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Fruit-pollen-latex cross-reactivity: implication of profilin (Bet v 2)

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Key words: cross-allergenicity; fruit allergy; latex; pollinosis; profilin; recombinant allergens.

Background: An association between allergy to fruits and latex, and between pollen and plant-derived food has been described. The cross-reactive structures responsible for these associations have not yet been completely elucidated.

Methods: IgE reactivity to the recombinant allergens Bet v 1 and Bet v 2, different pollens, natural latex, papain, and bromelain was investigated in 29 patients with allergy to fruits or vegetables who lived in an area without birch trees.

Results: Exactly 79.3% of patients were allergic to grass pollen, and two of them had clinical allergy to latex. Serum IgE reactivity (CAP) to birch pollen was found in 65% of patients, to Bet v 2 in 51.7%, to Bet v 1 in 3.4%, to latex in 58.6%, to bromelain in 51.7%, and to papain in 17.2% of patients. All subjects with positive IgE to Bet v 2 had also reactivity to latex, grass, olive tree, birch, and mugwort pollens. The six patients not allergic to pollen did not show IgE reactivity to latex, Bet v 1, or Bet v 2. A significant correlation was found between CAP to latex with Bet v 2 ($r=0.86$, $P<0.001$), with birch ($r=0.86$, $P<0.001$), and with ryegrass ($r=0.81$, $P<0.001$). Immunoblotting using nine sera with positive CAP to birch pollen showed IgE-binding to a 15-kDa band that was recognized by antiprolin monoclonal antibody. Bet v 2 CAP could be inhibited up to 52% by ryegrass and up to 23% by mugwort. CAP to latex was almost completely inhibited by ryegrass pollen with sera from five subjects without symptoms due to latex, whereas no inhibition was observed with serum from one patient with allergy to latex.

Conclusions: Patients with allergy to plant-derived food and associated pollinosis showed a high frequency of IgE reactivity to Bet v 2, which may cause positive serum IgE determinations to latex and birch pollen due to the presence of cross-reactive

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epitopes. IgE reactivity to Bet v 2 may serve as an indicator of broad sensitization.

In recent years, an association between allergy to plant-derived food and pollen sensitization has been described, mainly implicating birch (1–3), ragweed (4, 5), mugwort (6–8), and grass pollens (9–11). With RAST inhibition assays and immunoblotting, several common allergens responsible for the cross-allergenicity between vegetable food and pollen have been described (10–21). The cross-reactive structures have been identified as the major allergen of birch pollen Bet v 1 (13, 14, 18, 20) as well as profilins (15, 20–23), which are referred to as plant panallergens (24).

On the other hand, allergy to fruits is common in patients with hypersensitivity to latex from *Hevea brasiliensis*, and the presence of common IgE epitopes in latex and fruits has been reported (25–29). Recombinant Bet v 1 and Bet v 2 (birch profilin) have been recently isolated (30, 31) and are now commercially available. Subsequently, IgE reactivity to these recombinant allergens in patients with allergy to plant-derived food has been investigated (32–35).

The aim of our study was to assess the prevalence of IgE reactivity to the recombinant allergens of birch pollen Bet v 1 and Bet v 2 in patients with allergy to fruits or vegetables who lived in an area without birch trees. We also investigated a possible sensitization to natural rubber latex in patients with allergy to plant-derived food. The existence of cross-reactivity between natural latex, grass pollen, rBet v 1, and rBet v 2 was studied by IgE-inhibition experiments. Moreover, the possible sensitization to plant proteases (papain and bromelain) among patients allergic to vegetable foods was also evaluated.

Material and methods

Patients

The study population was selected from outpatients referred to the Allergy Department of Ramón y Cajal Hospital in Madrid. Twenty-nine patients who described immediate clinical symptoms consistent with type I allergy after the ingestion of fruits, nuts, or vegetables were selected between October 1996 and February 1997. Diagnosis of allergy to fruits and vegetable foods was established on clinical manifestations and on the positivity of skin prick tests or specific IgE determination (CAP) with the implicated

vegetal foodstuffs. A comprehensive medical history was obtained from each patient, including questions on exposure and symptoms upon contact with latex products.

Eighteen out of the 29 patients (62%) had experienced systemic reactions after the ingestion of vegetable foodstuffs consisting of urticaria, angioedema, asthma, or anaphylaxis. Eleven patients presented with oral allergy syndrome (OAS) without any systemic manifestation.

The sera from eight patients with asthma and/or rhinoconjunctivitis due to grass and olive pollen and without allergy to fruits or vegetables were used as controls.

Skin prick tests

Skin tests were performed by the prick-prick method (2) with the fresh fruits and vegetables suspected of causing allergy according to clinical history. A standard allergy prickler lancet was used (Dome/Hollister-Stier, Miles Ltd, Basingstoke, UK). Twenty-seven fruits and vegetables were tested: melon, peach, apple, banana, chestnut, kiwi, avocado, tomato, cabbage, fig, strawberry, pear, orange, custard apple, plum, cherry, sunflower seed, almond, hazelnut, peanut, walnut, celery, potato, carrot, garlic, onion, and boiled chickpea. Moreover, commercially available extracts of almond, hazelnut, and walnut were tested (Bencard, Worthing, UK).

Skin prick tests were also performed with a battery of common inhalant allergens that included environmentally relevant pollens in our area such as *Lolium perenne*, *Phleum pratense*, mixed grass pollen, and *Olea europaea* (ALK-Abelló, Madrid, Spain). Commercially available extract of latex from *H. brasiliensis* (Alyostal, Stallergènes SA, France) was also tested. Histamine phosphate at 10 mg/ml and normal saline were used as positive and negative controls, respectively. A wheal with a mean diameter 3 mm or greater with erythema, occurring 15 min after testing, was defined as a positive reaction, the skin prick test with saline being negative at the same time.

Total and specific IgE determination

Total serum IgE was measured by Pharmacia CAP System IgE FEIA (Pharmacia Diagnostics, Uppsala, Sweden). Specific IgE antibodies against r (recombinant) Bet v 1 and rBet v 2 (birch profilin) were measured with a kit (CAP technique) generously supplied by Pharmacia. Specific IgE against the fruits and vegetables implicated in allergic symptoms by clinical history were measured in every patient by Pharmacia CAP System RAST FEIA (Pharmacia

Diagnostics, Sweden). Specific IgE (CAP) against birch (*Betula verrucosa*), mugwort (*Artemisia vulgaris*), ryegrass (*L. perenne*), and *O. europaea* pollens, and against latex, papain, and bromelain were measured in sera from every patient and from a control group of eight pollen-sensitive subjects. CAP scores higher than 0.35 kU/l were regarded as positive.

CAP inhibition assays

CAP inhibition studies were carried out to investigate a possible cross-reactivity between rBet v 2 and pollen from *L. perenne* and *A. vulgaris*. Sera from seven patients allergic to fruits or vegetables were pooled (patients 7, 9, 13, 15, 17, 18, and 19). CAP to rBet v 2 was used in the solid phase, and *L. perenne* 2.5% w/v and *A. vulgaris* 2.5% w/v pollen extracts (Bencard, Worthing, UK) were used as inhibitor allergens. For assessment of the specificity of the CAP inhibition assays, control inhibition experiments were carried out with *L. perenne* pollen both in the solid phase and as inhibitor allergen.

The possible cross-reactivity between latex and *L. perenne* was also investigated with individual sera from patients 4, 15, 17, and 18 and sera from control subjects 1 and 3. CAP against latex was used in the solid phase and *L. perenne* in the liquid phase as inhibitor allergen. Volumes of 20 μ l of four progressive 10-fold dilutions of the inhibitor allergens in PBS and 20 μ l of PBS as negative control were preincubated with 50 μ l of serum for 15 min, and then the assay was continued by the standard CAP technique.

Results were expressed as the percent inhibition of CAP obtained with the inhibitor allergen as compared with a control assay with PBS inhibitor.

SDS-PAGE and immunoblotting

Proteins from birch pollen and latex extracts were separated by SDS-PAGE according to Laemmli (36) in 12.5% polyacrylamide gel (20 μ g protein applied per lane) under reducing conditions. After running, the separated proteins were electrophoretically transferred to PVDF membranes (Immobilon-P, Millipore Corp., Bedford, MA, USA) by the method of Towbin et al. (37). Unreacted membrane sites were blocked with 90% defatted dry milk in Tris-buffered saline (TBS) for 1 h at 37°C. After three washes with TBS, blots were incubated with 2.5 ml of undiluted human serum (16 h at 4°C). The blots were washed three times with TBS and incubated with 2.5 ml of 1:250 peroxidase-conjugated rabbit immunoglobulins to human ϵ -chains (Dako,

Glostrup, Denmark). Bound peroxidase was detected by the addition of 3 ml of freshly prepared 0.06% w/v of 4-chloro-1-naftol solution (Bio-Rad Laboratory, Richmond, CA, USA) and 0.01% H₂O₂ in TBS. After 30 min, the blots were rinsed in distilled water and air-dried. Antigen-antibody binding was identified by purple staining. Electrophoretic and immunoblotting bands were detected and evaluated by image analysis in the Bio-Image System (Millipore Corp., Bedford, MA, USA).

For profilin immunoblotting, monoclonal antibody (3G4) anti-sunflower profilin (Ifidesa-Aristegui, Bilbao, Spain) was used (38). After washing, blots were incubated in the same conditions with anti-mouse polyvalent immunoglobulins peroxidase-conjugated (Sigma Chemical Co., St Louis, MO, USA) diluted 1:1000 and developed as described above.

Immunoblotting inhibition

Aliquots of undiluted serum from patient 4 were preincubated for 12 h with equal volumes of *L. perenne* pollen extract, at a final concentration of 0.1 mg protein/ml. Mixtures were then centrifuged and supernatants incubated with latex-blotted PVDF strips, followed by immunodetection, as described in the immunoblotting section.

Statistics

SPSS for Windows was the statistical package used for analysis of data. Spearman's rank correlates were used for estimation of the regression coefficients, since our data were not normally distributed. As for statistical significance, the *P* value was <0.05.

Results

Clinical data and skin tests

Clinical data and results of skin prick tests with fruits and vegetables are shown in Table 1. The mean age of patients allergic to vegetable foods was 26.5 $\pm$ 9.8 years (range 16–51 years). Twenty-three patients were female (79.3%) and six patients were male (20.7%). All patients showed a positive skin prick test against at least one of the vegetable foodstuffs tested. Twenty-seven out of the 29 patients (93%) had allergic symptoms and positive skin tests with two or more fruits or vegetables, and only two patients (nos. 2 and 27) were allergic to only one fruit (peach and kiwi, respectively).

Table 1. Demographic and clinical data of patients with allergy to plant-derived food

Patient no.	Age (years)	Sex	Pollen allergy			Fruit allergy		
			Symptoms	Pollen	Years	Symptoms	Fruit (SPT +)	Years
1	18	F	A	G, O	12	OAS, U, Ax	Me, B, Av, Ch, K	?
2	27	F	RC	G	>10	OAS	P	7
3	17	M	RC	G, O	10	OAS	P, Me	<10
4	20	F	RC	G, O	10	OAS, Ax	P, Me, K	<1
5	37	F	A	G, O	10	OAS, AE	F, K	<1
6	23	F	No	G, O	–	OAS, U, AE	Sb, P, Ap	7
7	24	F	RC	G, O	15	U, AE	Me, K, Cp	?
8	20	F	RC	G, O	2	OAS	Me, Ap, B Po, Pe, W, O	1
9	46	F	A	G, O	20	OAS, A	K, Sb	14
10	19	F	A	G, O	4	U, AE	P, B, K, H	?
11	30	F	A	G, O	15	OAS	Me, B, Cu Pn, Al, H	<15
12	51	M	A	G, O	>30	A	Ga, On	?
13	43	M	A	G, O	?	OAS	P, B, Pl	?
14	25	F	RC	G	3	Ax	P, Ap, Pe	>3
15	24	M	RC	G, O	11	OAS	Me, O, Sb K, Pe	4
16	19	M	A	G, O	4	OAS	Pn, W, P	<4
17	27	M	A	G, O	7	U, AE	P, Che	<1
18	16	F	RC	G, O	6	OAS	Me, P, Ap B, K, Pe	3
19	30	F	A	G, O	20	OAS	Me, B, Sf	?
20	31	F	A	G, O	16	OAS, U, AE	Me, P, Ap, W	?
21	16	F	A	G, O	9	OAS	Me, B, K	1
22	27	F	A	G, O	8	OAS	Sb, K, Cu	3
23	30	F	A	G, O	10	AE, A, OAS	Ch, B	<1
24	24	F	No	No	–	AE, Ax	K, P, Al T, W	10
25	20	F	No	No	–	OAS, AE	C, T, B, Pn	2
26	48	F	No	No	–	AE, A, Uc	Ca, Ce	4
27	22	F	No	No	–	AE	K	3
28	18	F	No	No	–	Ax	Ch, K, Ap H, W, P	5
29	17	F	No	No	–	U, AE, A	Ap, P, Ch	1

G: grass, O: olive, SPT: skin prick test, A: asthma, RC: rhinoconjunctivitis, OAS: oral allergy syndrome, U: urticaria, AE: angioedema, Ax: anaphylaxis, Uc: contact urticaria. Ap: apple, Al: almond, Av: avocado, B: banana, C: cabbage, Ca: carrot, Ce: celery, Cp: chickpea, Cu: custard apple, Ch: chestnut, Che: cherry, F: fig, Ga: garlic, H: hazelnut, K: kiwi, Me: melon, O: orange, On: onion, P: peach, Pe: pear, Pl: plum, Pn: peanut, Po: potato, Sb: strawberry, Sf: sunflower seed, T: tomato, W: walnut.

Skin prick tests to olive and grass pollens were positive in 23 patients (79.3%) (patients 1–23 as shown in Tables 1 and 2). All these patients had seasonal rhinoconjunctivitis with or without asthma, with the sole exception of patient 6, who

showed positive skin tests to grass and olive pollens but did not report seasonal allergic symptoms. Six patients (nos. 24–29) were allergic to fruits or vegetables without associated sensitization to grass or olive pollens. Clinical and immu-

Table 2. Results of IgE determinations (kU/l) in patients with allergy to plant-derived food

Patient no.	Total IgE	<i>L. perenne</i>	<i>O. europaea</i>	rBet v 1	rBet v 2	Birch	Mugwort	Latex	Papain	Bromelain	Fruits/vegetables
1	310	>100	48.20	<0.35	13.40	2.32	8.04	4.70	<0.35	4.48	Me:1.5, B:1.32, Av:1.9
2	21	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	P:1.37
3	>2000	>100	75.30	<0.35	54.30	29.50	38.60	34	7.14	24.40	P:43, Me:18.9
4	664	>100	17	0.57	2.71	4.67	6.39	31.70	1.60	6.51	K:16.8, Ch:8.5, Av:9.7, B:5.6
5	130	1.74	34.60	<0.35	<0.35	1.2	0.75	<0.35	3.80	<0.35	F:7.56
6	115	<0.35	1.18	<0.35	<0.35	<0.35	1.04	<0.35	<0.35	<0.35	P:6.69, Sb:0.40
7	357	73.70	14.60	<0.35	4.22	3.26	1.50	3.21	<0.35	<0.35	Me:1.01, Cp:0.75
8	26	19.4	1.94	<0.35	4.62	2.28	0.50	1.34	<0.35	<0.35	Po:0.75
9	192	>100	23.50	<0.35	4.12	4.71	2.21	0.87	<0.35	<0.35	K:1.25
10	362	>100	15.30	<0.35	<0.35	7.37	11.20	2.57	<0.35	3.52	B:25.3, H:6.3, K:0.92
11	60	43.10	2.60	<0.35	3.62	1.77	2.50	1.38	<0.35	1.57	P and Me <0.35
12	207	43.10	3	<0.35	<0.35	<0.35	0.79	<0.35	<0.35	1.33	On:1.24
13	254	89.20	11.80	<0.35	9.53	3.15	2.96	2.71	<0.35	<0.35	P, B, and Pl <0.35
14	109	67.20	1.71	<0.35	3.32	1.64	0.63	1.22	<0.35	0.46	P:1.09
15	381	>100	32	<0.35	23.5	12.2	5.7	11.10	<0.35	1.33	Sb:1.25, O:0.66
16	192	49	2.28	<0.35	<0.35	<0.35	0.64	<0.35	<0.35	1.04	P:6.32, Pn:3.52
17	876	>100	41.50	<0.35	5.31	10.5	24.5	7.41	1.20	39.10	Che:5.84, P:79.7
18	247	>100	29.90	<0.35	10.70	9.07	8.62	5.57	0.91	1.25	P:14.2, Me:1.9, B:1.3
19	337	87.80	18.60	<0.35	22	10.5	9.16	5.29	<0.35	1.73	Me:0.48
20	267	12.20	1.59	<0.35	<0.35	0.52	2.75	0.48	<0.35	0.53	P:16.9, W:13, Ap:1.8, Me:0.9
21	91	54.80	8.73	<0.35	6.52	5.1	3.39	0.64	<0.35	<0.35	Me, B, and K <0.35
22	876	32.5	9.22	<0.35	4.41	3.75	2.84	1.82	<0.35	<0.35	Sb, K, and P <0.35
23	154	23	1.44	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	0.80	Ch:0.72, B:0.40
24	82	<0.35	<0.35	<0.35	<0.35	<0.35	7.75	<0.35	<0.35	<0.35	P:18.90, W:9.57
25	407	<0.35	<0.35	<0.35	<0.35	4.2	9.6	<0.35	<0.35	<0.35	Pn:67, H:29, C:2.3, To:10
26	38	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	Ca:2.3, Ce:2.6
27	328	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	K:0.81
28	62	<0.35	<0.35	<0.35	<0.35	<0.35	0.70	<0.35	<0.35	<0.35	H:6.7, Ap:1.4
29	148	<0.35	<0.35	<0.35	<0.35	<0.35	39	<0.35	<0.35	0.76	P:1.2, Ch:0.6

For abbreviations, see Table 1.

nologic data from control subjects are shown in Table 3. Only in two patients (nos. 6 and 14) out of 16 patients for whom both the onset of seasonal allergic symptoms and fruit allergy were known did fruit allergy symptoms precede symptoms of pollinosis.

Neither the patients nor the control subjects were occupationally exposed to latex. Only two out of the 29 patients allergic to fruits or vegetables (nos. 4 and 22) had symptoms indicative of latex allergy. Patient 4 had experienced several episodes of urticaria and angioedema upon contact with latex products in the last 7 years. Patient 22 had had several episodes of contact urticaria due to latex over 5 years. In both patients, latex allergy developed before symptoms of allergy to fruits commenced.

The remaining 27 patients allergic to vegetable food and the eight control pollen-sensitive subjects without associated fruit allergy showed no symptoms with latex products. Skin tests to latex were positive in five patients (nos. 3, 4, 7, 8, and 21).

Total and specific IgE determinations

Specific IgE against fruits and vegetables was positive in 25 out of the 29 patients (Table 2). In the four patients who had positive skin test to fresh fruits and negative CAP results to these foodstuffs (patients 11, 13, 21, and 22), fruit allergy presented as OAS. Total IgE and specific IgE against *L. perenne*, *O. europaea*, rBet v 1, rBet v 2, birch, and mugwort

Table 3. Demographic and clinical data and results of IgE determinations (kU/l) in control subjects allergic to pollen

Control	Age (years)/sex	Pollen allergy			IgE	<i>L. perenne</i>	<i>O. europaea</i>	rBet v 1	rBet v 2	Birch	Latex	Mugwort	Papain	Bromelain
		Symptoms	Pollen	Years										
1	26/M	RC	G, O	4	605	99.7	18.1	<0.35	13.3	9.36	13.3	4.6	<0.35	1.47
2	25/F	RC	G, O	2	78	22.4	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	0.72
3	16/F	RC	G, O	2	382	>100	14.3	<0.35	7.3	4.29	1.44	2.57	<0.35	0.88
4	36/M	RC	G, O	9	562	89.3	10.7	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
5	25/M	A	G, O	5	168	33.6	1.48	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
6	27/F	RC	G, O	8	180	27.2	0.64	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
7	32/F	A	G, O	2	1027	>100	9.69	<0.35	<0.35	<0.35	<0.35	<0.35	0.37	0.41
8	17/F	A	G, O	5	287	37.2	1.12	<0.35	<0.35	<0.35	<0.35	1.63	<0.35	0.58

RC: rhinoconjunctivitis, A: asthma, G: grass, O: olive.

pollen, and latex, papain, and bromelain in patients allergic to vegetable foods and control subjects are shown in Tables 2 and 3, respectively.

Only one patient (3.4%) allergic to plant-derived food had specific IgE (class 1) against rBet v 1. However, specific IgE (CAP) against rBet v 2 was equal to or higher than class 2 in 15 out of the 29 patients (51.7%). All the patients with positive specific IgE against rBet v 2 were also sensitized to grass and olive pollens and suffered from seasonal allergic symptoms. Patients who showed a positive CAP against rBet v 2 had also a positive CAP against birch pollen. Although specific IgE against birch pollen was positive in patients 5, 10, 20, and 25, CAP against rBet v 1 and rBet v 2 was negative in these patients. Twenty-five out of the 29 patients (86%) had a positive CAP against mugwort. Sensitization to this pollen was observed in patients with allergy to fruits with or without associated hypersensitivity to grass and *O. europaea* pollens. The 15 patients with positive specific IgE against rBet v 2 had a positive CAP against mugwort pollen.

Seventeen out of the 29 patients allergic to vegetable foods had a positive CAP against latex (58.6%), and all of them showed concomitant sensitization to grass and olive pollens. However, only two of these patients (nos. 4 and 22) had symptoms of allergy with latex products. Interestingly, the 15 patients who had a positive CAP against rBet v 2 had also a positive CAP to latex and birch, mugwort, grass, and olive pollens. Moreover, specific IgE against rBet v 2, birch pollen, mugwort, and latex was detected in two of the eight control pollen-sensitive subjects (Table 3). None of the six patients allergic to fruits without associated grass and olive pollinosis (nos. 24–29) showed specific IgE against rBet v 1, rBet v 2, or latex. Fifteen of the 29 patients allergic to vegetable foods (51.7%) had specific IgE against bromelain

and five to papain (17.2%). Four patients (nos. 3, 4, 17, and 18) were sensitized to both papain and bromelain and also showed specific IgE to rBet v 2, birch pollen, mugwort, and latex. Positive CAP to papain was found in one control, and IgE reactivity to bromelain was positive in five control subjects (Table 3).

In Spearman's ranking, IgE antibodies to rBet v 2 were found to be correlated with the CAP results for latex ($r=0.86$, $P<0.001$), birch pollen ($r=0.79$, $P<0.001$), *L. perenne* pollen ($r=0.65$, $P<0.01$), olive pollen ($r=0.64$, $P<0.01$), and mugwort pollen ($r=0.44$, $P<0.05$), and specific IgE to birch pollen with IgE to latex ($r=0.86$, $P<0.001$). Moreover, IgE antibodies to *L. perenne* pollen were found to be correlated with the CAP results for latex ($r=0.81$, $P<0.001$), birch pollen ($r=0.79$, $P<0.001$), olive pollen ($r=0.76$, $P<0.001$), and mugwort pollen ($r=0.76$, $P<0.001$).

CAP inhibition assays

CAP results against rBet v 2, *L. perenne*, and mugwort (*A. vulgaris*) with a pooled serum from seven subjects were 4.66, 70.5, and 4.15 kU/l, respectively. CAP against rBet v 2 could be inhibited up to 52% by *L. perenne* pollen and up to 23% by mugwort pollen. In addition, CAP to *L. perenne* pollen was almost completely inhibited (97%) in the control experiments with this pollen as inhibitor allergen. CAP to latex using individual serum from patients 15, 17, and 18 was inhibited by 89%, 93%, and 92.5% by *L. perenne* pollen, respectively (Fig. 1). However, with serum from patient 4, who had clinical allergy to latex products, CAP against latex could not be significantly inhibited by *L. perenne* pollen. In control subjects, *L. perenne* could inhibit CAP to latex by 94.4% using serum from control subject 1, and by 59% using serum from control subject 3 (Fig. 1).

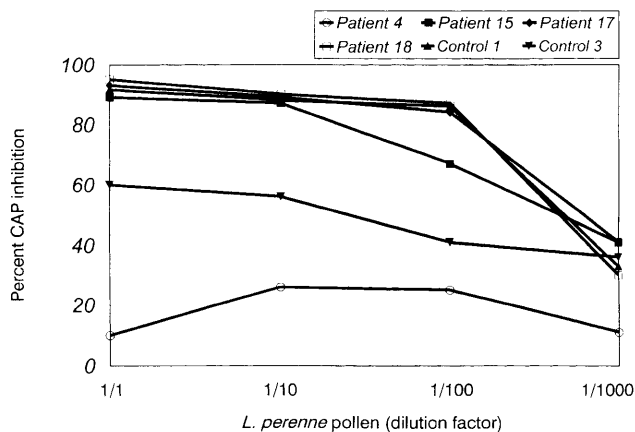


Figure 1. Inhibition of CAP to latex with *L. perenne* pollen with serum from subjects without clinical allergy to latex (patients 15, 17, and 18 and control subjects 1 and 3) and with serum from patient 4, who had clinical allergy to latex.

Immunoblotting

Immunoblots with serum from seven patients (nos. 1, 4, 7, 8, 9, 17, and 21) and two control subjects (nos. 1 and 3) showed IgE-binding bands of molecular weight (MW) of 12–16 kDa in the extract of birch pollen (Fig. 2) that were recognized by all but one subject (patient 21, lane 9). These IgE-binding bands of 12–16 kDa were recognized by anti-profilin monoclonal antibody (Fig. 2, lane 10). Moreover, several bands in a wide range of MW were recognized by some individual sera. Immunoblots of natural latex using sera from four patients (nos. 4, 15, 17, and 18) and two control subjects (nos. 1 and 3) showed IgE-binding bands only in patient 4 (Fig. 3, lane 6).

These bands had apparent MW of 69.8, 58.4, 49.9, 46.7, 40, and 15.2 kDa, respectively.

Immunoblotting inhibition

Preincubation of serum from patient 4 with *L. perenne* pollen could not completely inhibit IgE-binding to latex allergens (Fig. 3, lane 7). However, a variable degree of inhibition was observed at bands of 69.8 kDa (40%), 46.7–49.9 kDa (90%), and 40 kDa (100%). The lowest inhibition occurred at bands of 58.4 kDa (0%) and 15.2 (21%) kDa. IgE-binding at the 15-kDa band probably corresponded mostly to the rubber elongation factor, and this may explain the low inhibition attained with *L. perenne* pollen.

Discussion

The patients allergic to plant-derived food were principally young women (79.3%), and most were sensitized to grass and olive pollens (79.3%). Seasonal respiratory allergy symptoms usually preceded clinical manifestations of allergy to plant-derived food. Pollinosis in the Madrid area is mainly caused by grass, the second pollen in importance being olive, which rarely causes monosensitization (39).

Although birch trees are not found in Madrid, 19 out of the 29 patients allergic to vegetables (65%) showed specific IgE against birch pollen. Unlike patients with birch pollinosis in northern Europe, in approximately 97% of whom sensi-

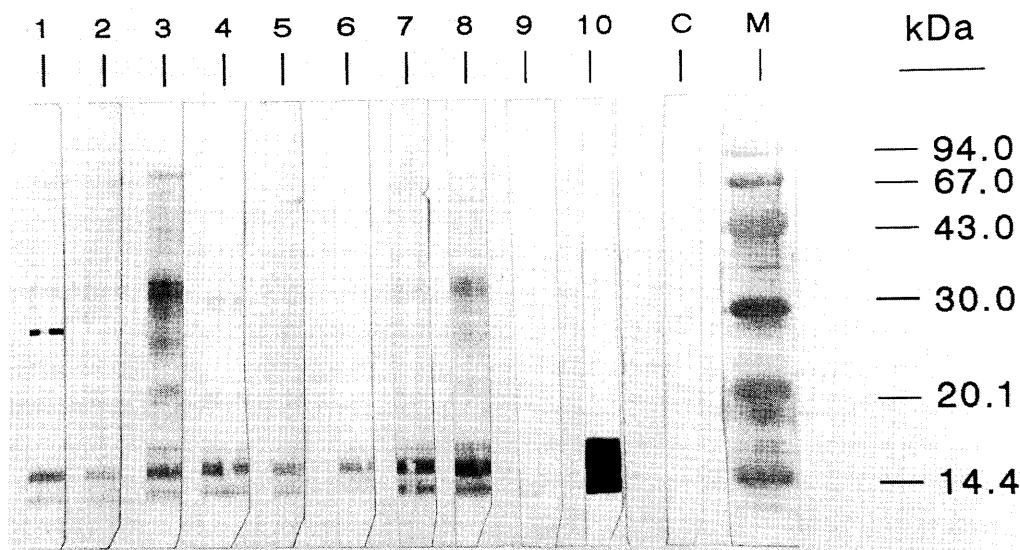


Figure 2. Immunoblots of birch pollen with serum from patient 1 (lane 8), patient 4 (lane 2), patient 7 (lane 6), patient 8 (lane 5), patient 9 (lane 4), patient 17 (lane 3), and patient 21 (lane 9) and from control subjects 1 (lane 7) and 3 (lane 1), showing IgE-binding bands of molecular weight 12–16 kDa. These bands were recognized by anti-profilin monoclonal antibody (lane 10). C: negative control serum, M: molecular weight markers.

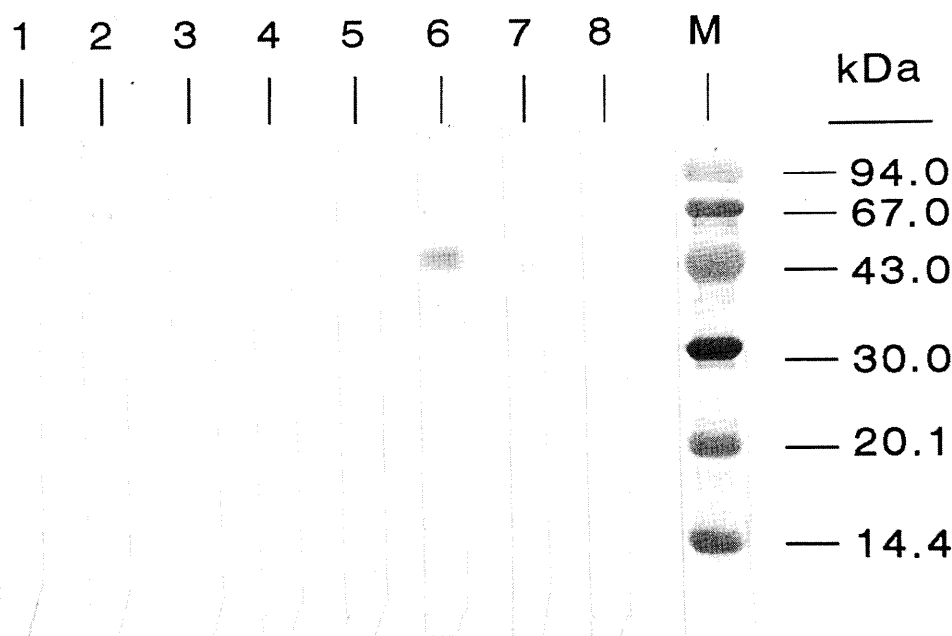


Figure 3. Immunoblots of natural latex with sera from patient 4 (lane 6), patient 15 (lane 4), patient 17 (lane 1), and patient 18 (lane 3) and pollen-allergic control subjects 1 (lane 5) and 3 (lane 2). IgE-binding bands were observed only in patient 4 (lane 6), who had clinical allergy to latex. Preincubation of serum from this patient with *L. perenne* pollen could not completely inhibit IgE-binding to latex allergens (lane 7). Negative control serum (lane 8). M: molecular weight markers.

zation to the major allergen Bet v 1 (17 kDa) is found (13, 18, 40), only one of our 19 patients had a positive CAP against rBet v 1 (5.2%). On the contrary, sensitization to birch profilin Bet v 2 (14 kDa) was found in 15 out of the 19 patients (79%) showing a positive CAP to birch pollen. These results, taken together with the results of immunoblotting assays, suggest that the positive CAP against birch pollen in these patients is quite probably due, not to a primary sensitization to birch pollen, but rather to the presence of IgE against birch profilin (Bet v 2), which shares epitopes with profilins from other pollens (24).

Interestingly, the 17 subjects who had a positive CAP to rBet v 2 (15 patients allergic to vegetable food and two control subjects) were also allergic to grass and olive pollens. In contrast, none of the six patients allergic to vegetables without grass and olive pollinosis had specific IgE against rBet v 2. Four out of these six patients had a positive CAP to mugwort, possibly due to the existence of other cross-reactive structures shared with plant-derived food. Specific IgE against mugwort pollen was found in 86% of our patients allergic to vegetables or fruits, in keeping with previous studies reporting a high prevalence of sensitization to mugwort among patients with allergy to plant-derived food (8, 9, 41). All subjects with positive CAP to rBet v 2 had also specific IgE to mugwort. Inhibition assays showed that *L. perenne* could inhibit the rBet v 2 CAP by as much as

52%, whereas mugwort pollen could inhibit rBet v 2 CAP only by 23%.

Profilins, which are present in extracts from several vegetables, have been shown to bind IgE (22) and cause cross-reactions between pollens and vegetables (10, 12, 14). van Ree & Aalberse (23), using profilin from *L. perenne*, observed that 30% of sera with positive RAST against grass pollen also had a positive RAST against profilin; this finding was more frequent (65% of the sera) in the patients who were allergic to both grass pollen and vegetables.

The frequency of sensitization to Bet v 1 and Bet v 2 varies widely in different European countries (42), probably because of the different environmentally relevant pollens in each region. It has been reported that 92–97% of the patients allergic to birch pollen are sensitized to Bet v 1, but sensitization to Bet v 2 or other profilins was found in only 9–20% of them (33, 43, 44). On the other hand, in patients allergic to grass, olive, or weed pollens, sensitization to Bet v 1 was present with a frequency varying from 4.3% (in our study) to 18.7% of the patients, and sensitization to Bet v 2 or profilins ranged from 11% to 75% (21, 45, 46).

Our findings are similar to those reported by van Ree et al. (21) in a study of 16 patients also living in Madrid and the surrounding area who were sensitized to grass and olive pollens. All the patients were allergic to fruits of the Rosaceae family, and only three of them had specific IgE

against Bet v 1, whereas 12 patients (75%) had specific IgE against profilin (from *L. perenne*) and a positive RAST against birch pollen. However, they did not find specific IgE against profilin in the patients who had allergy to fruits without grass pollinosis (21, 47). Ebner et al. (32) found that sera from four patients allergic to birch pollen with IgE to Bet v 2 reacted to a 14-kDa allergen in extracts of vegetables, and IgE-binding to this band could be inhibited by rBet v 2. Pauli et al. (33) reported an association between sensitization to Bet v 2 and sensitization to grass and weed pollen, and they also observed that sensitization to Bet v 2 is responsible for positive skin responses to birch in some patients with grass or weed pollinosis.

Immediate hypersensitivity to several fruits and nuts, principally banana, avocado, chestnut, and papaya, is frequently found in patients allergic to latex (26–29). However, there has been less investigation of latex sensitization in patients allergic to fruits and vegetables without obvious exposure to latex products. In our 29 patients allergic to fruits and vegetables, 17 (58.6%) had specific IgE (CAP) against latex, although only two out of these 17 had symptoms with latex products. A positive CAP to latex was also observed in two out of eight control pollen-allergic patients who had neither fruit allergy nor clinical hypersensitivity to latex products. It is to be noted that all patients who had specific IgE against rBet v 2 also had a positive CAP against latex and *L. perenne*, *O. europaea*, and *A. vulgaris* pollens. On the contrary, none of the six patients allergic to fruits without associated allergy to grass or olive pollen had specific IgE against profilin or latex. Vallier et al. (48) have recently reported that 59% of patients having specific IgE against ragweed profilin also had a positive CAP to latex in spite of the fact that they did not have any symptoms caused by latex. Moreover, these investigators observed that ragweed profilin could inhibit CAP to latex by 80%, suggesting that profilin, which was also present in natural latex, was the structure responsible for the cross-reaction (48).

In our study, with sera from patients with specific IgE to latex without symptoms of latex allergy, the CAP to latex was almost completely inhibited by *L. perenne* pollen. In contrast, with serum from a patient who had clinical allergy to latex products, CAP and immunoblotting against latex could not be significantly inhibited by *L. perenne* pollen. Moreover, a highly significant correlation was found between specific IgE to *L. perenne* pollen and latex. Fuchs et al. (49), using sera from five patients with accidentally diagnosed latex allergy, have recently observed that grass, ragweed, and mugwort pollens are able to inhibit CAP to latex. As the natural history of this sensitization is unknown, future studies will be necessary in order to determine whether these patients may later develop clinical allergy to latex.

Taken together, these observations suggest that profilin may well be the antigen causing cross-reactions between pollens, fruits, vegetables, and natural latex. Therefore, Bet v 2 can serve as a marker allergen for broad sensitization, but other cross-reactive structures need to be identified.

Specific IgE antibodies to papain and bromelain have been found in patients allergic to fruits and latex (17, 50–53). The existence of cross-reactive allergenic structures in plant-derived products, such as latex, fruits, and enzymes, may explain extensive allergenic reactivity. The role that plant enzymes (such as papain and bromelain) may play in cross-reactivity among fruits, pollens, and latex requires further research.

It is our belief that IgE reactivity to birch profilin may be the consequence of a primary sensitization induced by profilins from grass or weed pollen due to cross-reactivity; therefore, "birch sensitization" as determined by serology may be attributable to other allergen sources (e.g., grass pollen). Moreover, a large number of subjects sensitized to grass, weed, and tree pollens or some plant-derived foods may well have anti-profilin IgE antibodies which are responsible for the positive CAP results to latex.

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