

Short communication

Evaluation of IgE antibodies to recombinant pollen allergens (Phl p 1, Phl p 2, and Phl p 5) in a random sample of patients with specific IgE to *Phleum pratense*

Background: We measured specific IgE levels against the recombinant allergens (RAs) rPhl p 1, rPhl p 2, and rPhl p 5 in patients allergic to grass pollen, and examined the existence of different patterns of IgE production to RAs. The seasonal variations of IgE levels to rPhl p 1, rPhl p 2, and rPhl p 5 were considered, too.

Methods: Blood was taken from 276 consecutive patients with allergy to grass pollen diagnosed by patient history and skin prick testing. Total and specific serum IgE was measured by the immunoenzymatic CAP FEIA System. Eosinophil cationic protein (ECP) and myeloperoxidase (MPO) were assessed by radioimmunoassay according to the instructions of the manufacturers.

Results: We observed eight different patterns of IgE production to rPhl p 1, rPhl p 2, and rPhl p 5 in patients with specific IgE to timothy grass. A significant difference between the values of IgE levels to timothy and the sum of each level of specific IgE to individual RAs was found ($P=0.039$, Wilcoxon matched pairs test) in the whole population ($n=276$ subjects). In four subgroups of patients, the sum of each level of specific IgE to individual RAs was equal to the levels of specific IgE to timothy grass extract. In one subgroup, the sum of IgE to RAs was lower than the levels of IgE to the natural counterpart ($P=0.013$). A lack of subjects in two subgroups did not permit comparison at all. Finally, three subjects with specific IgE to timothy did not show specific IgE to RAs. Out of 276 patients, blood was taken from two different groups of subjects at different time points: November–January and May–July, respectively. The median values were as follows: total IgE = 139 kU/l, IgE to timothy = 10.2 kUA/l; IgE to rPhl p 1 = 3.6 kUA/l, to rPhl p 2 = <0.35 kUA/l, and to rPhl p 5 = 1.1 kUA/l; ECP = 8.25 µg/l; MPO = 303.08 µg/l (before exposure to grass pollen); total IgE = 159 kU/l, IgE to timothy = 57.2 kUA/l; IgE to rPhl p 1 = 22.1 kUA/l, to rPhl p 2 = 5.9 kUA/l, and to rPhl p 5 = 3.9 kUA/l; ECP = 16.21 µg/l; MPO = 413.09 µg/l (during the pollen season). There were significant variations of specific IgE levels between the patients exposed to pollen and the unexposed patients. Moreover, there were statistical differences in the IgE, ECP, and MPO levels in sera before and during the pollen season $P<0.035$, $P<0.017$, and $P<0.0062$, respectively.

Conclusions: The results suggest that RAs allow establishment of the patient's IgE-reactivity profile, encourage future research, and encourage manufacturers to produce further RAs for precise diagnosis and substantially improved immunotherapy injection of only those allergens against which significant amounts of specific IgE are produced.

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Key words: eosinophil cationic protein (ECP); IgE; myeloperoxidase (MPO); Phl p 1; Phl p 2; Phl p 5; recombinant allergens.

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Accepted for publication 10 September 1999

Grass-pollen extracts used for diagnosis and therapy are mixtures of different grass species and contain, beside nonallergenic components, a number of relevant and minor allergens (1, 2). For diagnostics and immunotherapy, the use of recombinant allergens

(RAs) seems to be a promising tool, since they are well-defined components and can be easily quantified (3).

It has previously been demonstrated that a panel consisting of a few RAs, rPhl p 1, rPhl p 2, rPhl p 5, and

profilin, was sufficient to diagnose grass-pollen allergy in a representative panel of patients allergic to grass pollen from different parts of the world (4). Another study demonstrated that in a high percentage of patients allergic to grass pollen (67.6%) a mixture of only four RAs accounted for more than 50% of IgE to nine different natural grass-pollen extracts (5). The group 4 allergen seems also to be important, although the levels of specific IgE are relatively low compared with groups 1 and 5 (6, 7).

This study aimed to describe the distribution of specific IgE to the *Phleum pratense* allergens rPhl p 1, rPhl p 2, and rPhl p 5 in order to study their relationship to each other, the possible existence of different patterns of IgE production to RAs, and the seasonal variations of IgE levels to RAs, in 276 consecutive patients allergic to timothy grass pollen.

Material and methods

Patients

The study involved 276 consecutive outpatients (147 males, aged 4–93 years, mean age 25.1 years; 129 females, mean age 26.2 years, range 7–69). All subjects had symptoms of rhinitis between April and July, and more than half also had symptoms of asthma and/or conjunctivitis. Because all patients lived in the province of Cuneo (Italy), they probably had similar exposure to grass pollen. None of the patients had received any form of specific immunotherapy to pollen extracts.

Skin prick test

All patients had positive responses to the skin prick test (SPT) performed with timothy grass-pollen extract (Neo Abelló, Madrid). Most of the patients (88.7%) displayed skin reactivity to various allergens (tree pollen, weed pollen, mites, animal danders, and molds). The results of SPT were evaluated by the EAACI guidelines (5).

In vitro tests

The 276 uniformly monthly distributed sera were obtained from March 1996 to March 1999 (mean month: 6.8) and contained timothy grass-pollen-specific IgE, as determined by the immunoenzymatic CAP method (Pharmacia, Uppsala, Sweden). Moreover, the sera were characterized in detail by determination of IgE antibodies to RAs (rPhl p 1, rPhl p 2, and rPhl p 5) by the CAP System (Pharmacia). Total IgE and specific IgE to nine common aeroallergens (house-dust mite, cat, mugwort, birch, pellitory of the wall, hazel, *Alternaria*, and *Cladosporium herbarum*) were also measured. Specific IgE levels were considered positive at levels of 0.35 kUA/l or higher (class 1).

Assessment of eosinophil cationic protein (ECP)

ECP assessment was done by radioimmunoassay (Pharmacia, Uppsala, Sweden) according to the instructions of the manufacturer. The normal range of ECP is 2.3–13.0 µg/l; total variation coefficients of the kit vary from 6.6% to 12%.

Assessment of myeloperoxidase (MPO)

MPO was measured in serum by radioimmunoassay, according to the manufacturer's instructions (Pharmacia). The normal range of MPO is 170–470 µg/l.

Statistics

Statistical analysis was performed with nonparametric tests (Wilcoxon matched pairs test, Mann–Whitney U test). The correlation index (r) was determined with the Spearman rank correlation coefficient matrix. Out of 276 subjects, 49 patients (17.8%) with specific IgE to timothy grass and 15 patients (5.4%) with specific IgE to RAs had IgE values above 100 kUA/l. In this case, the formula for missing data as described by Armitage (9) was used.

$$y_{lij} = \frac{rR_i + cC_j - T}{(r-1)(c-1)}$$

Results

We did not find sex differences in specific IgE to timothy extract and RAs (data not shown). The vast majority of the subjects with IgE to timothy grass also had specific IgE to rPhl p 1 (254 subjects, 92.03%). Specific IgE binding to rPhl p 5 and rPhl p 2 was detected, respectively, in 240 (86.96%) and in 164 (59.42%) out of 276 patients.

Varying patterns of IgE against different RAs, with relative percentage of sensitizations to other allergens, and median values of IgE levels to timothy and RAs are summarized in Table 1. In four subgroups of patients (rPhl p 1 + rPhl p 2 + rPhl p 5 +; rPhl p 1 + rPhl p 2 – rPhl p 5 +; rPhl p 1 + rPhl p 2 – rPhl p 5 –; rPhl p 1 + rPhl p 2 + rPhl p 5 –), the sum of each level of specific IgE to individual RAs was equal to the levels of specific IgE to timothy grass extract ($P > 0.05$) (Wilcoxon matched pairs test); rPhl p 1 – rPhl p 2 – rPhl p 5 + subgroup showed a sum of IgE to RAs lower than the value of IgE levels to the natural counterpart ($P = 0.013$). A lack of subjects in two subgroups (rPhl p 1 – rPhl p 2 + rPhl p 5 +; rPhl p 1 – rPhl p 2 + rPhl p 5 –) did not permit comparison at all. In the last subgroup (rPhl p 1 – rPhl p 2 – rPhl p 5 –) with specific IgE to timothy, IgE to RAs was lacking in sera.

Table 1. Varying patterns of IgE production against different recombinant allergens

	<i>n</i> (%)	Median kUA/l (2.5th–97.5th percentile)					Sum vs timothy	% PP
		rPhl p 1	rPhl p 2	rPhl p 5				
	276 (100)	9.5 (<0.35–>100)	1.7 (>0.35–64.0)	3.7 (<0.35–64.0)	24.9 (0.6–>100)	<i>P</i> =0.039	26.5 (0.9–>100)	88.7
rPhl p 1 + rPhl p 2 + rPhl p 5 +	149 (54.0)	23.1 (1.3–>100)	7.9 (0.5–>100)	20.1 (0.4–78.4)	53.1 (3.4–>100)	<i>P</i> =0.61	59.8 (2.7–>100)	91.3
rPhl p 1 + rPhl p 2–rPhl p 5 +	73 (26.4)	5.3 (0.6–49.4)	<0.35	2.0 (<0.35–61.8)	8.6 (1.5–93.7)	<i>P</i> =0.11	9.3 (1.0–>100)	91.8
rPhl p 1 + rPhl p 2–rPhl p 5–	20 (7.2)	2.1 (0.4–27.9)	<0.35	<0.35	2.1 (0.4–27.9)	<i>P</i> =0.53	2.9 (0.7–26.9)	95.0
rPhl p 1–rPhl p 2–rPhl p 5 +	16 (5.8)	<0.35	<0.35	0.4 (<0.35–33.7)	0.4 (<0.35–33.7)	<i>P</i> =0.013	3.2 (0.4–24.7)	87.5
rPhl p 1 + rPhl p 2 + rPhl p 5–	12 (4.3)	7.0 (0.4–35.3)	3.3 (1.0–20.0)	<0.35	9.5 (1.4–44.8)	<i>P</i> =0.38	14.1 (1.5–47.7)	100.0
rPhl p 1–rPhl p 2–rPhl p 5–	3 (1.1)	<0.35	<0.35	<0.35	<0.35	<i>P</i> =0.1	1.1 (0.4–1.4)	65.7
rPhl p 1–rPhl p 2 + rPhl p 5 +	2 (0.7)	<0.35	2.3 (1.1–3.5)	12.8 (0.4–25.1)	15.1 (4.0–26.3)	–	17.8 (4.8–30.9)	100.00
rPhl p 1–rPhl p 2 + rPhl p 5–	1 (0.4)	<0.35	1.1	<0.35	1.1	–	15.4	100.00

n: number of positive sera; Sum: sum of each level of specific IgE to individual recombinant allergens; PP: patients with specific IgE to timothy pollen and other aeroallergens.

To determine the seasonal variation of IgE reactivity to RAs, we took blood from two different groups of patients (out of 276) at different time points: November–January and May–July, respectively. The median value of serum total IgE and specific IgE levels were, as expected, lower during the November–January period. The results are summarized in Table 2 and Fig. 1. The median values of serum ECP and MPO are shown in Table 2 and Fig. 1, too.

In Spearman's ranking, IgE antibodies to the native timothy allergen were shown to be correlated ($P<0.05$) with the CAP results for Phl p 1 ($r=0.91$), Phl p 2 ($r=0.72$), Phl p 5 ($r=0.76$), and the sum of RAs ($r=0.97$) in the whole population.

Discussion

Seasonal variation of total and specific IgE levels to natural extracts are well recognized (10); our data confirm significant seasonal variations of total IgE and specific IgE against RAs, as well as a significant increase of inflammation markers (ECP, MPO) during the pollen season. In contrast, given that blood was taken from two different groups of patients at different time points, the claim of seasonal IgE rises is not supported by this study.

In the present study, we were able to observe eight different patterns of IgE production to rPhl p 1, rPhl p 2, and rPhl p 5 in patients allergic to timothy grass

pollen. Out of 276 allergic patients, more than half simultaneously had specific IgE against three RAs. These findings may reflect the impact of large amounts of grass pollen in this geographic area. On the other hand, it might be argued that IgE production to individual RAs may be under genetic control; therefore, eight varying patterns of IgE against RAs may represent corresponding phenotypes. However, we cannot exclude the possibility that the serum specific IgE to individual RAs might be increased during the pollen season, although the specific IgE levels were not detectable in the sera after the pollen season.

In a few sera, the sum of IgE directed against the three investigated RAs was lower than the IgE levels directed against timothy grass-pollen extract. This finding indicates that additional RAs need to be included in the grass-pollen allergy test panel. In fact, the use of a more complete panel of recombinant timothy grass pollen allergens for component-resolved diagnosis was recently reported (11).

Thus, it appears that RAs allow establishment of patients' IgE reactivity profile (allergogram) (12). A patient's allergogram may accurately direct the choice of a selected natural extract with the relevant RAs against which significant amounts of specific IgE are produced. Therefore, natural extracts with definite components may be used for successful specific immunotherapy. It appears reasonable to assume that future allergy diagnosis and specific therapy can

Table 2. Levels of total IgE and specific IgE to rPhl p 1, rPhl p 2, rPhl p 5, and timothy grass before and during seasonal exposure to grass pollen

	Sampling time period			
	November–January		May–July	
	Median kUA/l (2.5th–97.5th percentile)		Median kUA/l (2.5th–97.5th percentile)	
<i>n</i> =54				
Total IgE				
Timothy grass	10.2	(0.5–>100)	57.2	(1.5–>100)
rPhl p 1	3.6	(<0.35–58.1)	22.1	(<0.35–>100)
rPhl p 2	<0.35	(<0.35–20.0)	5.9	(<0.35–97.0)
rPhl p 5	1.1	(<0.35–55.7)	3.9	(<0.35–56.3)

n: number of positive sera.

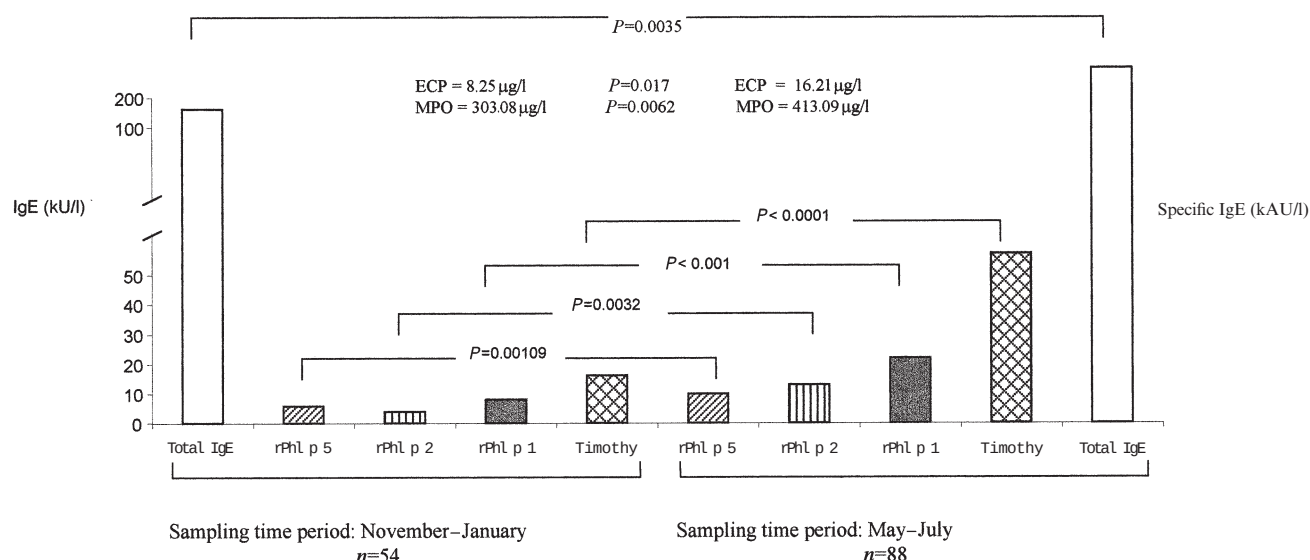


Figure 1. Median value of serum total IgE and specific IgE to recombinant allergens rPhl p 1, rPhl p 2, rPhl p 5, and their natural counterpart (timothy grass) in two groups of sera obtained from patients before and during seasonal exposure to grass pollen. Median values of ECP and MPO were evaluated, too. Mann-Whitney U test was used.

be improved by a component-based system using RAs (12). On the other hand, studies indicate that single post-translation modifications can alter the IgE

reactivity of allergens (13). This has to be taken into account when RAs are used for diagnostics and immunotherapy.

References

- KING TP, HOFFMAN D, LÖWENSTEIN H, MARSH DG, PLATTS-MILLS TAE, THOMAS W. Allergen nomenclature. Bull World Health Organization 1994;**72**:797–806.
- PETERSEN A, BUFE A, SCHRAMM G, SCHLAACK M, BECKER WM. Characterization of the allergen group VI in timothy grass pollen (Phl p 6). II. cDNA cloning of Phl p 6 and structural comparison to grass group V. Int Arch Allergy Immunol 1995;**108**:55–59.
- SCHEINER O, KRAFT D. Basic and practical aspects of recombinant allergens. Allergy 1995;**50**:384–391.
- LAFFER S, SPITZAUER S, SUSANI M, et al. Comparison of recombinant timothy grass pollen allergens with natural extract for diagnosis of grass pollen allergy in different populations. J Allergy Clin Immunol 1996;**98**:652–658.
- NIEDERBERGER V, LAFFER S, FRÖSCHIL R, et al. IgE antibodies to recombinant allergens (Phl p 1, Phl p 2, Phl p 5 and Bet v 2) account for a high percentage of grass pollen-specific IgE. J Allergy Clin Immunol 1998;**101**:258–264.
- LAFFER S, VRTALA S, DUCHÈNE M, et al. IgE-binding capacity of recombinant timothy grass (*Phleum pratense*) pollen allergens. J Allergy Clin Immunol 1994;**94**:88–94.
- FAHLBUSH B, MULLER WD, RUDESCHKO O, et al. Detection and quantification of group 4 allergens in grass pollen extracts using monoclonal antibodies. Clin Exp Allergy 1998;**28**:799–807.
- DREBORG S, FOUCARD T. Skin tests used in type I allergy testing. Position paper. Subcommittee on Skin Tests of the European Academy of Allergology and Clinical Immunology. Allergy 1989;**10**:1–59.
- ARMITAGE P. Statistical methods in medical research. Oxford: Blackwell Scientific, 1971.
- YUNGERLING JW, GLEICH GJ. Seasonal changes in serum and nasal IgE concentrations. J Allergy Clin Immunol 1973;**51**:174–179.
- VALENTA R, LIDHOLM J, NIEDERBERGER V, et al. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). Clin Exp Allergy 1999;**29**:896–904.
- VALENTA R, VRTALA S, LAFFER S, SPITZAUER S, KRAFT D. Recombinant allergens. Allergy 1998;**53**:552–561.
- PETERSEN A, SCHRAMM G, SCHLAACK M, BECKER WM. Post-translational modifications influence IgE reactivity to the major allergen Phl p 1 of timothy grass pollen. Clin Exp Allergy 1998;**28**:315–321.