine monocot pollen extracts after preabsorption of sera from different populations with recombinant pollen allergens

	Natural grass pollen extracts									Mean (all extracts)
	Ant o	Ave s	Cyn d	Lol p	Phr a	Poa p	Sec c	Tri s	Zea m	
Austria (n = 81)	65%	69%	47%	67%	55%	70%	64%	63%	45%	60%
Canada (n = 12)	65%	62%	50%	73%	76%	69%	69%	64%	57%	65%
Denmark (n = 26)	76%	75%	54%	68%	69%	71%	70%	71%	57%	68%
France (n = 22)	73%	72%	46%	73%	55%	68%	69%	70%	45%	63%
Japan (n = 14)	71%	58%	54%	50%	38%	53%	62%	59%	47%	55%
Sweden (n = 22)	48%	43%	34%	51%	37%	49%	47%	43%	27%	42%
Switzerland (n = 16)	61%	64%	44%	59%	57%	66%	62%	68%	43%	58%
Mean (all sera)	65%	66%	47%	64%	55%	66%	63%	63%	45%	59%

Sera from 193 patients from different countries allergic to grass pollen were preabsorbed with a mixture of four recombinant pollen allergens (rPh1 p 1, rPh1 p 2, rPh1 p 5, and rBet v 2). Average percent inhibition achieved in each population is displayed for each of the natural extracts.

ninety-three sera collected from patients in different parts of the world who were allergic to grass pollen were preabsorbed with recombinant allergens. The remaining levels of grass pollen-specific IgE were measured by ELISA with nine different grass pollen extracts as a substrate. A surprisingly high level of average grass pollen-specific IgE (59%) was directed against the mixture of only four recombinant allergens. In certain populations a lower degree of inhibition (Sweden: 42%; Japan: 55%) was observed, which might reflect sensitization to different grass species.

Differences in the average percentage of inhibition were also observed regarding the natural extracts tested. Inhibition of IgE binding to pollen extracts prepared from species more closely related to timothy grass was higher (e.g., *An. odoratum*: 65%, *Av sativa*: 66%, *L. perenne*: 64%, *Po. pratensis*: 66%, *S. cereale*: 63%, and *T. sativum*: 63%) than to extracts from more distantly related species (*C. dactylon*: 47%, *Ph. australis*: 55%, and *Z. mays*: 45%).

To reveal whether the lower inhibition of IgE binding to these species reflects the presence of different allergens or is due to immunologic differences among the major allergens, we studied the contents of allergen groups in the extracts with defined antibodies by IgE inhibition experiments. The results of this analysis indicated that *C. dactylon*, *Ph. australis*, and *Z. mays* lacked immunologically active group 2 allergens. Group 5 allergens were not detectable in *C. dactylon* and *Z. mays*, which may at least in part explain the lower degree of inhibition achieved with the cocktail of recombinant allergens. Absence of allergen groups in certain grass species has been reported before^{11, 17} and may be the reason for the

significantly lower IgE binding capacity of natural extracts from *C. dactylon*, *Ph. australis*, and *Z. mays* that we observed in this study (data not shown). The heterogeneity of natural grass pollen extracts regarding their major allergen contents emphasizes the need for thorough standardization of such extracts by use of specific antibodies and, as exemplified in this study, with recombinant allergens.

Our results encourage the use of recombinant allergens that account for high levels of specific IgE for specific immunotherapy.³³ The idea of using few cross-reactive components for hyposensitization is not new and may now be applied for recombinant allergens as well. It was demonstrated that therapy with birch pollen extract alone was equally effective compared with immunotherapy performed with allergen mixtures prepared from several tree pollens.³⁴

In conclusion, our study demonstrated that in a high percentage of patients allergic to grass pollen (67.6%) a mixture of only four recombinant allergens accounted for more than 50% of IgE to nine different natural grass pollen extracts. These data encourage the use of recombinant pollen allergens not only for diagnosis but perhaps also for patient-tailored immunotherapy of grass pollen allergy.

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