

IgE to Bet v 1 and profilin: Cross-reactivity patterns and clinical relevance

Marjolein Wensing, MD,^a Jaap H. Akkerdaas, MSc,^b W. Astrid van Leeuwen, BSc,^b
Steven O. Stapel, PhD,^b Carla A. F. M. Bruijnzeel-Koomen, MD,^a
Rob C. Aalberse, PhD,^b Bert J. E. G. Bast, PhD,^c André C. Knulst, MD,^a
and Ronald van Ree, PhD^b *Utrecht and Amsterdam, The Netherlands*

Background: Individuals with pollen allergy often have IgE against plant-derived foods. This can be due to cross-reactive IgE against Bet v 1 and homologues, profilins, and/or cross-reactive carbohydrate determinants.

Objective: The aim of this study was to correlate sensitization to Bet v 1 and profilin with individual recognition patterns to plant foods and clinical relevance.

Methods: Fifty-two patients with pollen allergy and IgE against at least one plant-derived food were included in the study. Adverse reactions to plant-derived foods were documented by using standardized interviews. Skin prick tests were performed for pollen (grass, birch, and mugwort) and 14 plant-derived foods. In addition, recombinant (r) Bet v 1 and rBet v 2 (profilin) were tested intracutaneously. Specific IgE against the abovementioned allergens were determined by means of RAST. Cross-reactivity was studied by means of RAST inhibition.

Results: Eighty-five percent of patients were sensitized to Bet v 1, and 71% were sensitized to profilin. Profilin was associated with a higher number of positive RAST results to plant-derived foods than Bet v 1. In contrast, Bet v 1 was associated with more positive skin prick test responses and more food-related symptoms. Sensitization to Bet v 1 was associated with IgE against apple, hazelnut, and peach, whereas sensitization to profilin was associated with positive RAST results to all investigated plant-derived foods except apple, peach, and melon.

Conclusions: IgE antibodies against Bet v 1 have a more limited spectrum of cross-reactivity than those against profilin, but they frequently give rise to clinically relevant cross-reactivities to food. In analogy to anticarbohydrate IgE, cross-reactive IgE against food profilins have no or very limited clinical relevance. (*J Allergy Clin Immunol* 2002;110:435-42.)

Key words: Bet v 1, profilin, cross-reactivity, diagnostic accuracy

IgE antibodies to plant-derived foods are frequently observed in patients with pollen allergy.¹⁻⁴ The association between pollen and plant food has resulted in the description of several clinical syndromes, such as the

Abbreviations used

CCD: Cross-reactive carbohydrate determinant
ICT: Intracutaneous test
OAS: Oral allergy syndrome
SPT: Skin prick test

parabirch,^{1,5} mugwort-celery-spice,⁶ and ragweed-banana-melon syndromes.³ As was extensively studied, these phenomena can be explained by cross-reactive IgE.⁷ Both pollen- and plant-derived foods contain structures that share homologous IgE-binding sites. Several pollen allergens that are responsible for the observed cross-reactivity patterns have been identified. Bet v 1 (the major birch pollen allergen) is a pathogenesis-related protein of 18 kd that is responsible for the oral allergy syndrome (OAS) in patients with birch pollinosis.^{8,9} It is an ubiquitous protein in plants, and many Bet v 1-related allergens have been identified. Mal d 1, the major apple allergen, was the first cloned Bet v 1 homologue in food.¹⁰ Thereafter, Pru av 1 from sweet cherry,¹¹ Pru ar 1 from apricot,⁹ Pyr c 1 from pear,¹² Api g 1 from celery,¹³ and Dau c 1 from carrot¹⁴ have been identified. Additional Bet v 1-related proteins were detected in parsley and potato.¹⁵ The major allergen from hazel pollen, Cor a 1, also a Bet v 1 homologue, was reported to have similar IgE-binding properties as the major hazelnut allergen.¹⁶

Another protein capable of inducing cross-reactive IgE is profilin, which was first identified as a minor allergen in birch pollen (Bet v 2).¹⁷ Profilins are present in almost all eukaryotic cells and are highly cross-reactive.^{18,19} Although only 10% to 20% of patients with pollen allergy are sensitized to profilin,^{17,19,20} they are believed to react to a broad range of pollen- and plant-derived food. Profilin is involved in the mugwort-celery-spice-syndrome⁶ (later extended to the birch-mugwort-celery-spice syndrome²¹), but it was also demonstrated that patients allergic to grass pollen react to profilins in celery (Api g 4) and carrot.²² Other homologues have been detected in, for example, hazelnut,¹⁶ apple,²³ potato,¹⁹ lychee,²⁴ and tomato.²⁵ Profilin from peanut, Ara h 5, was produced as a recombinant allergen.²⁶ The clinical relevance of a sensitization to profilin remains unclear. More severe systemic symptoms have been attributed to profilins,²⁴ but strong evidence to support this is still lacking. Profilin-specific IgE has also been reported to be of little clinical relevance.²⁷

From ^athe Department of Dermatology/Allergology and ^cthe Department of Immunology, University Medical Centre Utrecht, Utrecht, and ^bthe Department of Immunopathology, Sanquin Research at CLB, Amsterdam. Received for publication January 7, 2002; revised April 15, 2002; accepted for publication May 9, 2002.

Reprint requests: Ronald van Ree, PhD, Department of Immunopathology, Sanquin Research, Plesmanlaan 125, 1066 CX, Amsterdam, The Netherlands.

© 2002 Mosby, Inc. All rights reserved.
0091-6749/2002 \$35.00 + 0 1/83/126380
doi:10.1067/mai.2002.126380

Despite the increasing knowledge base concerning these cross-reactive structures and their availability as purified natural or recombinant proteins, it is still not clear to what extent IgE antibodies against these structures can predict cross-reactivity patterns and their clinical relevance.²⁷ This study aimed at diagnosing sensitization to cross-reactive structures in a population sensitized to pollen and at correlating these results to the individual cross-reactivity patterns and clinical histories for plant-derived foods. We used recombinant (r) Bet v 1 and rBet v 2 in skin tests and RASTs. Skin tests and RASTs to 14 plant foods were performed, and their clinical relevance was documented according to case histories. Serologic cross-reactivity was further studied with RAST inhibition assays.

METHODS

Patients

Fifty-two adult patients (41 female and 11 male patients) who came to the outpatient clinic of the University Medical Centre Utrecht between December 1997 and December 1998 were included. All patients were sensitized to birch, grass, and/or mugwort pollen and had IgE against at least one plant-derived food, as judged by a positive skin test response ($\geq 2+$), a positive RAST result (≥ 0.7 IU/mL), or both. In addition, 5 nonatopic and 3 atopic subjects without IgE against plant-derived foods and food-related symptoms were selected as control subjects for intracutaneous tests (ICTs) with rBet v 1 and rBet v 2. The study was reviewed and approved by the local ethics committee. Written informed consent was obtained from all subjects before enrollment in the study.

Case history

Immediate adverse reactions after ingestion of plant foods were documented with standardized interviews. The following symptoms were scored: OAS-like symptoms, rhinoconjunctivitis, urticaria, angioedema, breathing difficulties, abdominal pain, nausea, and anaphylactic shock.

Recombinant birch pollen allergens and natural profilins

Recombinant Bet v 1 and rBet v 2 were obtained from BIOMAY (Linz, Austria). Natural (n) Bet v 2 and Lol p 12 were purified, as described elsewhere.²² In addition, a proteinase K-treated grass pollen (*Lolium perenne*) extract was used for RAST inhibition as a source of IgE-binding cross-reactive carbohydrate determinants (CCDs).²⁸

Skin prick tests

Skin prick tests (SPTs) were performed on the flexor aspect of the forearm with a standardized prick needle (ALK Lancet) and documented according to the method of Dreborg and Frew.²⁹ Histamine dihydrochloride (10 mg/mL) was used as a positive control, and the glycerol diluent of the SPT extracts was used as a negative control (ALK-Abelló, Nieuwegein, The Netherlands). Patients were tested with extracts for hazelnut, maize, melon, paprika, peach, peanut, rice, celery, wheat, tomato, carrot, and birch, grass, and mugwort pollen (ALK-Abelló). Raw apple (Granny Smith), potato (both with peels), and buckwheat were tested with the prick-to-prick technique.²⁹ These foods were chosen to ensure that the most important allergenic botanic families of plant food are represented (Rosaceae, Betulaceae, Leguminosae, Solanaceae, Compositae, cereals, and Cucurbitaceae). SPT responses were regarded as positive when the

wheel diameter was one half of the histamine wheel diameter or larger ($\geq 2+$), provided that the histamine wheel was 5 mm or larger and the wheel of the negative control was less than 3 mm in diameter.

Intracutaneous tests

ICTs were performed on the flexor aspect of the patient's arm. The test sites were placed 5 cm apart. Histamine dihydrochloride and a 0.9% NaCl solution served as a positive and negative control, respectively (ALK-Abelló). Stock solutions of rBet v 1 and rBet v 2 (100 μ g/mL) were stored in aliquots at -20°C until use. For ICTs, 2 dilutions, 20 and 2 μ g/mL (in 0.9% NaCl), were injected intradermally. These dilutions were selected according to previous studies.^{20,30} For Bet v 1, however, these dilutions were changed to 2 and 0.2 μ g/mL in the course of the study because 20 μ g/mL resulted in very large wheals. This change had minimal effect on the scoring of positive ICT results for rBet v 1 because all patients with a positive ICT result to 20 μ g/mL also displayed a positive ICT result to 2 μ g/mL rBet v 1. The results were recorded according to the method of Dreborg and Frew.²⁹ Test results were regarded as positive when the wheal diameter was 5 mm or larger and the erythema diameter was 10 mm or larger.

RAST and RAST inhibition

RASTs were performed as previously described.³¹ The same extracts were used as mentioned in an earlier study.²² In brief, 100 μ g of purified protein or a 4-mg extract (dry weight) was coupled to 100 mg of CNBr-activated Sepharose 4B (Amersham Pharmacia Biotech, Uppsala, Sweden). Per test, 50 μ L of serum was added to 0.5 mg of purified protein sepharose, 0.5 mg of pollen extract sepharose, or 1.5 mg of food extract sepharose in a final volume of 300 μ L (phosphate-buffered saline/0.3% [wt/vol] BSA/0.1% [vol/vol] Tween 20) and incubated overnight. After washing, immunodetection was performed with iodine 125-labeled sheep anti-human IgE. RAST results were expressed in international units per milliliter by using an in-house standard of mouse-human chimeric IgE antibodies against Der p 2. These antibodies were calibrated against the World Health Organization international reference for IgE and tested in different dilutions for binding to sepharose-coupled rDer p 2. The resulting standard curve was used to calculate RAST results in international units per milliliter.³² The detection limit of the assay is 0.1 IU/mL specific IgE. Patients were designated as sensitized if specific IgE levels were 0.7 IU/mL or greater.

For RAST inhibition, sera were preincubated for 2 hours with 50 μ L of pollen extracts (5 mg/mL) before addition of sepharose. RAST inhibition was performed with birch pollen extract, grass (*Lolium perenne*) pollen extract, and/or proteinase K-digested grass pollen extract. The rationale behind this protocol is the assumption that selective inhibition with birch pollen indicates Bet v 1 as the dominant cross-reactive structure, whereas inhibition by both grass and birch pollen suggests involvement of profilin, CCDs, or both. Protease-digested grass pollen extract was used as a source of CCDs to distinguish between profilin and CCDs as the basis of cross-reactivity.

Statistics

For calculations of significant associations between sensitizations to cross-reactive structures and results of skin tests, RASTs, and case histories of the plant-derived foods, χ^2 tests were used. When tests were 2-sided, by using the Fisher exact test, P values of less than .05 were regarded as significant. χ^2 Tests were also used for testing the association of cross-reactive structures and the amount of false-positive RAST results. The Kruskal-Wallis test was used to calculate differences between the Bet v 1-specific and/or profilin-sensitized group concerning the number of positive SPT responses, RAST results, and case histories for plant-derived foods, after which pair-

TABLE I. Sensitization (positive ICT result, positive RAST result, or both) to rBet v 1 and profilin according to pollen sensitization

	Birch	Grass	Birch and grass	Birch and mugwort	Grass and mugwort	Birch, grass, and mugwort	Total
Bet v 1	8		2	1		3	14
Profilin		1			5	1	7
Both	1		4			25	30
None		1					1
Total	9	2	6	1	5	29	52

wise differences were calculated with the Mann-Whitney *U* test ($P < .017$ was regarded as significant after applying the Bonferroni correction). The Spearman rank test was used to calculate correlations between the level of Bet v 1- or profilin-specific IgE and the number of positive SPT responses, RAST results, and case histories and the specific IgE levels of the plant-derived foods. A *P* value of less than .05 was regarded as significant.

RESULTS

Patients

Fifty-two patients (41 female and 11 male patients) with a mean age of 33.4 years (range, 18-57 years) were included in the study. All patients were sensitized to birch, grass, and/or mugwort pollen and had IgE antibodies against at least one plant-derived food (mean of 8 foods per patient). Forty-nine patients had clinical symptoms of rhinoconjunctivitis in the corresponding pollen seasons, and 42 patients had plant food-related symptoms (mainly oral allergy syndrome [OAS], urticaria, and gastrointestinal symptoms in 68%, 8%, and 7%, respectively) to one or more plant foods (mainly apple, hazelnut, and tomato in 79%, 69%, and 57%, respectively). Thirty-nine patients had a history of asthma, and 37 patients had a history of atopic dermatitis.

Classification on the basis of recognition of Bet v 1 and profilin

Sensitization to rBet v 1 was found in 44 patients by using RASTs (geometric mean [standard error], 19.2 IU/mL [16.0-22.4 IU/mL]) and in 47 patients by using ICTs, corresponding well with sensitization to birch pollen ($n = 45$). IgE against rBet v 2 was found in only 10 patients by means of RASTs (0.4 IU/mL [0.3-0.5 IU/mL]), whereas 22 patients had a positive ICT result to rBet v 2. RASTs were also performed with natural Bet v 2 and its homologue from grass pollen, Lol p 12. In this case 27 and 32 patients were given a diagnosis of sensitization to profilin, respectively. IgE responses to rBet v 2 were greater than 4 times lower than those to natural Bet v 2 (1.8 IU/mL [1.4-2.2 IU/mL]) and almost 10 times lower than those to Lol p 12 (3.6 IU/mL [3.0-4.3 IU/mL], $P < .001$). Still, 2 patients had a positive ICT result with rBet v 2 without a positive RAST result to any of the profilin preparations used in this study. ICT results with rBet v 1 and rBet v 2 were negative in 5 nonatopic and 3 atopic control subjects. For further analyses, sensitization to profilin was defined as a positive RAST result (≥ 0.7 IU/mL) to natural Bet v 2, Lol p 12, or both, resulting in 37 profilin-sensitized patients.

TABLE II. Numbers of positive SPT responses, RAST results, and case histories to plant foods in Bet v 1-sensitized patients, profilin-sensitized patients, or both

	n	No. of positive responses (mean)		
		SPT	RAST	Case history
Bet v 1	14	4.43	6.79	4.36
Profilin	7	2.43	8.86*	1.14
Both	30	3.47	10.17	4.63*

*Significant differences, as calculated by means of Kruskal-Wallis and additional Mann-Whitney *U* analysis.

Table I summarizes the recognition of cross-reactive allergens in relation to the different patterns of pollen sensitization. As can be expected, sensitization to profilin was almost absent in patients monosensitized to birch or grass pollen (2/11). The majority of profilin-reactive patients were polysensitized to the 3 pollen species (26/37).

IgE responses to potentially cross-reactive plant-derived foods

The total number of positive responses to plant-derived foods, as judged by means of SPTs and RASTs, and the number of foods inducing clinical food allergy was counted per patient. Mean values for the 3 groups that were distinguished on the basis of recognition of Bet v 1, profilin, or both are displayed in Table II. When Bet v 1-sensitized patients were cosensitized to profilin, a broader spectrum of foods was recognized on the basis of serum IgE levels ($P = .01$). IgE titers against Bet v 1 did not differ significantly in this group compared with that in patients monosensitized to Bet v 1 (geometric means, 15.8 vs 11.7 IU/mL; $P > .2$). In addition, the IgE titer against profilin was positively correlated with the number of positive food RAST results ($R_s = 0.554$, $P < .01$). This dose-response relation was not observed for Bet v 1. Cosensitization to Bet v 1 and profilin was associated with significantly more positive case histories compared with monosensitization to profilin ($P = .003$). IgE titers against profilin were similar in both groups (4.5 vs 4.7 IU/mL, respectively; $P > .2$). The higher clinical effect of IgE against Bet v 1 was further supported by the comparison of patients monosensitized to either Bet v 1 or profilin, although in this case significance was not reached ($P = .031$). In addition, IgE titers against Bet v 1 correlated with the number of positive SPT responses and case histories ($R_s = 0.318$ and 0.285, respectively; $P < .05$).

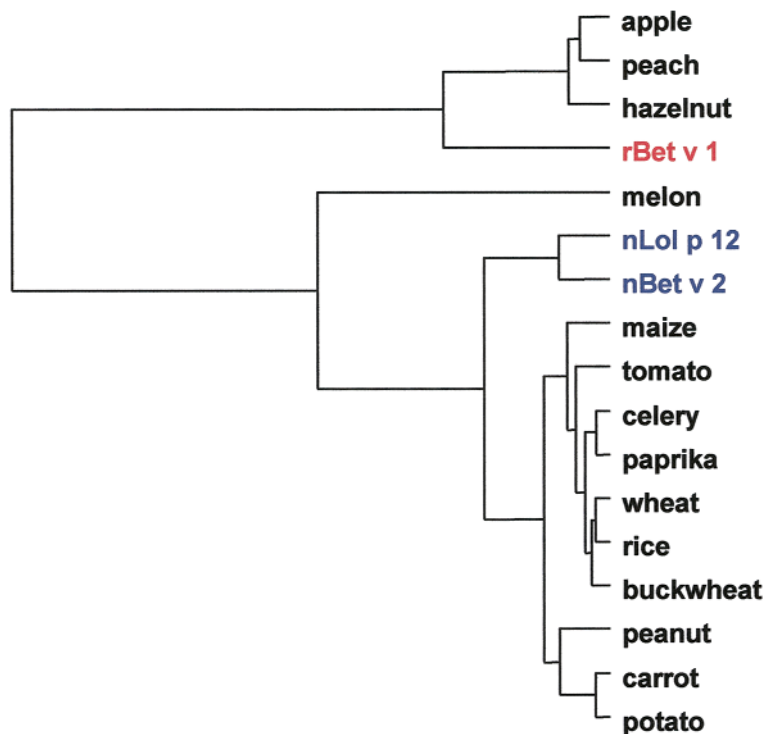


FIG 1. Correlations between specific IgE titers of Bet v 1, profilin, and 14 plant-derived foods calculated by using the Spearman rank test. (Figure continued on next page)

Association of cross-reactive structures with individual plant-derived foods

Is sensitization to Bet v 1 or profilin associated with IgE against specific foods? This question was first addressed by means of Spearman rank correlation analysis of all RAST results for cross-reactive structures and for foods (Fig 1). Bet v 1 was strongly correlated with apple, peach, and hazelnut ($0.69 < R_s < 0.79$) and only very weakly with carrot ($R_s = 0.28$). Profilin showed the highest correlation with potato, carrot, celery, buckwheat, paprika, and tomato ($R_s > 0.63$). Only for apple, peach, and melon were no significant correlations with profilin found. When patients were again subdivided into 3 groups according to the recognition of Bet v 1, profilin, or both, these correlations were confirmed by using χ^2 tests (Fig 2). In this analysis only positive case histories for apple, peach, and melon were linked to Bet v 1 sensitization ($P = .001$, $P = .031$, and $P = .038$, respectively). For other foods, no significant correlations were found between IgE to Bet v 1 or profilin and clinical histories.

Comparison of SPT results with reported clinical histories indicated a very low sensitivity of SPT materials for peach, tomato, paprika, and melon (<26%). In contrast, comparison of RAST results with clinical histories indicated high numbers of false-positive results. This was especially observed for the cereals with specificities of less than 15%. Mean numbers of false-positive RAST results were calculated for patients subdivided on the basis of profilin recognition. Sensitization to profilin

resulted in significantly more false-positive RAST results (6.92 vs 4.14, $P = .043$).

Serologic cross-reactivity studied by means of RAST inhibition

RAST inhibition assays were performed with sera of 15 patients with different recognition profiles of the 2 cross-reactive structures to confirm cross-reactivity as an explanation for the observed associations. Four representative examples are described below. Food RAST results of patient 2 with selective recognition of Bet v 1 (3.2 IU/mL) were inhibited by birch pollen but not by grass pollen extract (Fig 3, A). This was true for 3 clinically relevant foods (apple, peach, and hazelnut), as well as for a food not reported to cause clinical food allergy in this patient, carrot. Monosensitization to profilin (patient 11, 3.7 IU/mL) was accompanied by 10 or more positive RAST results for foods, as well as for latex, all without clinical relevance. IgE binding to these foods was in some cases inhibited by grass pollen extract (apple, hazelnut, celery, carrot, peanut, maize, and rice). No significant inhibition was observed with proteinase K-digested grass pollen extract. RAST results for tomato, potato, paprika, buckwheat, wheat, and latex were not inhibited by grass pollen (Fig 3, B). Possibly IgE antibodies against the latter foods are cross-reactive to latex rather than to grass pollen. Serum availability did not allow us to test this hypothesis, but the suggestion was supported by a food RAST inhibition with latex for patient 53 (Fig 3, C) that demonstrated a very similar spectrum of positive food

	Potato			apple			buckwheat			Hazelnut			maize			Melon			paprika		
	SP T	RAS T	CH	SP T	RAS T	CH	SP T	RAS T	CH	SP T	RAS T	CH	SP T	RAS T	CH	SP T	RAS T	CH	SP T	RAS T	CH
Bet v 1 (n=14)	10	5	7	11	13	10	6	5	0	14	13	8	2	5	1	0	4	5	1	4	7
Profilin (n=7)	5	6	1	0	3	0	2	7	0	3	2	2	0	4	0	0	0	0	2	3	1
Both (n=30)	18	23	11	21	29	23	12	18	1	20	25	19	2	19	1	0	12	13	1	20	14
Total	33	34	19	32	45	33	20	30	1	37	40	29	4	28	2	0	16	18	4	27	22

	peach			peanut			rice			celery			wheat			Tomato			carrot		
	SP T	RAS T	CH	SP T	RAS T	CH	SP T	RAS T	CH	SP T	RAS T	CH	SP T	RAS T	CH	SP T	RAS T	CH	SP T	RAS T	CH
Bet v 1 (n=14)	0	12	5	3	6	5	1	5	0	5	6	1	2	6	2	3	3	7	4	8	3
Profilin (n=7)	0	3	0	1	4	2	1	5	0	1	6	0	1	6	0	0	6	2	1	7	0
Both (n=30)	1	25	14	5	22	11	0	20	0	15	23	6	2	22	3	3	20	14	4	27	9
Total	1	40	19	9	32	18	2	30	0	21	35	7	5	34	5	6	29	23	9	42	12

FIG 2. Number of positive test results and case histories to 14 plant-derived foods subdivided according to Bet v 1 sensitization, profilin sensitization, or both. Significant associations (tested by means of χ^2 analysis) are indicated by arrows. Yellow cells indicate Bet v 1-related data, and blue cells indicate profilin-related data. CH, Case history.

RAST results as that seen for patient 11. Celery and carrot RAST results were inhibited by grass pollen extract, in contrast to peach, tomato, potato, peanut, paprika, and cereals. Especially peach, tomato, and paprika were strongly inhibited (>85%) by latex extract.

Patients with cosensitization to Bet v 1 and profilin (eg, patient 6) showed a surprising division between foods (Fig 3, D). Apple, peach, and hazelnut were selectively inhibited with birch pollen extract, indicating that cross-reactive IgE to profilin plays no significant role for these foods. Most other foods were inhibited equally by grass and birch pollen, indicating a dominant role for profilin. Some of these foods were reported to cause clinical food allergy (tomato and paprika), thereby supporting possible clinical relevance of antiprolin IgE in selected cases. Some food RAST results were completely inhibited by birch pollen and only partially by grass pollen (celery, carrot, peanut, and potato), suggesting that here both Bet v 1 and profilin are involved in cross-reactivity.

DISCUSSION

In this study skin tests and RASTs for the most important pollen-plant food cross-reactive structures were performed in 52 patients with pollen allergy. Most patients were sensitized to birch, grass, and mugwort pollen (n = 29), already indicating sensitization to cross-reactive allergens. Indeed, the link between multiple pollen sensitization and IgE against profilin and CCDs was confirmed.³³ All patients had IgE antibodies against at least one plant-derived food, of whom 42 patients reported food-related symptoms to one or more of the investigated plant-derived foods. Food-related symptoms were verified by means of case history only by using a standardized interview. Double-blind, placebo-controlled food

challenges were not included because the study was conducted during the normal routine practice of the outpatient clinic. Reliability of clinical histories on the basis of the standardized interview was supported by 2 different parallel studies aiming at determining threshold levels for hazelnut and peanut (unpublished data). In these studies 29 (94%) of 31 and 26 (100%) of 26 positive clinical histories (mainly OAS) were confirmed by means of double-blind, placebo-controlled food challenge. These investigations were performed by the same physician as in the present study. The vast majority of our study population reported OAS. In several studies by other groups, it was already shown that diagnosis of OAS by means of case history is reliable because symptoms are easily recognized and described.³⁴

As was previously described, birch pollen sensitization was reliably diagnosed, as compared with whole birch pollen extract, by using ICT or RAST with rBet v 1.^{20,30} In contrast, rBet v 2 proved to be a relatively poor reagent for studying sensitization to profilins. Comparison of RAST results with rBet v 2 and its natural counterpart confirmed earlier observations that the recombinant profilin has significantly lower IgE-binding capacity,³⁵ possibly because of improper folding of the recombinant molecule. For similar reasons, the prevalence of sensitization to profilin in patients with pollen allergy might have been underestimated when based on recombinant Bet v 2 reagents.^{20,36} The prevalence of sensitization to profilin in patients with pollen allergy reported in this study is much higher (71%) than that in other studies thus far published (10%-20%).¹⁷⁻¹⁹ In our study patients with pollen allergy were only included if they also had IgE antibodies against one or more plant-derived foods. This of course results in a bias toward cross-reactive (eg, profilin) sensitization.²²

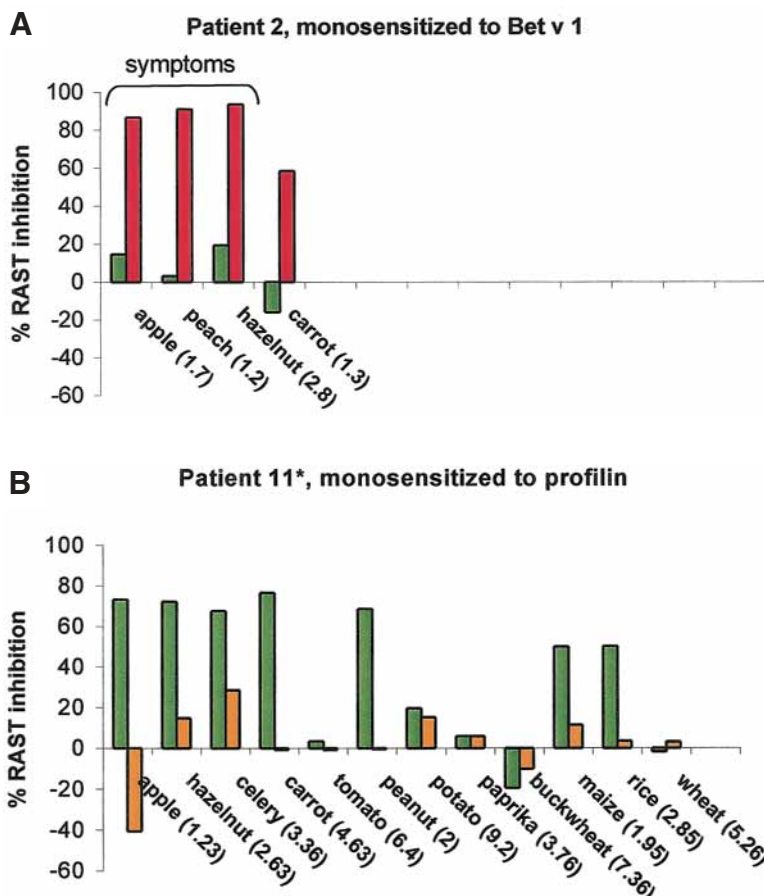


FIG 3. RAST inhibition assays of 4 patients. *Patients 11 and 53 are also sensitized to latex. RAST values are given in brackets. *Green bars*, Inhibition with grass pollen extract; *red bars*, inhibition with birch pollen extract; *orange bars*, inhibition with proteinase K-treated grass pollen extract (CCD); *blue bars*, inhibition with latex extract.

This study once more confirmed the poor quality of several commercial plant food extracts for SPTs.^{37,38} Peach, paprika, and melon proved to have especially low sensitivity (<26%). Prick-to-prick testing still seems to be the technique of choice in these cases. When SPTs resulted in false-negative results, food RAST results were frequently false positive. False-positive food RAST results appeared to be present more often in patients with IgE against profilin compared with in patients with IgE against Bet v 1 ($P = .043$).

The aim of this study was to investigate whether sensitization to a given cross-reactive structure can predict (individual) cross-reactivity patterns with regard to the plant-derived foods and their clinical relevance. By subdividing the study population according to Bet v 1, profilin, and Bet v 1 plus profilin sensitization, several significant differences and associations were detected. Significantly more positive food RAST results were found in the Bet v 1 plus profilin-sensitized group compared with in the Bet v 1-sensitized/profilin-negative group, suggesting that profilin-specific IgE broadens the spectrum of cross-reactivity compared with anti-Bet v 1 IgE (Table II).^{18,27} It can not be ruled out completely that this higher degree of cross-reactivity is (partly) caused by

higher anti-Bet v 1 titers in the Bet v 1 plus profilin-cosensitized group compared with in the Bet v 1-monosensitized group, although the observed difference was not significant (geometric mean, 15.8 vs 11.7 IU/mL). Support for a truly broader spectrum of cross-reactivity of antiprofilin IgE comes from the observation that the profilin-monosensitized group tends to recognize more foods than the Bet v 1-monosensitized group, even though IgE titers against Bet v 1 were higher (9.8 vs 4.7 IU/mL, $P = .04$). Higher titers of antiprofilin IgE were linked to more positive RAST results, implying that absolute levels of IgE do have influence on the spectrum of cross-reactivity. Finally, Spearman rank correlation analysis of all RAST results confirmed a much broader spectrum of cross-reactivity for profilin than for Bet v 1. Despite the high degree of cross-reactivity, specificities of antiprofilin IgE were found to be highly variable. In some cases latex, instead of pollen, appeared to be the cross-sensitizer. IgE against profilins did not always cross-react with apple, peach, and hazelnut, although these foods contain profilins recognized by other patients with pollen allergy.

This study shows that the broad spectrum of cross-reactivity of IgE antiprofilin is not necessarily translated

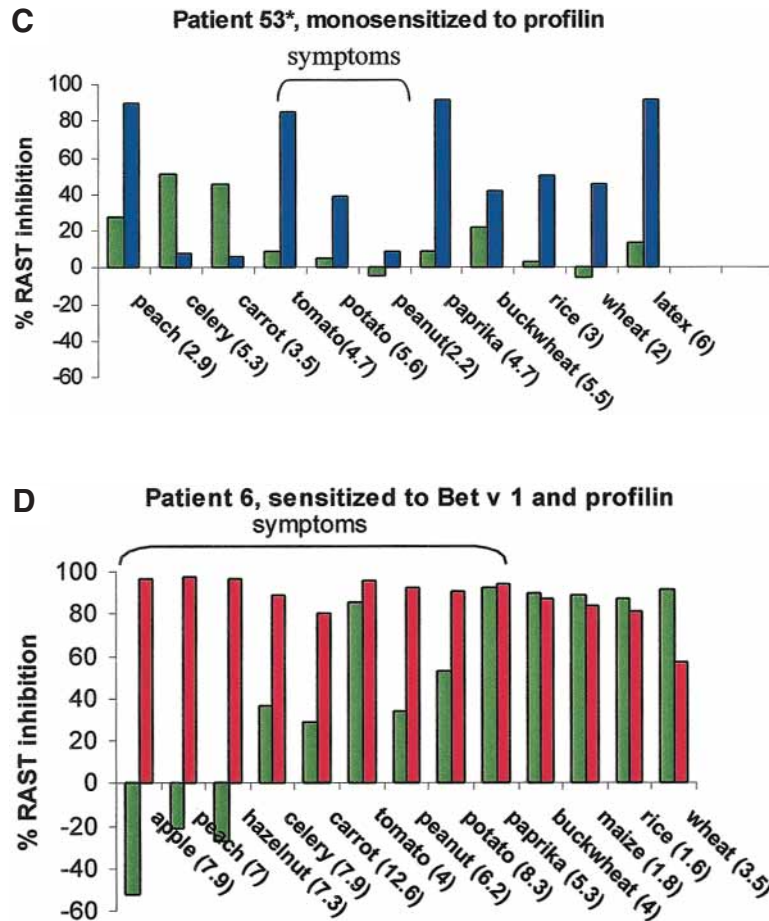


FIG 3. (Continued from previous page)

into a broad spectrum of clinical food allergies. Only in rare cases are food profilins linked to symptoms. The opposite is true for Bet v 1. IgE antibodies against Bet v 1 are less cross-reactive but are of higher clinical relevance. Apple, peach, and hazelnut were confirmed to be important Bet v 1-related foods. One could argue that the observed difference in biologic activity (SPT) and clinical relevance of anti-Bet v 1 and antiproliferin IgE is simply explained by the IgE titers (19.2 vs 3.6 IU/mL, respectively). Indeed, higher titers of anti-Bet v 1 IgE were correlated to more positive SPT responses and clinical histories. On the other hand, when all food RAST values and clinical histories were compared, no clear pattern was observed. For some foods, clinical food allergy was accompanied by higher mean RAST values, and for other foods, this was accompanied by lower mean values. When all food RAST results were analyzed together, mean values were significantly lower when linked to positive clinical histories (1.7 vs 2.5 IU/mL, $P < .001$). These observations suggest that there is no simple dose-response relationship between food-specific IgE (as determined by RAST) and clinical relevance. This was further supported by RAST inhibitions. Quantitatively

similar food RASTs, traced back to Bet v 1 cross-reactivity, can be both clinically relevant and irrelevant (Fig 3, A). For profilin, the balance was shown to be in favor of clinically irrelevant IgE antibodies. However, also for profilin, similar food RAST results were linked to different clinical presentations (Fig 3, D). Clinical food allergy is the result of allergen-induced cross-linking of IgE molecules bound to high-affinity IgE receptors on effector cells. Valency and affinity of this interaction determine whether efficient mediator release (ie, clinical food allergy) is induced.³⁹ A recent study has confirmed a close correlation between the affinity for IgE-allergen binding and biologic activity.⁴⁰ Most likely, the clinical relevance of pollen-plant food cross-reactions is dependent on the number of cross-reactive epitopes (valency) and the affinity of IgE recognition.

In summary, this study has confirmed, in a well-defined group of pollen-food sensitized patients, that profilin is responsible for broader cross-reactivity than Bet v 1. It was clearly demonstrated that profilin is rarely linked to clinical food allergy. This illustrates that the reported poor biologic activity of CCDs is not unique for carbohydrate determinants. Furthermore, this study has

shown that this lack of clinical relevance of profilin can not simply be explained by low titers of IgE.

We thank Frank Rijnja, pharmacist, for preparing the dilutions of the cross-reactive structures.

REFERENCES

- Calkhoven PG, Aalbers M, Koshte VL, Pos O, Oei HD, Aalberse RC. Cross-reactivity among birch pollen, vegetables and fruits as detected by IgE antibodies is due to at least three distinct cross-reactive structures. *Allergy* 1987;42:382-90.
- Dreborg S, Foucard T. Allergy to apple, carrot and potato in children with birch pollen allergy. *Allergy* 1983;38:167-72.
- Anderson LB, Dreyfuss EM, Logan J, Johnstone DE, Glaser J. Melon and banana sensitivity coincident with ragweed pollinosis. *J Allergy* 1970;45:310-9.
- Eriksson NE. Food hypersensitivity reported by patients with asthma and hay fever. *Allergy* 1978;33:189-96.
- Ebner C, Birkner T, Valenta R, et al. Common epitopes of birch pollen and apples—studies by Western and Northern blot. *J Allergy Clin Immunol* 1991;88:588-94.
- Wüthrich B, Dietschi R. Das 'Sellerie-Karotten-Beifuss-Gewürz-Syndrom'. Hauttest- und RAST-Ergebnisse. *Schweiz Med Wochenschr* 1985; 115:358-64.
- van Ree R, Aalberse RC. Pollen-vegetable food crossreactivity: serological and clinical relevance of crossreactive IgE. *J Clin Immunoassay* 1993;16:124-30.
- Breiteneder H, Pettenburger K, Bito A, et al. The gene coding for the major birch pollen allergen Bet v 1, is highly homologous to a pea disease resistance response gene. *EMBO J* 1989;8:1935-8.
- Breiteneder H, Ebner C. Molecular and biochemical classification of plant-derived food allergens. *J Allergy Clin Immunol* 2000;106:27-36.
- Vanek-Krebitz M, Hoffmann-Sommergruber K, Laimer da Camara Machado M, et al. Cloning and sequencing of Mal d 1, the major allergen from apple (*Malus domestica*), and its immunological relationship to Bet v 1, the major birch pollen allergen. *Biochem Biophys Res Commun* 1995;214:538-51.
- Neudecker P, Schweimer K, Nerkamp J, et al. Allergic cross-reactivity made visible. Solution structure of the major cherry allergen Pru av 1. *J Biol Chem* 2001;276:22756-63.
- Karamloo F, Scheurer S, Wangorsch A, May S, Hausteind D, Vieths S. Pyr c 1, the major allergen from pear (*Pyrus communis*), is a new member of the Bet v 1 allergen family. *J Chromatogr B Biomed Sci Appl* 2001;25:281-93.
- Breiteneder H, Hoffmann-Sommergruber K, O'Riordain G, et al. Molecular characterization of Api g 1, the major allergen of celery (*Apium graveolens*) and its immunological and structural relationships to a group of 17-kDa tree pollen allergens. *Eur J Biochem* 1995;233:484-9.
- Hoffmann-Sommergruber K, O'Riordain G, Ahorn H, et al. Molecular characterization of Dau c 1, the Bet v 1 homologous protein from carrot and its cross-reactivity with Bet v 1 and Api g 1. *Clin Exp Allergy* 1999;29:840-7.
- Hoffmann-Sommergruber K, Vanek-Krebitz M, Radauer C, et al. Genomic characterization of members of the Bet v 1-family: genes coding for allergens and pathogenesis-related proteins share intron positions. *Gene* 1997;197:91-100.
- Hirschwehr R, Valenta R, Ebner C, et al. Identification of common allergenic structures in hazel pollen and hazelnuts: a possible explanation for sensitivity to hazelnuts in patients allergic to tree pollen. *J Allergy Immunol* 1992;90:927-36.
- Valenta R, Duchêne M, Pettenburger K, et al. Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. *Science* 1991;253:557-60.
- Valenta R, Duchêne M, Ebner C, et al. Profilins constitute a novel family of functional plant pan allergens. *J Exp Med* 1992;175:377-85.
- Ebner C, Hirschwehr R, Bauer L, et al. Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). *J Allergy Clin Immunol* 1995;95:962-9.
- Pauli G, Oster JP, Deviller P, et al. Skin testing with recombinant allergens rBet v 1 and birch profilin, rBet v 2: diagnostic value for birch pollen and associated allergies. *J Allergy Clin Immunol* 1996;97:1100-9.
- Bauer L, Ebner C, Hirschwehr R, et al. IgE cross-reactivity between birch pollen, mugwort pollen and celery is due to at least three distinct cross-reacting allergens: immunoblot investigation of the birch-mugwort-celery syndrome. *Clin Exp Allergy* 1996;26:1161-70.
- van Ree R, Voitenko V, van Leeuwen WA, Aalberse RC. Profilin is a cross-reactive allergen in pollen and vegetable foods. *Int Arch Allergy Immunol* 1992;98:97-104.
- van Ree R, Fernández-Rivas M, Cuevas M, van Wijgaarden M, Aalberse RC. Pollen-related allergy to peach and apple: an important role for profilin. *J Allergy Clin Immunol* 1995;95:726-34.
- Fäh J, Wüthrich B, Vieths S. Anaphylactic reaction to lychee fruit: evidence for sensitisation to profilin. *Clin Exp Allergy* 1995;25:1018-23.
- Petersen A, Vieths S, Aulepp H, Schlaak M, Becker WM. Ubiquitous structures responsible for IgE cross-reactivity between tomato fruit and grass pollen allergens. *J Allergy Clin Immunol* 1996;98:805-15.
- Kleber-Janke T, Cramer R, Appenzeller U, Schlaak M, Becker WM. Selective cloning of peanut allergens, including profilin and 2S albumins, by phage display technology. *Int Arch Allergy Immunol* 1999;119:265-74.
- van Ree R, Akkerdaas JH, van Leeuwen WA, et al. New perspectives for the diagnosis of food allergy. *ACI Intern* 2000;12:7-12.
- van Ree R, Hoffman DR, van Dijk W, et al. Lol p XI, a new major grass pollen allergen, is a member of a family of soybean trypsin inhibitor-related proteins. *J Allergy Clin Immunol* 1995;95:970-8.
- Dreborg S, Frew A, editor. Allergen standardisation and skin tests. EAACI position paper. *Allergy* 1993;48(suppl 14):49-82.
- Menz G, Dolecek C, Schönheit-Kenn U, et al. Serological and skin-tests diagnosis of birch pollen allergy with recombinant Bet v 1, the major birch pollen allergen. *Clin Exp Allergy* 1996;26:50-60.
- Aalberse RC, Koshte V, Clemens JG. Immunoglobulin E antibodies that cross-react with vegetable foods, pollen and Hymenoptera venom. *J Allergy Clin Immunol* 1981;68:356-64.
- Schuurman J, Perdok GJ, Lourens TE, Parren PW, Chapman MD, Aalberse RC. Production of a mouse/human chimeric IgE monoclonal antibody to the house dust mite allergen Der p 2 and its use for the absolute quantification of allergen-specific IgE. *J Allergy Clin Immunol* 1997;99:545-50.
- Mari A. Multiple pollen sensitization: a molecular approach to the diagnosis. *Int Arch Allergy Immunol* 2001;125:57-65.
- Ortolani C, Ballmer-Weber BK, Skamstrup Hansen K, et al. Hazelnut allergy: a double-blind, placebo-controlled food challenge multicenter study. *J Allergy Clin Immunol* 2000;105:577-81.
- van Ree R, van Leeuwen WA, Akkerdaas H, Aalberse RC. How far can we simplify in-vitro diagnostics for Fagales tree pollen allergy? A study with three whole pollen extracts and purified natural and recombinant allergens. *Clin Exp Allergy* 1999;29:848-55.
- Elfman L, Svensson M, Lidholm J, Pauli G, Valenta R. Different profiles in specific IgE to rBet v 1 and rBet v 2 in patients allergic to birch pollen from six countries. *Int Arch Allergy Immunol* 1997;113:249-51.
- Rudeschko O, Fahlbusch B, Henzgen M, et al. Investigation of the stability of apple allergen extracts. *Allergy* 1995;50:575-80.
- Ortolani C, Spano M, Pastorello EA, et al. Comparison of results of skin tests (with fresh foods and commercial food extracts) and RAST in 100 patients with oral allergy syndrome. *J Allergy Clin Immunol* 1989;83:683-90.
- van Ree R. Factors modulating allergen-induced histamine release. In: Bousquet J, Yssel H, editors. Immunotherapy in asthma. New York: Marcel Dekker; 1999. p. 399-409.
- Pierson-Mullany LK, Jackola DR, Blumenthal MN, Rosenberg A. Evidence of an affinity threshold for IgE-binding in the percutaneous skin test reaction. *Clin Exp Allergy* 2002;32:107-16.