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Short Communication

Distribution of specific serum IgE to recombinant pollen allergens (rPhlp1, rPhlp2, rPhlp5, and rBetv2) and their relationship to each other and to their natural counterparts in patients allergic to grass pollen

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Abstract

Recombinant pollen allergens rPhlp1, rPhlp2, rPhlp5, and rBetv2 may function as effective markers of atopy and account for a substantial proportion of grass pollen-specific IgE. The purpose of the present study was to determine the frequency of IgE antibodies to rPhlp1, rPhlp2, rPhlp5 and rBetv2 in patients allergic to grass pollen. Blood was taken from 436 patients, aged 4–70 years, with allergy to grass. The sample was stratified by 10-year age groups. Specific serum IgE were measured by the immunoenzymatic CAP Fluoroenzyme Immunoassay System. Specific IgE binding to rPhlp1, rPhlp5, rPhlp2, was, respectively, detected in 388 (88.9%), 353 (80.9%) and 265 (60.7%) of 436 patients. Sera from 252 patients (57.7%) showed IgE binding to rPhlp1, rPhlp2 and rPhlp5; sera from 102 patients (23.3%) reacted to rPhlp1 and rPhlp5, but not rPhlp2; 30 sera (6.8%) reacted to rPhlp1 but not rPhlp2 and rPhlp5; 22 sera (5.1%) reacted only to rPhlp5; one serum reacted to rPhlp2 (0.2%); and nine sera (2%) did not react to recombinant allergens. It was found that patients in the age group 0–20 years had higher IgE levels to timothy grass and rPhlp1, rPhlp2 and rPhlp5, than patients in the older age group (41–60 years). We found, as expected, seasonal variation of IgE levels to recombinant allergens and natural allergen. Allergens rPhlp1, rPhlp2 and rPhlp5 were extremely positively correlated with timothy grass and rBetv2, but not rBetv1. These results encourage the use of recombinant pollen allergens for diagnosis and to improve specific immunotherapy in the near future.

Key words: rBetv2, recombinant allergens, rPhlp1, rPhlp2, rPhlp5, timothy grass.

Introduction

Allergens constitute one of the most well-defined groups of biomedically important proteins.

Proteins and glycoproteins derived from pollen grains are the major source of allergens in the external environment in temperate climates and between 10 and 25% of the population show symptoms of hay fever and allergic asthma.[1]

Conventional allergen extracts, a heterogeneous mixture of allergenic and non-allergenic proteins, will allow neither identification of reactivities to the individual allergens nor measurement of the amount of specific IgE against certain allergens.[2] For allergologists, further advances in allergen biotechnology will result in the development of new products for the diagnosis of allergic diseases. Currently *in vitro,* as well *in vivo*, allergy diagnosis can be improved by a component-based system using recombinant allergens (RA).[3]

Recently, the complementary DNA coding for the major timothy grass allergens rPhlp1,[4] rPhlp2,[5] rPhlp5[6] and timothy grass pollen profilin[7] were isolated.

The purpose of the present study was to estimate the frequency of IgE antibodies to RA (rPhlp1, rPhlp2, rPhlp5 and rBetv2) and their natural counterparts, in patients allergic to grass pollen. In this way, we hoped to establish whether the determination of RA-specific IgE could be a helpful tool in the diagnosis of grass pollinosis.

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METHODS

The study involved 436 patients (240 males, mean age 25.6 years, range 4–66, 196 females, mean age 26.5 years, range 4–70) diagnosed as having rhinoconjunctivitis and/or asthma caused by grass pollen. They all had positive responses to skin-prick test (SPT) performed with timothy grass pollen extract (Neo Abellò, Madrid, Spain). The results of SPT were evaluated by European Academy of Allergology and Clinical Immunology guidelines.[8] None of the patients had previously undergone specific immunotherapy. Out of 436 patients, two subgroups of subjects were selected during two periods, November–January ($n = 77$) and May–July ($n = 139$), respectively, to evaluate seasonal variations of specific IgE.

In vitro tests

The RA were supplied by Pharmacia, Uppsala, Sweden.

Specific antibodies to RA (rPhlp1, rPhlp2, rPhlp5, rBetv1 and rBetv2) and other allergens were measured by the immunoenzymatic CAP Method (Pharmacia). Specific IgE tests were considered positive at levels 0.35 kU/L or higher (≥ class 1).

The distribution of specific IgE to RA and their natural extract were described as the median and stratified by 10-year age groups.

Statistics

The Mann–Whitney rank sum test was used for unpaired observations. The coefficient of correlation was calculated as Spearman's rho. The significance level chosen was $P < 0.05$.

Table 1 Frequencies of sensitization to recombinant allergens rPhlp1 rPhlp2 and rPhlp5 in patients allergic to grass pollen

rPhlp1	rPhlp2	rPhlp5	Total patient sensitizations
+	+	+	252 (57.7%)
+	–	+	102 (23.3%)
+	–	–	30 (6.8%)
–	–	+	22 (5.1%)
+	+	–	17 (3.8%)
–	–	–	9 (2%)
–	+	+	3 (0.7%)
–	+	–	1 (0.2%)

n = 436; IgE ≥ 0.35 kU/L.

Table 2 Median value of serum-specific IgE to different allergens

	Median (25th –75th percentile)							
	0–70 years ($n = 436$)	0–10 years ($n = 34$)	11–20 years ($n = 140$)	21–30 years ($n = 118$)	31–40 years ($n = 91$)	41–50 years ($n = 27$)	51–60 years ($n = 13$)	61–70 years ($n = 13$)
rPhlp1	13.62 (<0.35–45.03)	11.82 (2.93–26.74)	15.82 (3.64–45.01)	6.42 (1.74–27.23)	6.01 (1.28–16.37)	5.79 (1.68–13.02)	2.21 (0.88–3.74)	2.67 (<0.35–6.08)
rPhlp2	2.44 (0.35–14.24)	2.71 (<0.35–14.22)	3.21 (<0.35–13.82)	0.92 (<0.35–7.71)	1.29 (<0.35–6.58)	1.76 (<0.35–3.53)	4.59 (<0.35–5.36)	<0.35 (<0.35–0.47)
rPhlp5	3.32 (<0.35–38.72)	3.7 (0.43–27.61)	8.11 (0.41–38.74)	3.24 (0.35–29.52)	1.89 (0.36–18.26)	0.41 (<0.35–6.09)	1.12 (<0.35–2.88)	<0.35 (<0.35–8.56)
rBetv2	0.53 (<0.35–4.53)	<0.35 (<0.35–<0.35)	1.52 (<0.35–4.54)	0.52 (<0.35–2.33)	<0.35 (<0.35–<0.35)	<0.35 (<0.35–0.49)	<0.35 (<0.35–<0.35)	<0.35 (0.35–0.35)
rBetv1	4.51 (<0.35–35.03)	<0.35 (<0.35–0.35)	<0.35 (<0.35–4.22)	3.24 (<0.35–22.81)	6.48 (<0.35–30.31)	5.18 (<0.35–23.12)	7.28 (0.51–35.04)	<0.35 (<0.35–8.02)
Timothy grass	24.71 (0.64–>100)	25.24 (4.73–>100)	27.51 (4.44–89.71)	19.21 (3.74–58.61)	10.42 (3.19–38.82)	4.27 (1.34–19.14)	4.06 (1.58–18.28)	2.03 (0.58–6.89)
Birch	7.62 (0.61–30.64)	3.32 (1.24–10.62)	5.12 (1.53–18.82)	6.29 (1.41–17.89)	8.88 (1.74–30.63)	4.09 (0.78–16.22)	2.78 (0.59–13.49)	2.93 (0.79–9.18)

Median values (kU/L)of serum-specific IgE to different recombinant allergens, timothy grass and birch extracts by 10-year age groups in 436 patients allergic to grass pollen, 269 (61.7%) of which had specific IgE to birch, too.

Table 3 Median value of serum specific IgE to recombinant allergens, rPhlp1, rPhlp2, rPhlp5, Betv2, Betv1, timothy grass and birch in two groups of sera obtained from patients before and during the seasonal exposure to grass pollen

	May–July (n = 139)	November–January (n = 77)	P value
rPhlp1	3.88 (0.82–16.33)	10.18 (3.13–32.88)	0.023
rPhlp2	0.97 (<0.35–7.38)	2.48 (<0.35–12.68)	0.113
rPhlp5	1.96 (<0.35–11.04)	2.41 (<0.35–25.38)	0.0451
Betv2	0.37 (<0.35–0.69)	<0.35 (<0.35–1.87)	0.002
Betv1	<0.35 (<0.35–0.35)	<0.35 (<0.35–0.37)	0.0126
Timothy grass	11.46 (2.57–50.13)	28.84 (4.41–83.77)	0.0009
Birch	0.74 (<0.35–6.97)	0.94 (<0.35–8.46)	0.579

Measurements are the median (25th–75th percentile), in kU/L. P values were determined using the Mann–Whitney U-test.

Table 4 Median values of serum specific IgE to recombinant allergens by 20-year age groups

	0–20 years (n=174)	41–60 years (n=40)	P value
rPhlp1	16.32 (2.88–45.03)	4.68 (1.68–13.04)	0.00005
rPhlp2	4.28 (<0.35–14.18)	2.38 (<0.35–5.38)	0.026
rPhlp5	2.21 (0.41–38.68)	0.78 (<0.35–6.09)	0.00009
Betv2	1.38 (<0.35–4.46)	<0.35 (<0.35–0.48)	0.346
Betv1	<0.35 (<0.35–4.18)	5.37 (<0.35–35.02)	0.0032
Timothy grass	27.09 (4.39–>100)	4.34 (1.31–19.14)	0.0351
Birch	4.12 (1.18–18.78)	3.88 (0.57–16.24)	0.023

Measurements are the median (25th –75th percentile), in kU/L. P values were determined using the Mann–Whitney U-test.

Table 5 Correlation between specific IgE to allergens from patients allergic to grass pollen

	n	r	P
rPhlp1 versus			
Timothy	230	0.90	<0.001
rBetv2	200	0.43	<0.001
rBetv1	197	0.08	>0.05
rPhlp2 versus			
Timothy	230	0.71	<0.001
rBetv2	200	0.31	<0.001
rBetv1	197	0.07	>0.05
rPhlp5 versus			
Timothy	230	0.79	<0.001
rBetv2	200	0.47	<0.001
rBetv1	197	0.18	>0.05

Results

We did not find sex differences in specific IgE to RA (data not shown). Sera of 57 subjects with CAP-negative IgE to seven common aeroallergens (mites, cat, timothy grass, birch, mugwort, pellitory of the walls and *Cladosporium herbarum*) did not show specific IgE to individual RA.

The vast majority of subjects with IgE to timothy grass also had specific IgE to rPhlp1 (388 subjects, 88.9%). Specific IgE binding to rPhlp5 and rPhlp2 was, respectively, detected in 353 (80.9%) and 265 subjects (60.7%) of 436 patients. The 228 patients (52.2%) allergic to grass had specific IgE to Betv2. Of 436 patients allergic to grass pollen, 269 (61.7%) subjects also had specific IgE to birch.

Of 436 patients, 252 (57.7%) showed IgE binding to rPhlp1, rPhlp2 and rPhlp5; sera from 102 patients (23.3%) reacted to rPhlp1 and rPhlp5, but not rPhlp2. The patients were divided into eight groups depending on the sensitization pattern of RA (Table 1).

Median values of specific IgE levels to rPhlp1, rPhlp2, rPhlp5, rBetv1, rBetv2, timothy grass and birch are summarized in Table 2.

Seasonal variations of IgE levels to RA and natural extracts are summarized in Table 3.

In the present study, we found that patients in the age group 0–20 years had higher IgE levels to timothy grass, rPhlp1, rPhlp2 and rPhlp5 than patients in the older age group (Table 4).

As expected, rPhlp1, rPhlp2 and rPhlp5 were extremely positively correlated with timothy grass and rBetv2 (profilin), but not rBetv1 (Table 5).

Discussion

Previously, on evaluating the results of IgE determinations to rPhlp1 in sera from allergic patients, Laffer *et al*. had found that group 1 allergens represent a target for IgE antibodies in more than 95% of patients allergic to grass pollen.[4,9] In our study, 88.9% of patients allergic to grass showed specific IgE to rPhlp1. In a recent study, it has been demonstrated that Phl1 exhibits a trypsin-like proteolytic activity when assayed with various test systems and substrates.[10] This observation strongly suggests a possible cause for the high IgE prevalence in individuals allergic to group 1 allergens. Group 2 allergens are recognized by IgE antibodies of 60% of patients allergic to grass pollen.[5] In our patients, 60.7% of sera had detectable specific IgE antibodies to rPhlp2. Seventy to eighty percent of patients allergic to grass pollen react to group 5 allergens.[6] Our findings showed that 80.9% of sera had IgE to rPhlp5. Finally, Vrtala *et al*. have found that 20% of patients allergic to grass display IgE reactivity to profilins.[11] In our patients, the percentage of sensitization to rBetv2 was 52.2%. This last difference may be due to non-uniform selection of study patients and to geographic differences in the study group.

Another finding of our study is that the patients affected by grass pollen allergy were divided into eight groups, depending on the sensitization pattern of rPhlp1, rPhlp2 and rPhlp5. In a group of patients allergic to grass of the same geographic area, the existence of eight varying patterns of IgE production to RA can evoke two considerations: (i) the degree of patient's sensitization to a single RA is due to the duration of exposure to major timothy grass pollen allergen; or (ii) certain patterns of sensitization to RA may reflect a genetic background. The levels of specific IgE of each recombinant allergen are lower than those of timothy grass (Table 2), because in the natural counterpart many allergens are simultaneously present. In fact, timothy grass contains an allergen that has not been detected in other grasses, namely Phlp6.[12] Moreover, group 4 allergens have been described in a number of grasses (*Phleum pratense, Lolium perenne, Dactylis glomerata*).[13]

Our findings indicate that subjects in the age group 0–20 years had higher IgE levels to timothy grass and RA than subjects in the older age group, whereas patients aged 41–60 years had higher IgE levels against birch and Betv1 than patients of 0–20 years of age.

Our findings that grass pollen sensitization is associated with higher IgE than birch allergens may be due to a higher exposure to grass. Another possibility is that a high specific IgE indicates more activation of the immune system, resulting in an increased risk of sensitization to specific allergens, especially in younger age groups. However, the delayed increase of specific IgE to birch allergens in the older age group may reflect a lower, but subcontinuous exposure to Betv1 than to the grass RA.

Seasonal variation of specific IgE levels to pollen allergens are well recognized[14] and our data confirm seasonal significant variations of specific IgE to pollen extracts and RA.

Further studies with a large number of patient sera as well as skin-prick test are necessary to further evaluate the immunologic data and especially confirm the usefulness of other RA in the diagnosis of grass pollen allergy.

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