

Short communication

Evaluation of recombinant allergens Bet v 1 and Bet v 2 (profilin) by Pharmacia CAP System in patients with pollen-related allergy to birch and apple

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Sixty-five patients presenting either rhinoconjunctivitis or asthma and sensitized to pollens of trees of the order Fagales were studied by the Pharmacia CAP system in order to assess specific IgE for the important birch pollen allergens Bet v 1 and Bet v 2. All 65 subjects reacted to at least one of the recombinant birch allergens: 43% to Bet v 1, 30.7% to Bet v 2, and 26% to both. Patients monosensitized to birch did not react to Bet v 2. Of patients with a history of oral allergy syndrome after eating apples, 16/28 (57%) reacted to Bet v 1; among 20 polysensitized subjects presenting oral allergy syndrome after consumption of apple, four reacted to Bet v 2 (20%). Among patients with IgE against both recombinant allergens, six (35.30%) presented symptoms of allergy after eating apples. Our results indicate that sensitization to Bet v 1 is specific for birch and apple allergies, whereas sensitization to Bet v 2 is common in polysensitized patients.

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Single recombinant allergens (RAs) may function as an effective marker of atopy and provide a useful tool for characterization of a patient's sensitivity to single allergens. For this purpose, the recently developed CAP system for Bet v 1 and Bet v 2 offers interesting possibilities.

Bet v 1, the major allergen of birch pollen (1), is known to belong to a ubiquitous family of pathogenesis-related proteins (2). It has been recognized that both Bet v 1 and a homologous 18-kDa apple protein are responsible for most allergenic cross-reactions between birch pollen and apples (3). A second cross-reactive allergen, Bet v 2, with a molecular mass of 14 kDa, has been cloned from birch pollen (4, 5) and from grass pollen (6). Bet v 2 was identified as a cytoskeleton-associated protein, profilin, present in all eukaryotic cells. An amino acid sequence homology between

birch and human profilin was found (5). Cross-reactivity with profilin in fruits was demonstrated (7, 8), though this is of little importance for patients with birch/fruit allergy (9, 10).

Nevertheless, a recent study (1) has demonstrated that profilin is an important allergen for patients with grass pollen-related allergy to fruits and other pollens. The relevance of profilin in fruit and vegetable allergy, as well as its possible relevance in latex-allergic patients, was demonstrated by Valier et al. (11).

Birch is the most widely cultivated tree in the province of Cuneo (Italy) and represents the second most important cause of pollen allergy in this area (12). The purpose of this work was to determine the IgE levels of our patients to Bet v 1 and Bet v 2 and to evaluate the incidence of their intolerance of apple.

Material and methods

The study involved 65 outpatients (35 males, 30 females; mean age 31.8 years, range 8–65) diagnosed as having rhinoconjunctivitis and/or asthma caused by birch and other inhaled allergens. They all had positive responses to skin prick test performed with the pollen extract of trees of the order Fagales (birch, hazel, alder) and had positive concordant CAP results. Patients suffering from birch pollen allergy are more often polysensitized than monosensitized (Table 1). Twenty-six (40%) of them reported an immediate adverse reaction after eating fresh fruits. The clinical pictures included oral allergy syndrome.

Skin tests

Skin prick tests were performed with a battery of commercially available (Neo Abelló, Madrid) common inhalant allergens (trees, *Dermatophagoides* mites, grass, mugwort, animal danders) and food allergens (apple, hazelnut).

IgE antibody determinations

Specific IgE antibodies to Bet v 1, Bet v 2, and other allergens were measured by the immuno-enzymatic CAP method (Pharmacia, Sweden).

Statistics

Statistical comparisons of the data were made with the Mann-Whitney U-test for nonparametric data. Results were expressed as median values and were taken to be significant when $P < 0.05$. Correlation coefficients were determined by Spearman's rank correlation test.

Results

Of 65 patients, 28 (43%) showed IgE binding to Bet v 1, but not Bet v 2 (group I). Sera from 20 patients (30.7%) reacted to Bet v 2, but not Bet v 1 (group II). Finally, 17 sera (26%) contained IgE antibodies to Bet v 2 in addition to Bet v 1 (group III). Of 28 patients, 16 (57%) with apple hypersensitivity belonged to group I. Other results are summarized in Table 1. In our study, most of the apple-allergic subjects were also sensitive to other fruit (melon, pear, kiwi, cherry, apricot, and persimmon), vegetables (carrot and celery), and hazelnuts (Table 1).

Median values of IgE levels to both RAs are summarized in Table 2.

In Spearman's ranking, IgE antibodies to Bet v 1 and Bet v 2 were shown to be correlated with the

CAP results for birch, hazel, grass, mugwort, and apple (Fig. 1).

Discussion

Our finding of a significantly increased level of IgE against Bet v 1 in patients with oral allergy syndrome to apple confirms the results of previous studies (1, 3). This is in full accordance with the finding that all epitopes of the major apple allergen are present on Bet v 1 (13). Bet v 1 may act as a pathogenesis-related protein, being synthesized *de novo* in situations of environmental stress. Environmental pollution might lead to augmented expression of allergens in pollens and other tissue of birch. (14)

Although cross-reactivity with Bet v 2 in fruit was also demonstrated (6, 7), our results showed this to be of minor importance for patients with Fagales/fruit hypersensitivity. The significant increase of IgE against Bet v 2 in patients in the study (groups II and III) is largely explained by profilin cross-reactive IgE against grass pollen (5).

Taken together, our results strengthen the concept that fruit and vegetables containing birch pollen-related allergens are potentially hazardous for subjects with specific IgE to either Bet v 1 or Bet v 2.

The availability of differential antibodies capable of distinguishing between two birch-related allergens should prove useful in further elucidating the biologic role of Bet v 1 and Bet v 2.

For screening purposes in allergic patients, the advantage of using RAs as markers of food allergy, in view of the high degree of cross-reactivity, may represent a starting point in the search for symptoms characteristically elicited by eating fresh fruits and vegetables. Moreover, patients with IgE to Bet v 2 could have severe reactions after exposure to latex (e.g., during surgical intervention). However, the existence of IgE antibodies to latex profilin is still a questionable factor in the sensitization of occupationally exposed patients.

The fact that a positive serologic reaction is not always correlated with oral allergy syndrome symptoms may be due to the occurrence of other reactions of various severity (i.e., urticaria, asthma) if the patient ingests fruits and vegetables. Moreover, local symptoms seem to be related to the concentration of mast cells in the oropharyngeal mucosa that leads to an interaction between the allergens released from the vegetable foods and the specific IgE bound on the mast-cell surface.

Taken together, the results of our study confirm that the clinical observations of an association between pollen and fruit and vegetable hypersensitivity have an important immunochemical foundation.

Table 1. Clinical characteristics of birch-allergic patients and specific IgE against Bet v 1 (group I); Bet v 2 (group II); Bet v 1 and Bet v 2 (group III); and birch (T3), apple (F49), hazelnut (T4), grass (G8), and mugwort (W6)

Group I	Clinical manifestations	Bet v 1 (kU/l)	T3 (kU/l)	Bet v 1/T3 ratio	T4 (kU/l)	F49 (kU/l)	G8 (kU/l)	W6 (kU/l)
1 AA	RC, OAS (apple, cherry)	29.1	24.8	0.85	NT	13.3	> 100	14.5
2 AS	RC	1.64	1.82	1.10	< 0.35	NT	> 100	2.03
3 BJ	RC	6.14	7.65	1.24	5.64	NT	75.9	< 0.35
4 BP	RC, OAS (apple)	45.7	39.7	0.86	47.2	5.48	< 0.35	< 0.35
5 BR	RC, OAS (apple)	48	2.08	0.04	0.01	1.54	< 0.35	< 0.35
6 BG	RC, BA, OAS (apple, hazelnut)	33.9	24.7	0.72	20.3	2.8	1.94	< 0.35
7 CS	RC	51.3	36.7	0.71	44	NT	3.67	< 0.35
8 FN	RC	3.54	6.62	1.87	2.83	NT	37	NT
9 FE	RC, BA	5.46	4.97	0.91	5.01	0.41	< 0.35	NT
10 GA	RC, OAS (apple, hazelnut)	7.69	6.78	0.98	5.08	2.72	< 0.35	< 0.35
11 GC	RC, AB, OAS (apple)	> 100	83.8	0.83	81.7	7.04	< 100	< 0.35
12 LF	RC, BA, OAS (apple)	49.9	NT	NT	29.2	2.06	< 0.35	< 0.35
13 LC	RC	1.13	0.563	0.49	< 0.35	0.01	44.7	< 0.35
14 MD	RC	15.9	NT	NT	NT	2.86	< 0.35	< 0.35
15 MV	RC, BA, OAS (apple)	3.79	NT	NT	NT	0.664	1.25	2.97
16 OM	RC	2.95	NT	NT	NT	0.56	< 0.35	< 0.35
17 OI	RC, BA, OAS (apple, cherry, peach)	> 100	67.1	0.67	63	NT	< 0.35	< 0.35
18 OI	RC	7.2	6.18	0.85	11.7	NT	< 0.35	< 0.35
19 PG	RC, AB, OAS (apple)	71.5	41.5	0.58	41.3	NT	< 0.35	0.448
20 PA	RC, OAS (apple)	16.4	14.7	0.89	NT	NT	< 0.35	< 0.35
21 PF	RC, BA	7.06	19.2	2.71	8.88	NT	< 0.35	< 0.35
22 RJ	RC	13.6	11.6	0.85	6.9	< 0.35	53.7	< 0.35
23 RG	RC, OAS (apple)	29.2	31.3	1.07	36	NT	57	< 0.35
24 SM	RC, BA, OAS (apple, cherry)	18	NT	NT	NT	2.26	< 0.35	< 0.35
25 TM	RC	70.6	NT	NT	65	NT	0.01	< 0.35
26 VM	RC, OAS (apple)	76.5	63.2	0.82	70.8	NT	14.7	< 0.35
27 ZL	RC, OAS (apple, hazelnut)	25.4	20.7	0.81	24.7	2.42	< 0.35	< 0.35
28 ZC	RC, OAS (apple)	67.5	NT	NT	NT	3.2	< 0.35	< 0.35

Group II	Clinical manifestations	Bet v 2 (kU/l)	T3 (kU/l)	Bet v 2/T3 ratio	T4 (kU/l)	F49 (kU/l)	G8 (kU/l)	W6 (kU/l)
1 AR	RC	1.93	2.11	1.09	2	NT	83.5	2.1
2 AA	RC	10.2	9.56	0.93	10	NT	> 100	8.45
3 BR	RC	21.2	19.9	0.93	20.9	NT	> 100	34
4 BC	RC, OAS (kiwi, celery)	12	6.22	0.51	7.29	5.2	72.3	3.7
5 CP	RC, BA	> 100	NT	NT	38.7	NT	> 100	19.8
6 CA	RC	6.42	5.71	0.88	5.71	NT	> 100	< 0.35
7 CG	RC, BA	1.99	1.66	0.83	1.83	< 0.35	20.2	1.51
8 CD	RC, OAS (apricot, persimmon, melon)	4.57	6.34	1.38	6.99	2.70	> 100	< 0.35
9 DM	RC	3.34	2.43	0.72	4.24	1.96	> 100	1.66
10 EP	RC	51.7	25.6	0.49	49.8	NT	> 100	< 0.35
11 FP	RC	4.62	1.97	0.42	3.07	< 0.35	> 100	NT
12 GA	RC	3.09	2.79	0.90	2.94	NT	55.5	3.92
13 GM	RC	8.63	3.64	0.42	4.41	NT	75.1	1.91
14 GE	RC, BA	1.54	1.15	0.74	1.29	NT	92.7	NT
15 LM	RC	2.13	< 0.35	0.16	2.65	NT	6.89	0.624
16 RL	RC, BA	3.05	< 0.35	0.11	1.98	NT	84.5	0.742
17 SD	RC, OAS (apricot)	0.669	0.378	0.56	0.787	NT	15.9	0.01
18 SA	RC	12.03	6.38	0.53	7.7	NT	80.3	< 0.35
19 TA	RC, BA	4.74	NT	NT	30	NT	> 100	11.6
20 TM	RC, OAS (apple, pear)	3.6	6.18	1.71	5.82	5.71	> 100	< 0.35

Group III	Clinical manifestations	Bet v 1 (kU/l)	Bet v 2 (kU/l)	T3 (kU/l)	T4 (kU/l)	F49 (kU/l)	G8 (kU/l)	W6 (kU/l)
1 BS	RC, OAS (apple, peach)	8.16	0.974	NT	NT	1.5	NT	NT
2 BS	RC	> 100	13	NT	NT	5.2	NT	NT
3 BM	RC, OAS (apple, carrot)	26.2	15.9	49.9	56.1	7.38	NT	< 0.35
4 CF	RC	15	4.46	13.9	10.7	NT	54.6	NT
5 CL	RC, OAS (hazelnut)	31.7	6.03	32.6	21	4.65	80.9	NT
6 CS	RC, BA	12.2	4.37	16.7	11	11.4	101	13.5

Table 1 cont. Clinical characteristics of birch-allergic patients and specific IgE against Bet v 1 (group I); Bet v 2 (group II); Bet v 1 and Bet v 2 (group III); and birch (T3), apple (F49), hazelnut (T4), grass (G8), and mugwort (W6)

7 CE	RC, BA, OAS (apple, peach, apricot, hazelnut)	68.4	3.11	60.4	34.9	18	2.1	0.811
8 C F	RC	24.2	4.3	25.3	22.7	1.11	14	1.92
9 DS	RC, BA	76.9	2.64	64.5	79	NT	27.8	21.7
10 DN	RC, BA	9.39	0.473	NT	8.69	NT	1.06	3.07
11 DF	RC, BA, OAS (apple)	53	2.93	13.4	23.4	2.23	6.79	NT
12 EE	RC	3.92	1.04	2.42	1.61	NT	15.9	2.5
13 GL	RC	0.558	1.06	NT	NT	NT	NT	12.1
14 MR	RC	1.11	5.94	4.36	3.83	NT	23.2	NT
15 PC	RC, BA	16.6	12.8	NT	NT	NT	101	NT
16 PM	RC, OAS (apple)	63.9	0.368	54.8	41.9	2.61	45.3	2.66
17 SI	RC, BA	27.6	0.507	NT	20.2	NT	101	NT

RC = rhinoconjunctivitis; BA = bronchial asthma; OAS = oral allergy syndrome; NT = not tested.

Table 2. Level of IgE (kU/l) to Bet v 1 and Bet v 2, T3 (birch), T4 (hazel), F49 (apple), G8 (*Poa pratensis*), and W6 (mugwort) (median value, 25th percentile and 75th percentile)

Group I (Bet v 1 = positive and Bet v 2 = negative)							
Patients with OAS			Patients with OAS versus Patients without OAS Mann-Whitney U test	Patients without OAS			
<i>n</i>	Median kU/l	(25th–75th percentile) kU/l		<i>n</i>	Median kU/l	(25th–75th percentile) kU/l	
Bet v 1	16	39.8	(21.7–69.5)	<i>P</i> = 0.002	12	6.6	(3.2–14.8)
T3	12	28.1	(17.7–52.4)	<i>P</i> = 0.008	9	6.6	(1.8–9.6)
T4	11	36.0	(20.3–63.0)	<i>P</i> = 0.0052	10	6.3	(1.0–10.3)
F49	11	2.7	(2.1–5.5)	<i>P</i> = 0.02	5	0.4	(< 0.35–0.5)
G8	7	14.7	(1.3– > 100)	NS	7	44.7	(3.7–75.9)
W6	3	3.0	(0.4–14.5)	–	1	–	(–)
Bet v 1/T3 ratio	12	1.2	(1.1–1.4)	NS	9	1.1	(0.5–1.2)

Group II (Bet v 1 = negative and Bet v 2 = positive)							
Patients with OAS			Patients with OAS versus Patients without OAS Mann-Whitney U test	Patients without OAS			
<i>n</i>	Median kU/l	(25th–75th percentile) kU/l		<i>n</i>	Median kU/l	(25th–75th percentile) kU/l	
Bet v 2	4	4.1	(2.1–8.3)	NS	16	4.7	(2.6–11.3)
T3	4	6.2	(3.3–6.3)	NS	12	3.2	(2.0–8.0)
T4	4	6.4	(3.3–7.1)	NS	16	4.3	(2.3–15.5)
F49	3	5.2	(2.7–5.7)	<i>P</i> = 0.04	3	< 0.35	(< 0.35–2.0)
G8	4	86.7	(44.1– > 100)	NS	16	96.9	(77.7– > 100)
W6	2	1.9	(< 0.35–3.7)	NS	11	2.1	(1.5–11.6)
Bet v 2/T3 ratio	4	1.2	(0.7–1.8)	NS	12	1.3	(1.1–2.0)

Group III (Bet v 1 = positive and Bet v 2 = positive)							
Patients with OAS			Patients with OAS versus Patients without OAS Mann-Whitney U test	Patients without OAS			
<i>n</i>	Median kU/l	(25th–75th percentile) kU/l		<i>n</i>	Median kU/l	(25th–75th percentile) kU/l	
Bet v 1	6	42.4	(8.2–58.5)	NS	11	15.0	(3.9–27.6)
Bet v 2	6	3.0	(0.4–4.6)	NS	11	4.3	(1.0–5.9)
T3	5	49.9	(13.4–52.4)	NS	6	15.3	(2.4–21.0)
T4	5	34.9	(21.0–38.4)	<i>P</i> = 0.04	8	10.9	(6.3–21.5)
F49	6	3.6	(1.5–6.0)	NS	3	1.1	(1.1–5.2)
G8	4	26.0	(4.4–63.1)	NS	9	27.8	(14.0–77.8)
W6	2	1.7	(0.8–2.7)	NS	6	7.6	(1.9–12.8)
Bet v 1/T3 ratio	5	1.1	(0.5–1.1)	NS	6	1.0	(0.3–1.1)
Bet v 2/T3 ratio	5	0.2	(0.0–0.2)	NS	6	0.3	(0.0–0.4)

n = number of positive sera; OAS = oral allergy syndrome; NS = nonsignificant.

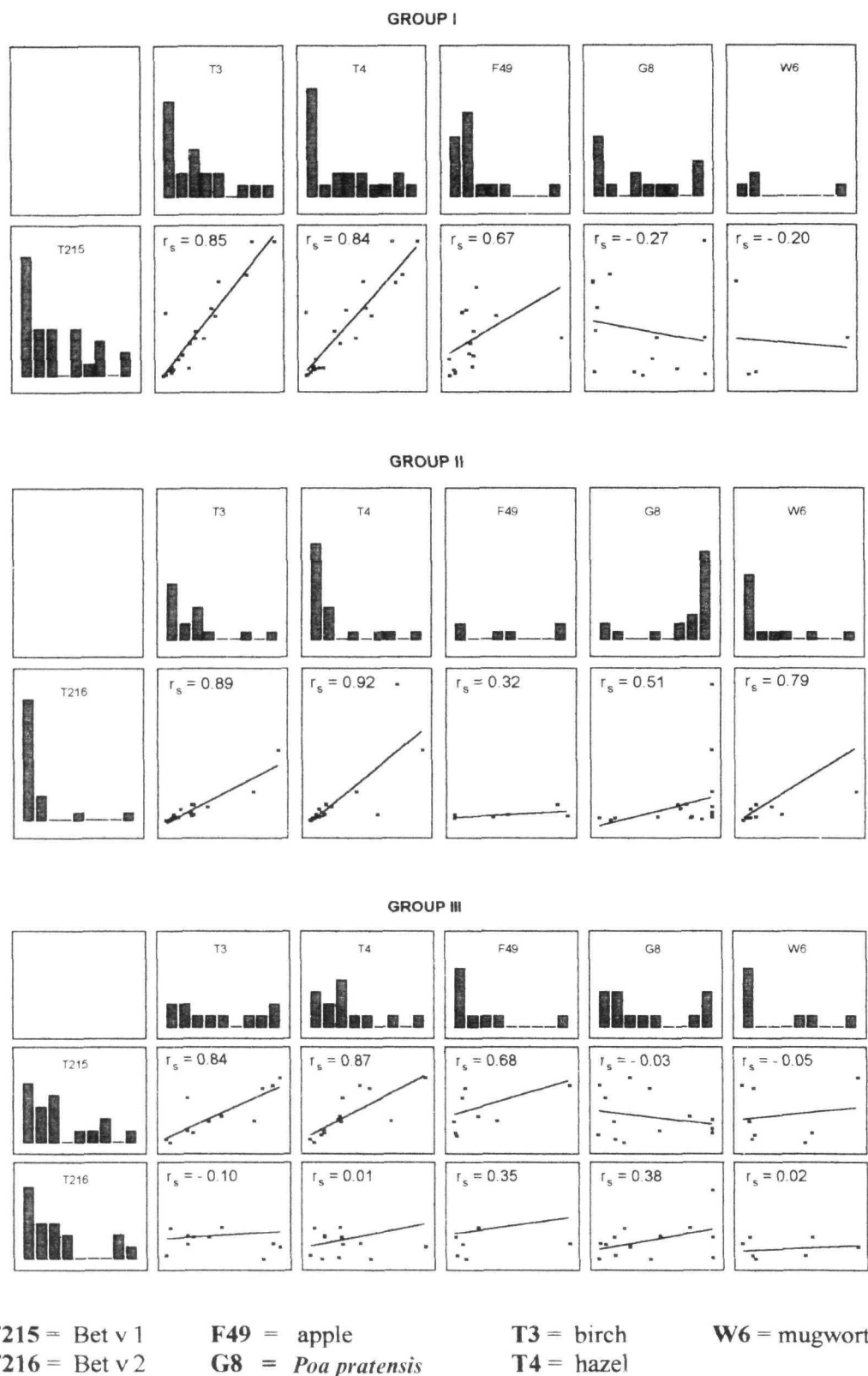


Fig. 1. Correlation between recombinant allergens and whole allergen extracts in patients with IgE against Bet v 1, but not Bet v 2 (group I); IgE against Bet v 2, but not Bet v 2 (group II); IgE against both recombinant allergens (group III).

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