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Current Understanding of Cross-Reactivity of Food Allergens and Pollen

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ABSTRACT: Pollen-allergic patients frequently present allergic symptoms after ingestion of several kinds of plant-derived foods. The majority of these reactions is caused by four distinct cross-reactive structures that are present in birch pollen. Proteins that share common epitopes with Bet v 1, the major birch pollen allergen, occur in pollens of several tree species: apples, stone fruits, celery, carrot, nuts, and soybeans. Approximately 70% of our patients who are allergic to birch pollen may experience symptoms after consumption of foods from these groups. In contrast, two minor allergenic structures—profilins and cross-reactive carbohydrate determinants (CCD)—that sensitize approximately 10–20% of all pollen-allergic patients are also present in grass pollen and weed pollen. Moreover, IgE-binding proteins related to the birch pollen minor allergen Bet v 6 have been found in many vegetable foods such as apple, peach, orange, lychee fruit, strawberry, persimmon, zucchini, and carrot. Frequently, the occurrence of cross-reactive IgE antibodies is not correlated with the development of clinical food allergy. In particular, the clinical relevance of sensitization to CCD is doubtful. Generally, pollen-related allergens tend to be more labile during heating procedures and in the digestive tract compared to allergens from classical allergenic foods such as peanut. However, recent DBPCFC studies have shown that both cooked celery and roasted hazelnuts still pose an allergenic risk for pollen-sensitized subjects. Since pathogenesis-related proteins share several common features with allergens and both the Bet v 1 and the Bet v 6-related food allergens are defense-related proteins, approaches to introduce such proteins as a measure to protect plants against diseases should be performed with caution as they may increase the allergenicity of these crops.

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POLLEN-RELATED FOOD ALLERGY

Fruits, vegetables, and nuts belong to the most important elicitors of food allergy in adults and adolescents.^{1–7} The majority of allergic reactions against these foods is highly associated with several pollen allergies.^{2,3,6–10} Approximately 15–20% of the population in developed countries are allergic to pollen,¹¹ and between 50 and 93% of birch pollen-allergic patients have IgE-mediated reactions to pollen-related foods.³ On the basis of these data, the prevalence of fruit, nut, and vegetable hypersensitivity can be estimated to be significantly higher than 1%. The overwhelming majority of pollen-related reactions to fruits is associated with birch pollen allergy. In addition, cross-reactive allergies to certain foods such as apple, peach, tomato, or peanuts have been found in a minority of subjects with grass pollen allergy.¹ Allergies to several birch pollen-related foods, in particular to celery tuber, carrot, and spices can occur in mugwort pollen-allergic patients without birch pollen allergy,¹⁰ but our recent studies have shown that this phenomenon is relatively rare.^{12,13} In 1970 allergy to watermelon and banana was reported for a minority of patients with ragweed pollen allergy,¹⁴ but there is a lack of clinical reports or studies confirming this finding. Recently, Enrique *et al.*¹⁵ reported an association between plane tree pollinosis and plant food allergy, with 50% of the pollen-allergic patients studied being allergic to at least one vegetable food. The foods most frequently implicated were hazelnuts; fruits such as peach, apple, melon, and kiwi; peanuts, maize; chick peas; and vegetables such as lettuce and green beans. Although *Parietaria* (*Urticaceae*) and trees from the *Oleaceae* family are common elicitors of pollen allergies in the Mediterranean area,¹⁶ there are no reports about specific food allergies associated with these diseases. A summary of the observed clinical associations between pollen and plant food allergy is given in TABLE 1. At the molecular level, these observations are based on cross-reactions of human IgE antibodies, which are directed against pollen allergens, with homologous allergens in plant food. Such cross-reactions can even occur between phylogenetically distantly related species such as birch and kiwi.¹⁷

Among pollen-associated food allergies, the birch pollen-related ones are most important and have been studied intensively. A comprehensive list of the foods most frequently causing oral reactions in birch pollen-allergic subjects was published in 1982 by Eriksson *et al.*² These authors presented results of a questionnaire answered by 380 subjects with birch pollen allergy. Fifty-three percent of the subjects reported that they had type I reactions to hazelnut, 47% to apple, 34% to peach, 29% to cherry, 27% to almond, 26%

TABLE 1. Allergy to plant foods in pollinosis patients

Pollen	Food
Birch	Apple, pear, cherry, peach, nectarine, apricot, plum, kiwi, hazelnut, other nuts, almond, celery, carrot, potato
Birch and mugwort or mugwort	Celery, carrot, spices, sunflower seed, honey
Grass	Melon, watermelon, orange, tomato, potato, peanut, swiss chard
Ragweed	Watermelon and other melons, banana, zucchini, cucumber
Plane	Hazelnut, peach, apple, melon, kiwi, peanuts, maize, chickpea, lettuce, green beans

to walnut, 26% to pear, 23% to carrot, 21% to plum, 20% to potato peel, 20% to brazil nut, and 14% to peanut. Apart from contact urticaria to potato peel, relatively mild oral allergy syndrome (OAS) was the most frequently reported symptom. Other authors reported slightly diverging frequencies, and there is evidence for regional differences in the spectrum of foods involved in pollen-related food allergy. For example, Etesamifar and Wüthrich⁵ evaluated case histories of 383 patients who were diagnosed as being allergic to at least one food and found the following prevalences: hazelnut, 36.8%; celery tuber, 36.3%; apple, 25.6%; carrot, 24.8%; peanut, 12.8%; almond, 10.7%; peach, 10.2%; and soybean, 9.1%. These data strongly suggest that pollen-related food allergies in adults are more prevalent than “classical” (i.e. nonpollen-related) food allergies. Also, celery allergy had a prevalence similar to hazelnut, whereas this food allergy is rarely mentioned in publications from Scandinavian authors, most likely due to differences in nutritional habits. In a recent project that was supported by the European Union (EU) and conducted in three different clinical centers in Milan, Copenhagen, and Zürich, all three centers were able to identify more than 20 patients with a positive double-blind, placebo-controlled food challenge (DBPCFC) to hazelnut in less than one year,¹⁸ whereas patients with confirmed celery allergy could be recruited only in Zürich,¹² another strong indicator for regional differences.

It is a common belief that patients with pollen-related food allergies always have mild oropharyngeal symptoms such as itching of the lips, tongue, and throat and, occasionally, swelling of the lips and tongue occurs. Recent DBPCFC studies on hazelnut, apple, and cherry allergy support this view.^{18–20} Other studies, in particular those on celery and carrot allergy in pollen-allergic subjects, reported systemic reactions in approximately 50% of the patients according to case histories, and up to 50% of the patients experienced

TABLE 2. Birch pollen allergens

	Bet v 1	Bet v 2	Bet v 3	Bet v 4	Bet v 6	Bet v 7	Bet v 8
	(17 kDa)	(14 kDa)	(24 kDa)	(9 kDa)	(35 kDa)	(18 kDa)	(65.3 kDa)
Homology	PRP	Profilin	Calmodulin	Ca-binding pol- len allergens	IFR/IRL	Cyclophilin	Pectin esterase
Cross-reaction to food	Yes	Yes	No	No	Yes	Likely	Likely
Prevalence (%)	>90	10–20	10	20	10	21	?

ABBREVIATIONS: PRP: pathogenesis-related proteins; IFR: isoflavone reductase; IFL: isoflavone reductase-like proteins.

TABLE 3. Data of Bet v 1-related allergenic food proteins with known cDNA sequences

Allergen	Source	Molecular mass (kDa)	pI	No. of amino acid residues	Sequence identity with Bet v 1a (%)	Sequence identity with PcPR1-1 (%)	GenBank Acct. No.
Mal d 1	Apple	17.5	5.5	158	58	40	X83672
Pru av 1	Cherry	17.7	5.8	159	59	41	U66076
Pru ar 1	Apricot	17.2	4.6	159	60	46	U93165
Pyr c 1	Pear	17.4	5.6	158	57	38	AF057030
Api g 1.01	Celery root	16.2	4.5	153	40	61	Z48967
Api g 1.02	Celery root	17.1	4.5	158	39	60	Z75662
Dau c 1.0103	Carrot	16.1	4.5	153	38	59	Z81361
Cor a 1.0401	Hazelnut	17.5	6.1	160	67	43	AF136945
SAM22	Soybean	16.6	4.4	156	48	36	X60043

NOTE: Sequence identities are given at the amino acid level.

systemic reactions under challenge even when DBPCFC was performed by a stepwise “spit and swallow” protocol, which was abrogated at the lowest dose of food reproducibly causing symptoms.^{12,21} Similarly, 15 birch pollen-allergic patients with severe reactions to a soybean-containing dietary product have been described recently, and a Bet v 1-related stress protein in soybean, SAM22, has been identified as the major allergen for these patients.²² In conclusion, these studies show that symptoms of pollen-related allergy to certain foods can be more severe than is commonly assumed.

This review will focus on cross-reactive structures shared by birch pollen and foods. According to our current knowledge on cross-reactive allergens, cross-reactivity between other pollen species and foods is caused by ubiquitous antigens. Hence, this approach will cover almost all known families of cross-reactive allergens in pollens and foods.

CROSS-REACTIVE STRUCTURES

Seven birch pollen allergens have been published,^{23–29} and six have been included in the official allergen list of the WHO/IUIS allergen nomenclature subcommittee (<http://www.allergen.org/List.htm>) (TABLE 2). Three of these allergens, Bet v 1, Bet v 2, and Bet v 6 are known to cross-react with food allergens. For two allergens, Bet v 7, a cyclophilin, and Bet v 8, a pectin esterase, IgE cross-reaction to homologous food proteins is likely, but has not yet been published in peer-reviewed papers. IgE antibodies specific for certain *N*-glycans of plant glycoproteins occur in 10–20% of pollen-allergic subjects (reviewed by Fötisch *et al.*³⁰), and these antibodies are highly cross-reactive with almost all foods of plant origin. The properties of the main cross-reactive allergen families in pollen and food are summarized in the following paragraphs.

The Bet v 1 Allergen Family

Allergens belonging to the Bet v 1 allergen family are the main cause of pollen-related food allergies. In our recent studies, between 59% and 96% of patients with food allergy to celery tuber, carrot, pear, cherry, and hazelnut were sensitized to members of this group of allergens.^{13,21,31–33} In addition, a Bet v 1-related protein in soybean, SAM22,³⁴ was identified as the most likely cause of severe reactions to a soy protein-containing dietary product in 11 out of 15 patients.²² Molecular characteristics of Bet v 1-related food allergens are summarized in TABLE 3. These allergens comprise 153–160 amino acid residues and have theoretical isoelectric points (pIs) in the acidic range between 4.4 (SAM22) and 6.1 (Cor a 1.0401). The amino acid sequence identity to Bet v 1, the major birch pollen allergen, varies between

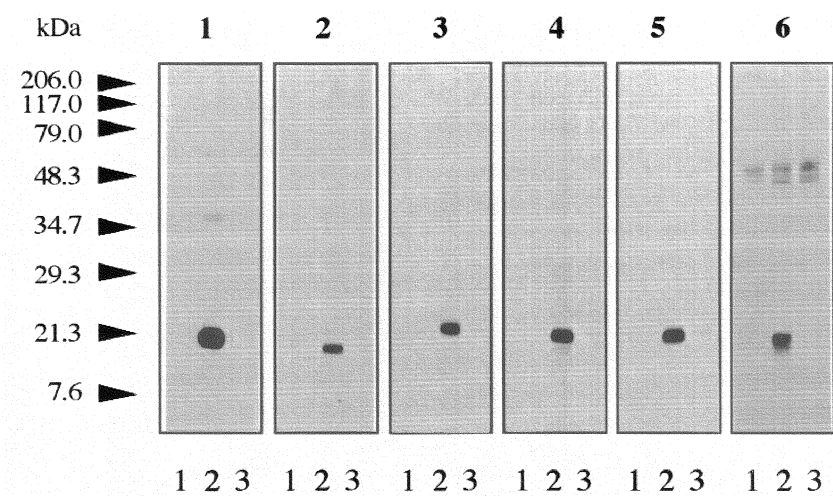


FIGURE 1. Immunoblot inhibition of IgE binding to Bet v 1a (column 1), Api g 1 (column 2), Mal d 1 (column 3), Pru av 1 (column 4), Pyr c 1 (column 5), and Cor a 1.0401 (column 6) on the solid phase. A serum pool from birch pollinotic subjects with cherry and celerly allergy was preincubated with rBet v 1a (15 µg) (lane 3) as an inhibitor. Serum from a nonallergic subject (lane 1) and samples without inhibitor (lane 2) were used as controls.³⁹ From Neudecker *et al.*³⁹ Used with permission.

67% (Cor a 1.0401) and 38% (Dau c 1.0103). In addition, the Bet v 1-related food allergens share between approximately 35% and 60% amino acid sequence identity with the PR10 group of pathogenesis-related proteins (PRP).³⁵ These PR-10 proteins are induced under stress conditions such as the presence of microbial pathogens, whereas the allergens (except SAM22) appear to be constitutively expressed in the fruit. As an example, sequence identities of Bet v 1-related allergens with a PR-10 protein from parsley³⁶ are given in TABLE 3. Interestingly, two different isoforms of Api g 1 have been cloned from celery, sharing only 52% amino acid sequence identity with each other, but both share approximately 40% identity with Bet v 1.^{37,38} We have expressed both forms as recombinant proteins in our laboratory and found Api g 1.01 to be the dominant IgE-binding protein in patients with DBPCFC-confirmed allergy to celery (unpublished data). In general, IgE antibodies directed against Bet v 1 are highly cross-reactive to food proteins belonging to this family, as demonstrated by the immunoblotting inhibition experiment depicted in FIGURE 1.³⁹

The IgE reactivity of Bet v 1 and its homologues in foods is highly conformation dependent. Fragments display virtually no IgE reactivity, and the allergenicity of the cherry allergen, Pru av 1, and the apple allergen, Mal d 1, was abolished as a mutation of the conserved amino acid serine in position

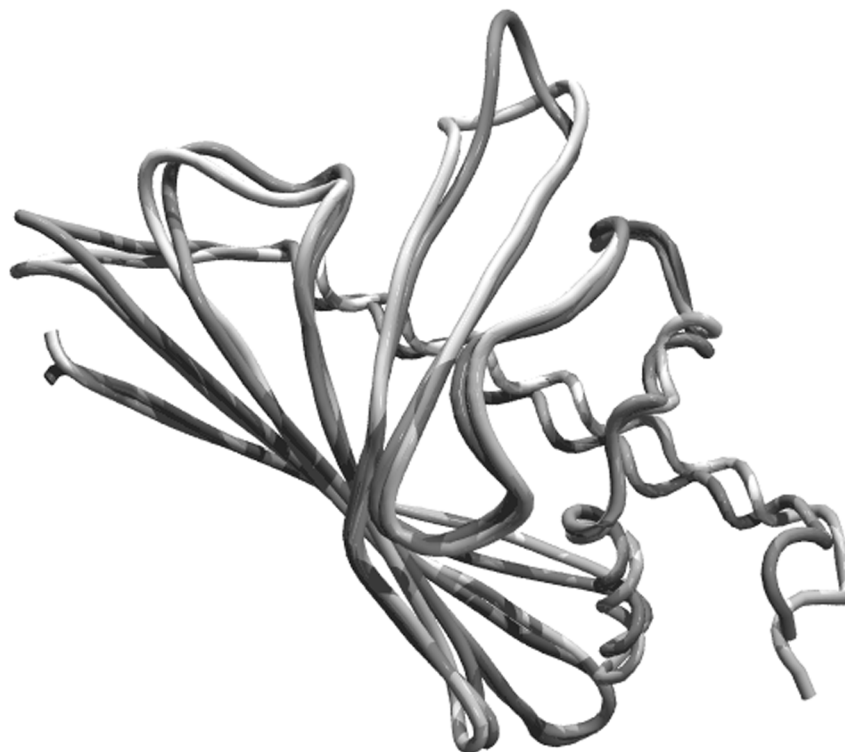


FIGURE 2. Backbone overlay of the lowest energy solution structure of Pru av 1 (gray)³⁹ with the crystal structure of Bet v 1 (white).⁴²

112 into proline led to proteins with an altered conformation.^{40,41} The structure of only one food allergen of this family is known (again, Pru av 1), and it has been analyzed by heteronuclear magnetic resonance spectroscopy.³⁹ As shown in FIGURE 2, the tertiary fold of Pru av 1 is very similar to Bet v 1,⁴² consisting of three helices, a seven-stranded anti-parallel sheet, and a large hydrophobic cavity. Mirza *et al.*⁴³ found the conserved P-loop of Bet v 1 recognized by a fab fragment of a monoclonal antibody directed against a putative IgE epitope of Bet v 1, with the side chain of amino acid glutamic acid in position 45 (E45) located in the cleft between the complementary determining regions (CDRs) of the light chain and the heavy chain of the antibody. Our mutational analysis confirms that E45 is also important for IgE binding to Pru av 1: The mutant Pru av 1 E45W carrying tryptophane in position 45 was correctly folded, and its IgE reactivity was reduced by approximately 40% (unpublished data). Hence, it can be concluded that the P loop is an important IgE epitope of the Bet v 1 family. Our ongoing work is focused on the identification of further IgE epitopes on Bet v 1-related food allergens.

Profilins

Profilins constitute a family of conserved proteins with molecular masses ranging from 12 kDa to 15 kDa, corresponding to polypeptides consisting of 124–153 amino acids. They are present in almost all eukaryotic cells. They probably function as important mediators of membrane-cytoskeleton communication. Profilins specifically bind to several ligands, that is, actin, phosphatidylinositol-4,5-bisphosphate (PIP2), and poly-L-proline. These characteristics enable them to participate in the regulation of actin polymerization and interact with the PIP2 pathway of signal transduction (reviewed by Vieths *et al.*^{44,45}). The tertiary structure of birch pollen profilin Bet v 2 was determined.⁴⁶ Similar to all known profilin structures, it consists of three helices, seven β -strands, and ten turns forming a seven-stranded β -barrel. As demonstrated for soybean profilin, the IgE-binding epitopes of profilin appear to be conformational.⁴⁷

Sensitization to profilins is observed in approximately 20% of the pollen-allergic population. IgE antibodies to pollen profilins are highly cross-reactive to profilins in vegetable foods. The allergenic profilins identified so far in pollen and plant foods are summarized in TABLE 4. In our own studies, we have identified allergenic profilins in apple, cherry, pear, lychee fruit, ba-

TABLE 4. Data of Bet v 2-related allergenic food profilins with known cDNA sequences

Allergen	Source	Molecular mass (kDa)	pI	No. of amino acid residues	Sequence identity with Bet v 2 (%)	GenBank Acct. No.
Pru av 4	Cherry	14.0	4.7	131	76	AF129425
Pyr c 4	Pear	14.0	5.1	131	83	AF129424
Mal d 4	Apple	14.1	5.3	131	82	AF129426
		14.1	4.7	131	74	AF129427
		14.0	4.5	131	78	AF129428
Api g 4	Celery root	14.3	4.5	134	80	AF129423
Gly m 3	Soybean	14	4.6	131	74	AJ223982
Ara h 5	Peanut	14	4.6	131	72	AF059616
Mus a ?	Banana	14.0	4.3	131	78	AF377948
Lit c ?	Lychee	14.0	5.0	131	81	AY049013
Ana c ?	Pineapple	14.2	4.5	131	71	AF377949
Cor a 2	Hazelnut	14.1	4.9	131	77	AF327622
		14.0	4.7	131	77	AF327623
Lyc e 1	Tomato	14.1	4.7	131	78	AY061819
		14.3	4.6	131	74	AJ417553
Cap a 2	Bell pepper	14.2	4.6	131	77	AJ417552

nana, pineapple, hazelnut, celery, carrot, peanut, and tomato. The prevalences of IgE binding ranged from 11% in 61 patients with a positive case history of pear allergy to 44% in subjects with allergy to fresh tomatoes and up to 70% in seven subjects allergic to lychee fruit^{13,21,31,32,48–51} (also, unpublished data). These data implicate that the rate of sensitization to profilins in foods containing Bet v 1 homologues (pear, apple, cherry) is similar to the prevalence of specific IgE antibodies to Bet v 2 in birch pollen allergy, whereas a higher frequency of sensitization occurs in allergies to foods lacking Bet v 1-related allergens (banana, lychee fruit). Because strong IgE reactivities to recombinant profilins from celery, cherry, and pear have been found in Bet v 2-sensitized subjects without clinical food allergy,⁵² it seems that a significant proportion of IgE responses to profilins is clinically insignificant.

Resulting from its ubiquitous distribution, the question must be raised why profilin has only been described as a panallergen from plants and why the literature lacks reports about allergenic profilins in food derived from animals or about autoimmune diseases caused by human profilins. Indeed, a weak immunoreactivity of IgE from a birch pollen-allergic patient with human profilin has been described by Valenta and colleagues.^{24,53} Human profilin was also able to trigger histamine release from sensitized basophils of allergic patients.

For a conclusive interpretation of the complex data regarding profilin allergy, some additional molecular information about profilins has to be taken into consideration. Despite their identical functions, the molecular properties of profilins from distantly related organisms are very different. The pI values of known profilins range between 4.3 and 9.2 (reviewed in Vieths *et al.*⁴⁵). The amino acid sequence identities among profilins from different taxonomical orders are very low. For example, profilins from organisms of six different orders have only four common amino acid residues in identical positions (reviewed in Vieths *et al.*^{44,45}). Mammalian profilins, however, share sequence identities of approximately 90%. Similarly, the identities among known allergenic plant profilins range between 70% and 80%, and these profilins share an identical sequence motif of eight amino acid residues (VERLG DY L) near their COOH-terminal end. Because of their common functions and ligand-binding properties, the tertiary structures have been found to be highly conserved even between bovine and amoeba profilins.⁵⁴

The close relationship among plant profilins on the one hand and their low sequence identity to mammalian profilins on the other hand may be responsible for the observed differences in allergenic potency between profilins from mammals and plants. Moreover, a high uptake of native (nondenatured) plant profilins via the respiratory route (pollen) and by oral ingestion (fresh fruits and vegetables) can be assumed, whereas animal profilins are usually consumed in a heat-denatured state (i.e. cooked). Weak IgE reactivities of pollen-allergic patients with animal and human profilin may be below the “symptom threshold.” It has been suggested, however, that autoimmune reactivity of birch pollen-allergic patients to human profilin may be responsi-

ble for maintaining a high IgE titer outside the pollen season, whereas IgE against Bet v 1 decreases in wintertime.^{24,53}

The role of profilins as allergens can be summarized as follows:

- (i) Sensitization to almost any fruits and vegetables can be caused by profilin, and an unknown proportion of these sensitizations result in food allergy.
- (ii) Allergies to, typically, birch pollen-related foods can not only be caused by members of the Bet v 1 allergen family, but also by profilins. Moreover, unusual cross-reactions exceeding the cluster of typical birch pollen-associated foods can be mediated by IgE antibodies directed against profilins.^{49,51}
- (iii) Mugwort pollen-associated food allergies can be caused by profilins.^{55–58} These allergies are frequently concomitant with cross-reactivity and co-sensitization to birch pollen profilin.
- (iv) Food allergies in patients suffering from grass pollinosis may also be mediated by profilins. This has been confirmed in Spanish patients with peach and apple hypersensitivity⁵⁹ and for other fruits in Dutch patients.⁶⁰ The particular nature of the “grass pollen food cluster” may be influenced by specific nutritional habits of Mediterranean countries.

Bet v 6-Related Allergens

Wellhausen and colleagues⁶¹ isolated a 35-kDa protein from birch pollen that bound IgE of 9/67 (12%) sera from birch pollen-allergic patients. In addition, IgE antibodies to this protein cross-reacted with protein bands of comparable size from apple, pear, orange, banana, mango, lychee fruit, and carrot.^{61,62} The allergen, Bet v 6, was cloned and sequenced^{27,63} and displayed a high degree of amino acid sequence identity of up to 81% with isoflavone reductase-homologous proteins (IFRH) and phenylcoumaran benzylic ether reductase (PCBER), as well as lower identities of 60% and 51% with isoflavone reductases (IFR) and pinorensin/lariciresin reductase (PLR), respectively (summarized in Karamloo *et al.*²⁷) (TABLE 5). These defense-related reductases (IFR, IFRH, PCBER, and PLR) appear to be evolutionarily derived from a common ancestor, and each catalyzes a rather similar conversion in the isoflavonoid and lignan pathways. Bet v 6 consists of 307 amino acid residues and has a calculated molecular mass of 34.1 kDa and a theoretical pI of 6.7. Recently, we have shown that both Bet v 6 and the pear allergen Pyr c 5, the first cloned food allergen belonging to this family, have PCBER enzymatic activity; both proteins were able to catalyze the NADPH-dependent reduction of 8–5'-linked lignans, such as dehydrodiconiferyl alcohol, to give isodihydrodehydrodiconiferyl alcohol.²⁷

TABLE 5. Degree of amino acid sequence identity of Pyr c 5 with other plant proteins

Protein/function	Origin	Accession number	Identity %
IFRH	<i>Glycine max</i>	AF202184	83
Bet v 6	<i>Betula verrucosa</i>	AF282850	80
IFRH	<i>Solanum tuberosum</i>	X92075	78
PCBER	<i>Forsythia intermedia</i>	AF242491	75
IFRH	<i>Medicago sativa</i>	AF201458	73
PCBER	<i>Populus trichocarpa</i>	AJ005804	71
IFRH	<i>Arabidopsis thaliana</i>	Z49777	72
PCBER	<i>Tsuga heterophylla</i>	AF242498	67
PCBER	<i>Pinus taeda</i>	AF242490	66
IFRH	Tobacco	D28505	64
IFRH	<i>Zea mays</i>	U33318	61
IFR	<i>Pisum sativum</i>	S72472	59
IFR	<i>Medicago sativa</i>	X58078	59
IFR	<i>Cicer arietinum</i>	X60755	58
IFR	<i>Glycine max</i>	AJ003245	56
PLR	<i>Tsuga heterophylla</i>	AF242501	51
PLR	<i>Forsythia intermedia</i>	U81158	51
PLR	<i>Thuja plicata</i>	AF242506	50
IFRH	<i>Lupinus albus</i>	U48590	47
IFRH	<i>Citrus paradisi</i>	Y12689	46

SOURCE: From Karamloo *et al.*²⁷ Used with permission.

ABBREVIATIONS: IFR, Isoflavone reductase; IFRH, Isoflavone reductase-homologue; PCBER, phenylcoumaran benzylic ether reductase; PLR, pinoretinol-lariciresinol reductase.

Both Pyr c 5 and Bet v 6 allergens had similar IgE-binding characteristics, as demonstrated by immunoblotting and enzyme allergosorbent test (EAST), and bound IgE from 10 sera of birch pollen-allergic patients including six pear-allergic subjects. EAST inhibition experiments with Pyr c 5 as the solid-phase antigen suggested that homologous allergens may be present in many vegetable foods such as apple, peach, orange, lychee fruit, strawberry, persimmon, zucchini, and carrot (TABLE 6). In extracts of pear, apple, orange, and persimmon, the presence of proteins of approximately 30–35 kDa containing Bet v 6 cross-reactive epitopes was demonstrated with two Bet v 6-specific monoclonal antibodies. Recombinant Pyr c 5 triggered a strong, dose-dependent mediator release from basophils of a pear-allergic subject, suggesting that Pyr c 5 has the potential to induce symptoms of type I allergies. Recombinant Pyr c 5 (rPyr c 5) and rBet v 6 showed identical IgE-binding properties in immunoblotting. Remarkably, rPyr c 5 strongly inhibited

TABLE 6. Maximal inhibition of IgE binding to rPyr c 5 allergen disks by various inhibitors at a protein concentration of 50 µg/ml

Inhibitor	% Inhibition	Inhibitor	% Inhibition
Birch pollen	89	Soya	79
Pear	82	Hazelnut	49
Pyr c 5	82	Potato	40
Bet v 6	80	Mango	36
Nectarine	76	Persimmon	35
Lychee	64	Melon	30
Orange	63	Banana	22
Apple	59	Grass pollen	18
Zucchini	58	Cherry	15
Carrot	55	Kiwi	13
Peach	52	Pea	9
Mugwort pollen	51	Milk	5
Strawberry	50		

SOURCE: From Karamloo *et al.*²⁷ Used with permission.

IgE binding to Bet v 6.²⁷ This is in contrast to Bet v 1–related food allergens that are weak inhibitors of IgE binding to Bet v 1, due to the fact that sensitization to this allergen family is primarily induced by inhalation of pollen.

Thus, in addition to many other defense-related plant proteins,³⁵ a new defense-related allergen family was identified.²⁷ Common features of pathogenesis-related proteins and allergens appear to be relatively high stability and solubility. All PCBER, PLR, IFR, and IFRH contain a glycine-rich motif that facilitates binding to NAD(P)H. Interestingly, this motif is similar to the P-loop motif of the Bet v 1 allergen family,^{42,64} which is not related to these reductases, and we have recently generated a monoclonal antibody against Bet v 6 that displays weak cross-reactivity to Bet v 1 (A. Hoffmann, unpublished data).

Although all pear-allergic patients studied by Karamloo *et al.*²⁷ had a history of multiple food allergies and all except one were sensitized to mugwort pollen, it is not clear at the moment whether the cross-reactions detected by inhibition assays are clinically relevant. Further investigations are required to evaluate the clinical significance of the new allergen family as a source of pollen-related food allergies and to highlight whether the related enzyme families IFR and PLR are similarly involved in the elicitation of allergic reactions.

Cross-Reactive Carbohydrate Determinants

Cross-reactive carbohydrate determinants (CCD), a fourth distinct structure related to allergen cross-reactivity, have been the subject of controversial discussions over many years. Allergen extracts are complex mixtures of proteins of which many are glycosylated. Approximately 20% of pollen-allergic patients show IgE binding to multiple bands with molecular masses higher than 30 kDa in plant extracts. The IgE reactivity to these structures can be significantly reduced by periodate treatment of the antigens but is resistant to proteolytic cleavage of the peptide chain.^{56,65–67} These observations point to the involvement of carbohydrate structures in IgE binding. Immunoblot analysis of electrophoretically separated allergen extracts has shown that these carbohydrate structures can be present on many different glycoproteins from one allergen source.^{31,56,66} Sera with anti-carbohydrate IgE show a very broad spectrum of cross-reactivity, including pollen, many fruits and vegetables, and also allergens from invertebrates such as molluscs and arthropods. Examples of allergens with IgE-binding carbohydrate moieties are phospholipase A 2 (PLA 2) from bee venom⁶⁸ or a β -fructofuranosidase from tomato.^{51,69} The antigenic structure of PLA 2 and of the tomato allergen was identified as an α -1,3-linked fucose of an *N*-linked oligosaccharide. In addition, β -1,2-linked xylose residues are thought to be IgE-binding constituents of *N*-glycans from food plants. Recently, van Ree *et al.*⁷⁰ investigated the *N*-glycans of the major peanut allergen Ara h 1, a vicilin, and Müller *et al.*,⁷¹ those of an IgE-binding vicilin-like protein from hazelnut. Both glycoproteins had major *N*-glycan structures containing xylose, but lacking a fucose residue. However, studies by Fötisch and Vieths³⁰ indicate that the majority of IgE antibodies against plant glycans is directed against structures containing the α -1,3-linked fucose: a carbohydrate ELISA with defined glycan structures covalently linked to the surface of microtiter plates was used to analyze sera of approximately 300 patients allergic to tree and grass pollen for IgE binding to both the bromelain-type glycan (with α -1,3-linked fucose and β -1,2-linked xylose) and the fibrin-type glycan (with only mannose residues). The IgE antibodies of 91 patients (of 319 tested) reacted with the bromelain-type glycan, but the IgE antibodies of only 17 patients (of 298 tested) reacted with the fibrin-type glycan, corresponding to 29.5% in the first and 5.7% in the second case (TABLE 7). Eighty of the patients who possessed IgE to the bromelain-type glycan were also tested with the defucosylated glycan structure, carrying only the β -1,2-linked xylose residue and the α -1,6-linked mannose at the proximal mannose. Only 10 of these patients (12.5%) showed detectable IgE binding to this structure. As shown by the low IgE prevalence to the human IgG glycan in TABLE 7, α -1,6-linked fucose, which is common in mammalian glycans, is a weak inducer of IgE responses. Because of their small size, the described CCD structures represent monovalent epitopes, and thus a single CCD structure is unable to cross-link receptor-bound IgE.

TABLE 7. IgE reactivity of pollen-allergic patients to CCD structures, tested by carbohydrate ELISA³⁰

<i>N</i> -Glycan of:	Structure	Abbreviation	% Positive patients
Bromelain	Man α 1-6(Xyl β 1-2)Man β 1-4GlcNAc β 1-4 (Fuc α 1-3)GlcNAc	MUXF ³	29.5
Brom., defuc. ^a	Man α 1-6(Xyl β 1-2)Man β 1-4GlcNAc β 1-4GlcNAc	MUX	12.5
Human IgG	Man α 1-6(Man α 1-3)Man β 1-4GlcNAc β 1-4 (Fuc α 1-6)GlcNAc	MMF ⁶	5.5
Fibrin	Man α 1-6(Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc	MM	5.7

SOURCE: From Fötisch & Vieths.³⁰ Used with permission.^aBrom., defuc. = bromelain, defucosylated.

Patients with confirmed food allergies have prevalences of CCD-specific IgE antibodies in the same range as grass and tree pollen-allergic patients. Sensitizations to glycans of pear,³¹ tomato,⁵¹ carrot,²¹ and celery¹³ were detected in 16 to 55% of the patients allergic to the foods. However, there is an ongoing debate on the clinical relevance of anti-CCD IgE,⁷² and in several reports it was concluded that anti-CCD IgE antibodies are unable to mediate allergic symptoms. For example, van der Veen *et al.*⁷³ investigated a panel of grass pollen-sensitized patients who were IgE positive but nonallergic to peanut and showed that anti-CCD IgE in these patients had no or only poor biological activity. Similarly Mari *et al.*⁷⁴ tested 136 bromelain-CAP-positive patients with pollinosis by skin test with a bromelain solution, and none of them showed a positive skin test response. Based on the results of such studies, it has been suggested that anti-CCD IgE responses in general are without clinical significance.^{67,72,75} The main reason given for poor biological activity of anti-CCD IgE is that many glycoproteins such as bromelain or Ara h 1 from peanut carry only one carbohydrate chain and therefore are incapable of inducing a histamine release by cross-linking of anti-carbohydrate IgE antibodies. Moreover, low-affinity IgE antibodies could be another reason for poor biological activity. However, proteins with multiple glycosylations such as tomato fructofuranosidase would theoretically be able to stimulate histamine release from sensitized mast cells. Also, a polyclonal IgE response to CCD and additional protein epitopes of a glycoprotein would be able to induce allergic symptoms. In our study on CCDs in celery extract,⁶⁶ a celery-allergic and CCD-positive patient showed no histamine release with the purified glycans of bromelain and fibrin and with BSA, but up to 25% mediator release with BSA conjugates of the bromelain glycan. These glycan-BSA conjugates carried 3–4 saccharide units per BSA molecule, and thus were multivalent in regard to their CCD epitopes. Similarly, we were able to demonstrate histamine release with BSA-CCD conjugates in a subgroup of CCD-

positive patients with confirmed allergy to carrots (unpublished data). These results underline the necessity of polyvalent epitopes for the biologic activity of the glycoproteins. Besides, we found a few celery-allergic patients whose only detectable IgE reactivity directed against celery was specific for CCD without any additional recognition of protein allergens of celery,¹³ and a similar result was obtained in a patient with confirmed food allergy to kaki fruit.⁷⁶ In summary, we believe that these data indicate that sensitizations to CCD may be clinically relevant in a subgroup of patients allergic to plant foods. However, mediator release experiments with purified glycoproteins carrying multiple *N*-glycosylations or plant-expressed recombinant glycoproteins are required to prove this hypothesis.

STABILITY UNDER CONDITIONS OF HEATING AND DIGESTION

Patients with birch pollen-related allergy to fruits such as apple, pear, and cherry usually tolerate these fruits after heat treatment. This corresponds to our *in vitro* data, which show that the apple allergen Mal d 1 and the cherry allergen Pru av 1 become rapidly inactivated during heat processing of the fruit⁷⁷ (also, unpublished data). A more complex situation exists with other pollen-related foods such as celeriac and hazelnut. Celery tubers are known to contain both a thermostable and a heat-labile allergenic component.¹⁰ We have demonstrated the complete inactivation of the major allergen Api g 1 in celery tuber by even short heating procedures.^{77,78} Similarly, the IgE-binding capacity of the major hazelnut allergen Cor a 1.0401³³ is strongly affected by roasting.^{71,79} By contrast, a heat-resistant IgE reactivity has been demonstrated for CCDs on glycoproteins in both celery and hazelnuts.^{71,78} Profilin in celery roots displayed a higher heat stability than Api g 1, but was more labile than CCD.⁷⁸ Notably, it is important to perform heating experiments with the food itself rather than with extracts, as the allergenicity of Api g 1 is not affected by a heat treatment of 30 min at 100°C when heating is applied to semipurified protein fractions.⁷⁷ Even the extremely labile Mal d 1 showed a residual IgE binding potential after a 30-min heating of apple extract.⁷⁷

The link between heat stability determined *in vitro* and clinical food allergy to heat-processed, pollen-related foods is not entirely clear, however. Recent DBPCFC studies confirmed that both cooked celery and roasted hazelnut can cause allergic symptoms in pollen-allergic subjects⁸⁰ (also, unpublished data). Although five out of six patients with a positive DBPCFC to cooked celery were sensitized to profilin and/or CCD, four of the patients were also IgE-positive to Api g 1, including one subject monosensitized to this allergen. Similarly, five patients with positive DBPCFC to roasted hazelnuts were all sensitized to rCor a 1.0401, including two patients with an ad-

ditional sensitization to the hazelnut profilin Cor a 2 (unpublished data). Obviously, these data are not in full accordance with *in vitro* data, indicating very low heat stability of Bet v 1-related food allergens. We think that it is possible that a cumulated dose of low amounts of Bet v 1 homologues not deactivated in cooked celery and roasted hazelnut may be responsible for the allergic reactions in patients highly sensitized to this allergen family. More importantly, the comparison of our clinical and *in vitro* data shows that the risk of clinical reactivity of a patient to heated, pollen-related food cannot be deduced directly from the sensitization pattern to individual pollen-related food allergens.

The fact that the majority of subjects with pollen-related food allergy presents local allergic reactions at the mucosa of the oropharyngeal tract suggests that the major allergens of the Bet v 1 family are labile to digestion. Two studies with somewhat contradictory results have been published, one on celery⁸¹ and one on hazelnut allergens.⁸² In the study by Jankiewicz *et al.*, a considerable stability of the celery allergens Api g 1 and Api g 4 to a two-step *in vitro* digestion procedure was found, although the total protein pattern of celery extract was strongly affected by the activity of the digestive enzymes. The second study was focused on a comparison of peanut and hazelnut allergens using the same experimental design.⁸² Four sera from hazelnut-allergic patients sensitized to the major allergen Cor a 1.0401 showed almost no IgE reactivity to hazelnut extract after 2 hr of gastric digestion of hazelnut extract. By contrast, IgE binding of serum from a patient sensitized to a low-molecular-weight, pollen-independent hazelnut allergen remained unaffected by gastric and combined gastric and pancreatic digestion. Four sera from peanut-allergic patients had almost identical IgE reactivity to untreated and digested peanut extract, underlining the high resistance of classical food allergens to digestion. Taken together, the *in vitro* digestion data may indicate that Api g 1 in celery is indeed more stable to digestion than Cor a 1.0401 in hazelnut, a view that corresponds to the high rate of systemic allergic reactions to this vegetable.

CONCLUSIONS

Homologues of the birch pollen allergens Bet v 1, Bet v 2, and Bet v 6, as well as plant glycoproteins carrying cross-reactive carbohydrate determinants, have been identified as important cross-reactive allergen families in plant foods. Bet v 1-related allergens cause adverse reactions to typical birch pollen-related foods such as apple, pear, cherry, hazelnut, carrot, and celery. They are particularly abundant in *Prunoideae*, *Maloideae*, and *Apiaceae*, and hence mediate a restricted cluster of cross-reactivities. Especially food allergies caused by Bet v 1 homologues in fruits have been found to be relatively mild, whereas more severe reactions have occurred to vegetables

such as celery¹² and carrot¹³ or soybeans,²² which contain allergens with a lower degree of sequence identity to Bet v 1 compared to the homologous fruit allergens.

Both profilins and CCDs are ubiquitous in plants. Thus, these structures can cause almost unlimited cross-reactivity patterns to all kind of plant foods, and sensitization to profilins and CCDs from various kinds of pollen such as grass, mugwort, or ragweed pollen can consequently lead to pollen–food cross-reactions. Until now, allergenic components responsible for specific associations of allergies to these pollens and foods remain unknown.

According to the low prevalence (approximately 10%) of specific IgE antibodies, Bet v 6-related proteins have minor importance as cross-reactive, pollen-related allergens in food. Further investigations, focusing on their distribution in the plant kingdom and on their clinical relevance as allergens, are required.

Clinically insignificant cross-reactivity is a major problem in *in vitro* and *in vivo* diagnosis of pollen-related food allergy. Low-affinity binding to cross-reactive food proteins or binding to monovalent epitopes may explain this observation, which still affects accurate diagnosis. It has to be noted, however, that not only IgE binding to CCDs,^{73,74} but also IgE reactivities to profilins^{13,20,21,52} and Bet v 1-related proteins^{13,20,21} are common in patients without clinical symptoms of food allergy.

Pathogenesis-related proteins have several features that predispose them to be allergenic, such as small size, high solubility, and stability at low pH and in digestion.³⁵ Both the Bet v 1 and Bet v 6 families are defense-related proteins. Also, nonspecific lipid transfer proteins, which belong to the PR-14 group of pathogenesis-related proteins, have been identified as imported food allergens without cross-reactivity to birch pollen in the Mediterranean area.^{83,84} This fact has to be taken into consideration in approaches to increase crop plant resistance by means of plant biotechnology.

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