

Original article

Association of allergic patients' phenotypes with IgE reactivity to recombinant pollen marker allergens

Background: During the last decade allergen molecules from several allergen sources have been produced by recombinant DNA technology. The aim of this study was to investigate whether IgE reactivity to recombinant pollen allergens with broad and narrow cross-reactivity is associated with clinical phenotypes of allergic sensitization.

Methods: Serum IgE reactivity to a panel of six recombinant birch and grass pollen allergens was measured by ELISA in pollen sensitized patients from Central Europe to define groups of patients with exclusive IgE reactivity to rBet v 1, with exclusive reactivity to major grass pollen allergens (rPhl p 1, rPhl p 2, rPhl p 5) and with IgE reactivity to cross-reactive pollen allergens (rBet v 2, rPhl p 7). Patients' clinical phenotypes were recorded. IgE responses to tree, grass and weed pollen as well as plant food extracts were evaluated *in vitro* by CAP-FEIA and clinical sensitivities were confirmed *in vivo* by skin prick testing.

Results: IgE reactivity to the recombinant major birch pollen allergen, rBet v 1, was associated with sensitization to pollen from birch, taxonomically related trees and to certain plant-derived food. Reactivity to the recombinant timothy grass pollen allergens, rPhl p 1, rPhl p 2, rPhl p 5, indicated sensitization to pollen from grasses. Patients reacting with the highly cross-reactive allergen rPhl p 7 were polysensitized to pollen from unrelated trees, grasses and weeds and rBet v 2-positive patients were polysensitized to pollen and plant-derived food from unrelated plants.

Conclusions: IgE reactivity to recombinant marker allergens is associated with clinical phenotypes of allergic sensitization and may be useful for the selection of treatment strategies.

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Key words: component-resolved diagnosis; cross-reactivity; pollen allergy; recombinant marker allergens; sensitization profile.

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Accepted for publication 4 September 2009

Diagnosis of Type I allergy is based on the documentation of a positive case history, *in vivo* provocation tests (e.g., skin prick test, nasal, oral and bronchial challenge) and the *in vitro* detection and measurement of allergen-specific IgE antibodies in patient's serum (1). Natural allergen extracts used for allergy diagnosis and immunotherapy have a number of disadvantages: they contain many different, also nonallergenic proteins and various ill-defined substances (e.g., carbohydrates, enzymes, and fats). The standardization of natural allergen extracts is difficult and the allergen contents and composition of

extracts vary from manufacturer to manufacturer and from batch to batch (2–6). Extracts can lack certain allergenic proteins which might have got degraded during the production process and storage and they can be contaminated with proteins from other allergen sources which can deliver false diagnostic results (7). Moreover, allergy tests performed with natural extracts determine only the allergen source a patient reacts with but do not give information about the nature and the number of the disease-eliciting molecules the patient is sensitized to (8).

The identification and characterization of allergenic molecules by cDNA cloning allows the large scale production of purified recombinant allergens. Biological and immunological studies of these recombinant proteins indicate that they are suitable tools for allergy diagnosis (9–12). Many allergens of related and even unrelated sources show structural similarity (13–15) leading to IgE cross-reactivity. Based on this fact allergenic proteins from different sources can be grouped into ‘immunologically related’ allergens (15, 16). Within such a group patients sensitized to one (13) allergen may also react to homologous proteins of other sources. Among immunologically related allergens the allergen containing most IgE binding epitopes can be used as the representative marker allergen (17) for diagnostic purposes.

Marker allergens from pollen can be species-specific and IgE reactivity to such marker allergens then indicates genuine sensitization to certain taxonomically closely related allergen sources (e.g., group 1, 2, 5 grass pollen allergens) (18–20). A second group of marker allergens is formed by allergens which occur in pollen of taxonomically related sources (e.g., trees of the order Fagales) and also in certain unrelated sources of plant-derived food (e.g., Bet v 1) (21–23). A third group of marker allergens represents highly cross-reactive proteins which occur in many distantly related allergen sources [e.g., the panallergen profilin (24) and the cross-reactive calcium binding pollen allergens (25–27)].

The aim of our study was to investigate a panel of recombinant pollen allergens regarding their diagnostic value as marker allergens in a Central European population. rBet v 1 (21) was used as a potential marker for allergy to pollen from birch and related trees (Fagales) and to certain plant-derived food, rPhl p 1, rPhl p 2 and rPhl p 5 (19) as markers for genuine grass pollen allergy and the profilin rBet v 2 (24) and the calcium-binding pollen allergen rPhl p 7 (27) as markers for broad sensitization.

To reveal if the *in vitro* reactivity to these marker allergens is associated with clinical phenotypes of allergic sensitization to certain allergen sources, clinical symptoms were recorded and skin responses and IgE reactivity to allergen extracts were studied.

Methods

Recombinant allergens

Recombinant allergens from birch pollen, rBet v 1 and rBet v 2, and timothy grass pollen, rPhl p 1, rPhl p 2 and rPhl p 5, were obtained from BIOMAY (Vienna, Austria). rPhl p 7 was expressed in *Escherichia coli* strain BL21/DE3 and purified to homogeneity as described (27).

Pollen allergic patients

Sera from patients suffering from pollen and/or plant-derived food allergy were analysed. Patients were characterized by a positive case

history of Type I allergy to pollen allergens and the presence of at least one of the following symptoms: conjunctivitis, asthma, urticaria, atopic dermatitis, and allergic symptoms after ingestion of food. Sera were first tested regarding their IgE reactivity to recombinant pollen allergens to identify a group of patients exclusively reacting with rBet v 1 (B1–B10), a group of patients reacting with the recombinant major grass pollen allergens rPhl p 1, rPhl p 2 and rPhl p 5 (G1–G11) and a group reacting with the cross-reactive pollen allergens rBet v 2 and/or rPhl p 7 (P1–P9) (Table 1). Skin prick testing with commercial allergen extracts was performed in the course of routine diagnosis after informed consent was obtained from the patients, to investigate which clinical phenotypes are associated with the IgE reactivity profiles to the marker allergens. The demographic and clinical data of the groups are displayed in Table 1.

Quantification of total serum IgE, of specific IgE to pollen and food extracts and of specific IgE to recombinant allergens

The serum samples of the allergic individuals were tested for their IgE reactivity to recombinant allergens by ELISA as described by Stern et al. (28). Total serum IgE levels (kU/l) and specific IgE levels (kUA/l) were determined using the CAP-FEIA system (Phadia, Uppsala, Sweden). Specific IgE levels were measured to tree pollen extracts from birch (*Betula verrucosa*), olive (*Olea europea*) and ash (*Fraxinus excelsior*), to grass pollen extracts from timothy grass (*Phleum pratense*), rye-grass (*Lolium perenne*), cocksfoot (*Dactylis glomerata*) and rye (*Secale cereale*) and to weed pollen extracts from mugwort (*Artemisia vulgaris*), common ragweed (*Ambrosia elatior*), English plantain (*Plantago lanceolata*) and *Parietaria officinalis*. Food-specific IgE levels were determined using extracts from apple (*Malus domestica*), hazelnut (*Corylus avellana*), peanut (*Arachis hypogaea*) and celery (*Apium graveolens*).

Skin prick testing

Skin prick tests were performed with commercially available extracts (ALK, Horsholm, Denmark) from all the above mentioned allergen sources as well as from alder pollen. Prick-to-prick tests were performed with apples (variety Golden Delicious) purchased at a local market. Histamine solution (ALK, Horsholm, Denmark) was used as positive control and 0.9% sodium chloride as the negative control (29). The mean wheal diameter (DM) was calculated as $(D1 + D2)/2$, where D1 represents the maximal longitudinal diameter and D2 the maximal transversal diameter.

Results

Serological testing with recombinant marker allergens rBet v 1, rPhl p 1, rPhl p 2, rPhl p 5, rPhl p 7 and rBet v 2 identifies patients with distinct reactivity profiles

IgE reactivity to the recombinant major pollen allergens, rBet v 1, rPhl p 1, rPhl p 2, rPhl p 5, and to the recombinant cross-reactive pollen allergens, rPhl p 7 and rBet v 2, was analysed by ELISA in sera from patients suffering from pollen and plant-derived food allergies. According to the IgE reactivity profiles, three groups of allergic patients could be discriminated from patients showing mixed IgE reactivity to the recombinant allergens (Table 1).

Table 1. Demographic, serological and clinical characterization of patients

Patients	Sex	Age (years)	Serum IgE reactivity determined by ELISA to				Allergies	Symptoms	Total IgE (kU/l)
			rBet v 1	rPhl p 1/rPhl p 2/rPhl p 5	rBet v 2/rPhl p 7				
B1	F	26	+	-/-/-	-/-	t, f	rc	158	
B2	F	48	+	-/-/-	-/-	t, a	rc, oas	172	
B3	F	24	+	-/-/-	-/-	t, f, a	rc	197	
B4	F	33	+	-/-/-	-/-	t, f	rc, as, oas	286	
B5	M	44	+	-/-/-	-/-	t, f	rc, oas	42	
B6	F	27	+	-/-/-	-/-	t, a, mi	rc, as, ad, oas	204	
B7	M	53	+	-/-/-	-/-	t	rc, as	177	
B8	F	38	+	-/-/-	-/-	t, f	rc, as	120	
B9	M	23	+	-/-/-	-/-	t, f, a	rc, oas	205	
B10	M	38	+	-/-/-	-/-	t, f	rc, oas	110	
G1	M	28	-	+/+/+	-/-	g, w, mi	rc, oas	108	
G2	M	26	-	+/+/+	-/-	g, f, a, mi	rc, as	131	
G3	M	24	-	+/+/+	-/-	g, f, a, mi	rc	163	
G4	M	27	-	+/+/+	-/-	g	rc	55	
G5	F	28	-	+/-/+	-/-	g, a	rc	195	
G6	M	31	-	+/+/+	-/-	g, w, mi	rc, as	283	
G7	M	28	-	+/+/+	-/-	g, a, mi	rc	86	
G8	F	22	-	+/+/+	-/-	g	rc	152	
G9	M	29	-	+/-/+	-/-	g, mi	rc	204	
G10	M	39	-	+/-/+	-/-	g, mi	rc	195	
G11	M	24	-	+/+/+	-/-	g	rc	31	
P1	M	28	-	+/+/+	-/+	t, g, w	rc	148	
P2	M	32	+	+/+/+	-/+	t, g, w, f	rc, ad	125	
P3	M	46	+	+/+/+	+/+	t, g, w, f, a, mi	rc, as, oas	1612	
P4	M	29	+	+/+/+	+/+	t, g, w, f, a	rc, as, u, oas	595	
P5	F	33	+	+/+/+	+/+	t, g, w, f	rc, as, ad, oas	243	
P6	M	24	+	+/+/+	+/+	t, g, w, f, a	rc, as, oas	380	
P7	M	26	+	+/+/+	+/+	t, g, w, f	rc, as, oas	>2000	
P8	M	29	+	+/+/+	+/+	t, g, w, f, a	rc, u, oas	215	
P9	F	35	+	+/+/+	+/+	t, g, w, f, a	rc, oas	168	

Total IgE measured by CAP-FEIA is expressed in kU/l; t, tree pollen; f, food; a, animals; mi, mites; g, grass pollen; w, weed pollen; mo, molds; rc, rhinoconjunctivitis; as, asthma; ad, atopic dermatitis; u, urticaria; oas, oral allergy syndrome; +, IgE reactivity to recombinant allergen(s); -, no IgE reactivity to recombinant allergen(s).

Group I comprised 10 individuals (B1–B10) with serum IgE reactivity solely to rBet v 1. Group II included eleven individuals with serum IgE reactivity to at least one of the three major grass pollen allergens (rPhl p 1, rPhl p 2 and rPhl p 5) (G1–G11). Eight of these patients contained IgE antibodies to all three timothy grass pollen allergens, whereas three (G5, G9 and G10) reacted with rPhl p 1 and rPhl p 5, but not with rPhl p 2. Group III consisted of nine individuals displaying IgE reactivity to the cross-reactive allergens rBet v 2 and/or rPhl p 7 (P1–P9). Among these patients all nine reacted with rPhl p 7, whereas seven (P3–P9) showed IgE reactivity to rBet v 2. All patients of this group also reacted with rPhl p 1, rPhl p 2 and rPhl p 5 and eight (P2–P9) had IgE antibodies to rBet v 1.

IgE reactivity to rBet v 1 identifies patients with clinical symptoms to pollen from birch and related trees as well as to birch pollen associated food

In vitro analysis of patients' responses to tree, grass and weed pollen as well as plant food extracts by CAP-FEIA revealed that all 10 individuals of group I, with IgE

reactivity solely to rBet v 1, but not to recombinant grass pollen allergens reacted with birch pollen extract in the CAP-FEIA system (Fig. 1A). Each patient of this group also displayed IgE reactivity to apple extract, eight (B1, B3, B4, B6–B10) to hazelnut extract and seven (B1, B3, B4–B6, B8, B9) to celery extract. In four individuals of group I (Fig. 2A; B1, B7, B9, B10) sensitivity to pollen and food extracts was further confirmed by *in vivo* skin prick test experiments. Positive wheal reactions to birch pollen, apple, hazelnut and to pollen from alder occurred in all four patients and thus confirmed *in vitro* data obtained by CAP-FEIA. Positive skin reactions to celery were not only induced in patients B1 and B9, who showed celery-specific IgE in CAP-FEIA, but also weakly in patient B7, who displayed no *in vitro* IgE reactivity to celery extract. No patient of group I displayed relevant *in vitro* or *in vivo* reactivity to any of the tested grass pollen extracts. The data thus suggest that IgE reactivity to rBet v 1 depicted patients allergic to pollen from birch and related trees as well as to certain plant-derived foods (e.g. apple, hazelnut, and celery). Clinical case history of group I patients further supported these findings (Table 1): All

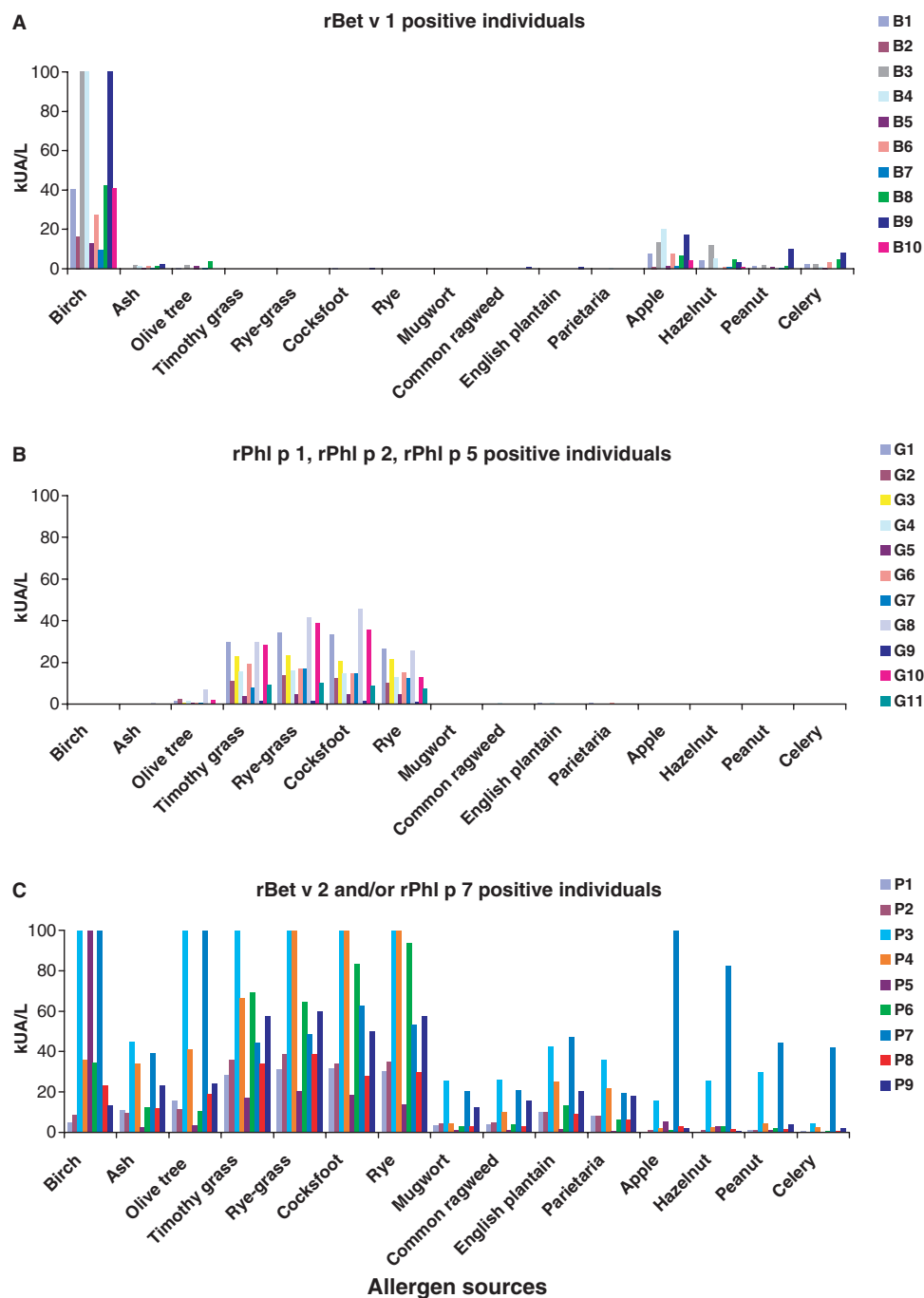


Figure 1. Serum IgE reactivity to allergen extracts in patients with IgE reactivity to rBet v 1 (A), to rPhl p 1, rPhl p 2, rPhl p 5 (B) and to rBet v 2 and/or rPhl p 7 (C). Specific IgE levels (kUA/L, y-axes) to extracts of tree pollen (birch, ash, and olive tree), grass pollen (timothy grass, rye-grass, cocksfoot, and rye), weed pollen (mugwort, common ragweed, English plantain, and *Parietaria*) and food (apple, hazelnut, peanut, and celery) were determined by CAP-FEIA measurements and are displayed for each patient.

10 patients of group I reported allergic symptoms (rhinoconjunctivitis) upon exposure with birch pollen and four of them (B4, B6, B7, B8) even suffered from asthmatic episodes during the birch pollen season. Seven patients of group I reported oral and pharyngeal itching after consumption of certain fruits, nuts and vegetables.

In addition to the strong responses observed in the majority of patients, some additional co-sensitizations to other pollen and food extracts were also observed among group I individuals. Weak IgE reactivity to peanut extract was detected by CAP-FEIA in six patients (B1, B3, B5, B7–B9) and was corroborated by positive wheal reactions

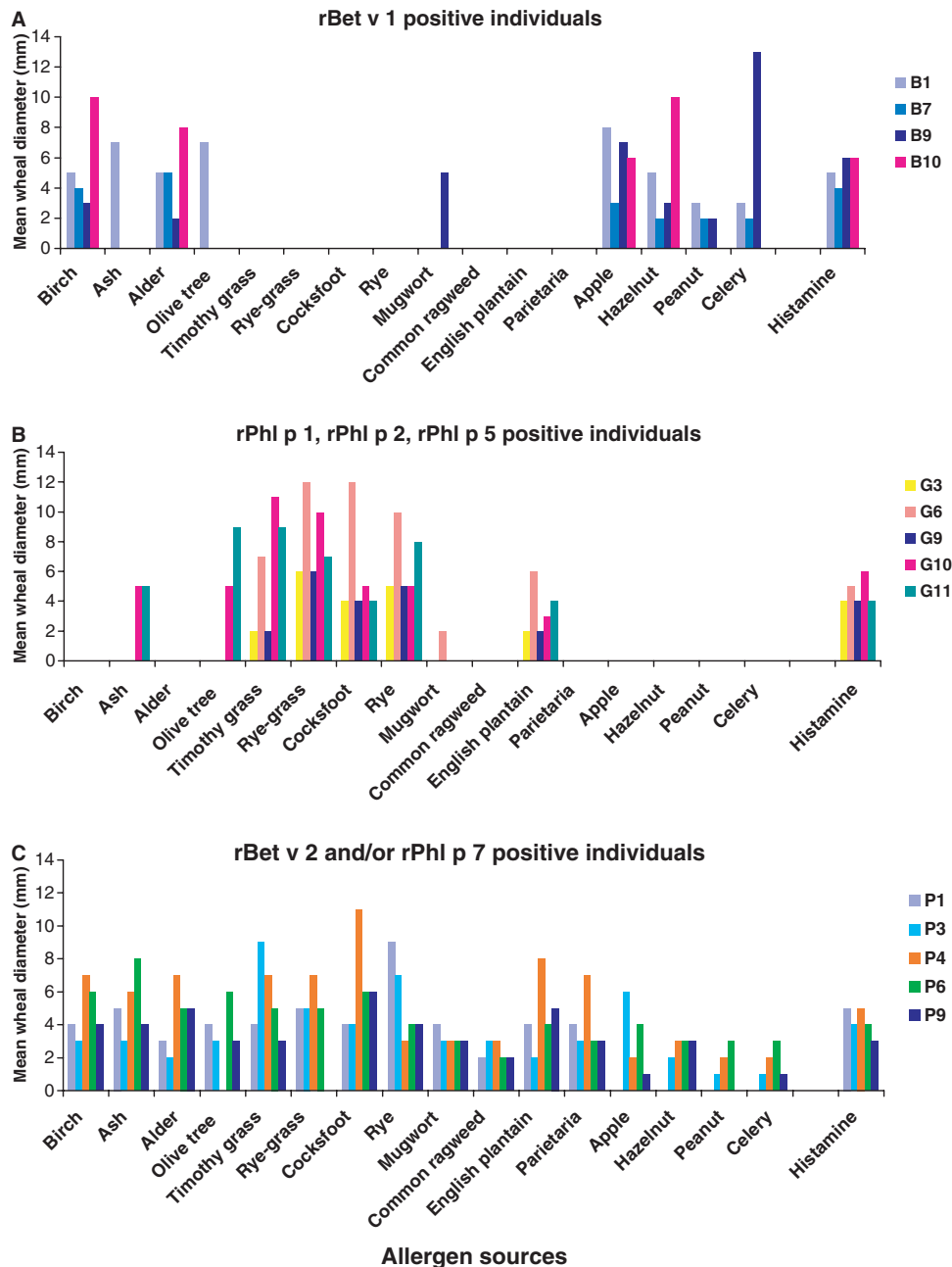


Figure 2. Immediate skin reactions in patients with IgE reactivity to rBet v 1 (A), to rPhl p 1, rPhl p 2, rPhl p 5 (B) and to rBet v 2 and/or rPhl p 7 (C). Patients were skin prick tested with tree pollen extracts (birch, ash, alder, and olive tree), grass pollen extracts (timothy grass, rye-grass, cocksfoot, and rye), weed pollen extracts (mugwort, common ragweed, English plantain, *Parietaria*) and food extracts (hazelnut, peanut, celery) and prick-to-prick tested to apple. Mean wheal diameters (mm, y-axes) are displayed for each tested patient.

to peanut in the skin prick tested patients (B1, B7, and B9). Furthermore, low IgE reactivity to ash pollen extract was observed in seven (B3–B9) and to olive pollen extract in five patients of group I (B1, B3, B5, B7, and B8). In patient B1 ash and olive pollen extract also induced a positive cutaneous reaction. Interestingly, patient B9 displayed a unique, pronounced immediate-type skin reaction upon application of mugwort extract, which was not reflected by IgE reactivity testing.

IgE reactivity to rPhl p 1, rPhl p 2 and rPhl p 5 identifies patients allergic to grass pollen

All 11 individuals of group II with serum IgE reactivity to the recombinant timothy grass pollen allergens rPhl p 1, rPhl p 2 and/or rPhl p 5, but not to rBet v 1, reacted in CAP-*FEIA* not only with timothy grass pollen extract, but also with pollen extracts of the other tested grass species (rye-grass, cocksfoot and rye; Fig. 1B). However, they showed

no IgE reactivity to extracts of birch pollen or plant-derived food. *In vivo* skin prick tests performed in five patients of group II (3, G 6, G9–G11) confirmed the *in vitro* data obtained by CAP-FEIA: all five patients showed positive skin prick reactions to pollen extracts of timothy grass, rye-grass, cocksfoot and rye (Fig. 2B), but no immediate-type skin reactions were induced after application of birch pollen or food extracts. The conclusion that IgE reactivity to rPhl p 1, rPhl p 2 and/or rPhl p 5 identified grass pollen allergic patients was also supported by analysis of the clinical history of the patients (Table 1). All patients reported allergic symptoms, mainly rhino-conjunctivitis, upon exposure to grass pollen. Two patients (G2, G6) reported even asthmatic episodes and the use of anti-asthmatic sprays during the grass pollen season.

Besides the described responses some patients of group II showed *in vitro* and *in vivo* reactivities to additional pollen allergen sources (Figs 1B and 2B).

IgE reactivity to the highly cross-reactive allergens rBet v 2 and rPhl p 7 identifies polysensitized patients

All nine patients of group III with IgE reactivity to rBet v 2 and rPhl p 7 displayed IgE reactivity to almost all tested pollen and food extracts (Fig. 1C). In five of these patients (P1, P3, P4, P6, and P9) also *in vivo* skin prick experiments were performed, which supported the *in vitro* results by showing that nearly all of these extracts were able to provoke immediate-type skin reactions in the patients (Fig. 2C). The assumption that IgE reactivity to the cross-reactive recombinant allergens rBet v 2 and rPhl p 7 identified patients polysensitized to many allergen sources was proven by the clinical data of group III patients (Table 1). They all had allergic symptoms upon exposure to pollen of different trees, grasses, weeds and after ingestion of food.

Discussion

Our results indicate that it may be possible to predict clinical phenotypes of allergic sensitization in patients allergic to pollen and plant-derived food based on serum IgE reactivities to selected recombinant birch and grass pollen allergens (rBet v 1, rBet v 2, rPhl p 1, rPhl p 2, rPhl p 5, and rPhl p 7). By screening sera from pollen allergic patients from Central Europe for IgE reactivity with the recombinant pollen allergens, we found that patients who exhibited IgE reactivity only to the major birch pollen allergen rBet v 1, but not to the other marker allergens, had a clinical sensitization to tree pollen of the order Fagales (e.g. birch, alder, hazel) and to certain foods (e.g. apple, celery, and hazelnut). This finding can be explained by cross-reactivity of Bet v 1 with allergens that are present in taxonomically related trees and certain plant derived food (17, 30). One out of

the 10 patients with exclusive rBet v 1 reactivity showed a positive skin test result with mugwort pollen extract, an allergen source that lacks a Bet v 1 cross-reactive allergen, but had no symptoms during the flowering season of mugwort.

The group of patients with exclusive serum IgE reactivity to the timothy grass pollen allergens rPhl p 1, rPhl p 2 and rPhl p 5 exhibited a clinical phenotype of predominant sensitization to grasses (e.g. timothy grass, rye grass, rye, and orchard grass). Two of the 11 patients reported clinical symptoms to weed pollen, but had no detectable IgE antibodies to any of the common weed extracts (mugwort, ragweed, and *Parietaria*). The major grass pollen allergens are known to occur exclusively in grass pollen and hence allow to identify patients with genuine sensitization to grass pollen (20). A further differentiation of the primary sensitizing grass species may be obtained by the presence or absence of reactivity to rPhl p 2 and rPhl p 5. Whereas rPhl p 1 is expressed in pollen of all grass species, rPhl p 2 and rPhl p 5 are known to be present only in a subgroup of grasses, the Pooideae (e.g. timothy grass, rye grass), but not in maize or Bermuda grass.

Reactivity to the birch pollen profilin, rBet v 2, and the calcium-binding allergen rPhl p 7 from timothy grass identified patients displaying a clinical phenotype of polysensitization to numerous pollen allergen and plant food allergen sources. This broad sensitization may result from cross-reactivity to profilin (24) and/or to the calcium binding allergen, Phl p 7 (27). Birch pollen profilin Bet v 2 represents a panallergen, present not only in pollen but in all eukaryotic cells, which can be responsible for the clinical symptoms caused by pollen of various plants and plant-derived food. By contrast, the Phl p 7 cross-reacting allergens occur exclusively in pollen from trees, grasses and weeds. These allergens might cause allergic symptoms upon pollen exposure from spring to autumn. It is equally possible that clinical symptoms to tree, grass, weed pollen and plant food observed in this group are caused by co-sensitization to major allergens such as Bet v 1, the major grass pollen allergens or other major allergens not tested by us.

The reactivity profile of patient P1 represents a good example for the possible predictive value of the recombinant allergens. Patient P1 shows IgE reactivity to rPhl p 7 and to the grass pollen marker allergens, but not to rBet v 1 and rBet v 2. The patient was polysensitized and displayed allergic symptoms upon exposure to tree, grass and weed pollen. As a result of the absence of IgE reactivity to rBet v 1 and rBet v 2, this patient had no allergic symptoms upon ingestion of food.

In this study, discrepancies between the results of CAP-FEIA and skin prick tests were observed. These discrepancies could be based on the fact that the extracts with which these two assays were performed originate from different commercial sources. Moreover, different processing of the allergen sources might have resulted in differences in the allergen contents.

The use of marker allergens for IgE reactivity testing enables the allergologist to consider allergen sources to which the patient may potentially react or develop reactivity, so that advice regarding possible allergen avoidance can be made. It is possible that at the time of diagnosis the patient does not yet suffer from clinical symptoms to all allergen sources despite showing *in vitro* reactions, because levels and/or affinity of specific IgE antibodies might be too low to trigger a clinical reaction (31, 32), but the sensitization process may be already in progress. This is supported by the observation that many allergic individuals report a clinical history with allergic symptoms to an increasing number of allergen sources in the course of illness years (33).

The results of our study also indicate that additional marker allergens should be included in diagnostic testing to explain the observed additional reactivity patterns (e.g. olive, ash, and peanut). Using Ole e 1 as a marker for Oleaceae sensitization (34), Parij 2 as a marker for weed pollen sensitization (35), allergen markers from the group of mite and mould allergens and for instance lipid transfer

proteins as markers for botanically unrelated food (36) it may be possible to detect additional genuine sensitizations to other allergen sources. The selection of additional marker allergens will need to be adjusted to the geographical region the patient comes from and resides, as environmental differences can result in exposure to different allergen sources (e.g., exotic plants and food) (37).

In conclusion, diagnosis with recombinant marker allergens may have important implications for recommendations regarding allergen avoidance and also for the selection of appropriate therapy strategies.

Acknowledgments

We would like to thank Nadja Balic for performing the CAP-FEIA analyses and Christian Lupinek for his valuable assistance with the preparation of the line art figures. This study was supported by grants F01804, F01815, and F01818 of the Austrian Science Fund (FWF) and by research grants of the Christian Doppler Association, Vienna, Austria of Biomay, Vienna, Austria and of Phadia, Uppsala, Sweden.

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