

Pollen-Vegetable Food Crossreactivity: Serological and Clinical Relevance of Crossreactive IgE

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Allergy to vegetable foods is frequently observed in patients with pollinosis. The common explanation for this phenomenon is crossreactive IgE, as can be demonstrated with radioallergosorbent test (RAST) inhibition. IgE against birch pollen allergens is often crossreactive with fruits from the family of the *Rosaceae*. Another example is IgE against celery and potato, which is usually found in sera from patients with grass or weed pollinosis. The individual allergens responsible for these types of crossreactivities were identified in the last few years. The most important allergen for the birch pollen-fruit crossreactivity is the major birch pollen allergen *Bet v I*. The counterpart of this allergen in fruits probably plays an important role in the clinical manifestations in patients with birch pollinosis, after the ingestion of fruits like apple. Another crossreactive allergen that was identified recently is profilin. Profilin is not only involved in birch pollen-fruit crossreactivity, but has a more widespread spectrum of crossreactivity covering almost all vegetable foods. As in the case of *Bet v I*, clinical symptoms related to profilin seem restricted to the consumption of raw vegetable foods. Besides *Bet v I* and profilin, carbohydrate structures on glycoproteins are also involved in pollen-vegetable food crossreactivity. Their clinical relevance is, however, doubtful.

Keywords: IgE; Crossreactivity; *Bet v I*; Profilin; Carbohydrates.

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Introduction

It has been known for years that birch pollinosis predisposes for symptoms referred to as the oral allergy syndrome (OAS), caused by various fruits of the family of the *Rosaceae*, for example, the apple (1-5). This concordance of allergic symptoms caused by allergens from unrelated origin is not a unique phenomenon. Mugwort pollen allergy is frequently accompanied by complaints related to the consumption of certain vegetable foods such as carrot and celery (6,7). Another well-known example of allergens that

are linked is ragweed and some fruits like melon and banana (8,9). Among allergens of animal origin, unexpected concordances are also found. Food allergy caused by snails seems to prevail among patients allergic to house dust mite (10). Allergy for certain insects might also be linked to house dust mites (11).

The common explanation for these observations is crossreactive IgE. For example, IgE binding to allergens from apple can usually be inhibited by birch pollen extract (12). This indicates that both allergen sources contain structures that share similar IgE binding sites. Is IgE against such crossreactive epitopes of clinical relevance? Identification of the individual allergens that are responsible for the observed crossreactivities is a major help in answering this question. Recent progress in allergen research has resulted in the identification of several crossreactive allergens, especially allergens of plant origin. In this paper we will focus on two of these allergens, *Bet v I* and profilin, and on carbohydrate structures on glycoproteins that are involved in IgE crossreactivity.

Bet v I

The first reports on the major allergen from birch pollen (now known under the name *Bet v I*) go back to the early 1970s (13,14). Since then the purification of the major allergens of birch pollen was reported in several papers (15-17). The production of monoclonal antibodies reactive with *Bet v I* further facilitated its purification (18). The end of the 1980s saw the cloning of *Bet v I* which resulted in the elucidation of the complete primary structure of the allergen (19). *Bet v I* was shown to be responsible for IgE crossreactivity between birch pollen and other tree pollen, like alder, hazel, and hornbeam (20,21). Pollen of these trees contains allergens that are highly homologous with *Bet v I* (22-25).

Apple allergy is frequently found among birch-pollen-sensitive patients (1-5). The existence of anti-

birch pollen IgE, crossreactive with apple, was first demonstrated by means of radioallergosorbent test (RAST) inhibition (12). The involvement of *Bet v I* in this crossreactivity was demonstrated by Calkhoven et al (26). IgE antibodies eluted from solid-phase bound apple extract bound to a band corresponding to *Bet v I* on a birch pollen immunoblot. The same was shown for peach and cherry. Ebner et al performed northern blotting with a *Bet v I* cDNA clone as a probe (27). Cross-hybridization of birch and apple allergen coding nucleic acids was demonstrated, confirming the existence of a *Bet v I*-like component in apple. It was shown that this apple allergen had a MW comparable to *Bet v I* (around 17 kD). In the same study, 83 birch-pollen-sensitive patients were analyzed by immunoblotting for reactivity with birch pollen and apple extracts. Of these patients, 63 demonstrated clinical symptoms after ingestion of apple. These symptoms were more severe during the pollen season, indicating that pollinosis and food allergy are indeed linked in some way. It was shown that 81/83 patients were *Bet v I*-positive. Only nine of these sera recognized other allergens in birch pollen than *Bet v I*. All 81 *Bet v I*-positive sera also recognized the apple equivalent of *Bet v I*. These results strongly suggest an involvement of crossreactive IgE against *Bet v I*-like structures in both birch pollinosis and food allergic symptoms like OAS, caused by fruits. Although other crossreactive structures in fruits were reported (28), *Bet v I* seems to be of major importance for the IgE reactivity with fruits from the family of the *Rosaceae*. However, this does not imply that every pollinosis patient with cross-reactive IgE against the group I allergen from tree pollen and its counterpart in fruit has symptoms following consumption of those foods. Of the 81 patients with IgE against *Bet v I* and its counterpart in apple, only 63 had apple-related symptoms. A recent study by Hirschwehr et al analyzed two groups of patients with tree pollinosis (29). One group had symptoms after ingestion of hazelnuts, the other did not. Both groups recognized the group I allergen from hazel pollen (*Corylus avellana*), *Cor a I*, and the crossreactive structure in hazelnuts. One possible explanation of the observed clinical difference between both groups could be the titers of crossreactive IgE. The authors did not analyze the anti-group I IgE quantitatively, since they performed immunoblotting. They did however report that symptoms after ingestion of hazelnuts in the food-allergic group were more severe during pollen season, when IgE titers tend to increase. The same was observed in the group of patients with apple-related symptoms (27). It is possible that the titers of crossreactive IgE in the group without food symptoms were below a threshold level. This should be analyzed by determining the IgE titers in a quantitative manner, for example, in a RAST for *Bet v I*.

Profilin

Calkhoven et al demonstrated that there was a second crossreactive allergen in birch pollen besides *Bet v I*, with an even larger spectrum of crossreactivity (26). This structure was shown to be crossreactive not only with fruits but also with other allergens like grass pollen and potato. It had a slightly lower molecular weight than *Bet v I*. In a study by Vallier et al, a crossreactive allergen of the same size was reported in mugwort, birch pollen, and celery (30). Van Toorenbergen et al demonstrated IgE binding by immunoblotting to a band of comparable molecular weight in various spices (31). Recently, Valenta et al cloned this allergen from birch pollen (32,33), shortly followed by its cloning from grass pollen (34). The 14 kD allergen was identified as profilin. Profilin from pollen can be isolated from pollen extract in a single-step affinity purification with Sepharose-coupled poly(L-proline) (33,35). Profilin is a highly conserved protein present in almost all eukaryotic cells. This property potentially gave the allergen a very broad spectrum of crossreactivity. We demonstrated recently that profilin is indeed an allergen in a large variety of vegetable foods, as well as in pollen (35). What is the role of profilin in pollen-vegetable food crossreactivity? From a group of sera that was submitted to our diagnostics department for testing with the most commonly inhaled allergens, 66 sera were selected on ground of a positive RAST for grass pollen. Sera from patients, for whom testing on food allergens was requested in addition to inhalant allergens, were excluded. In this way a selection was made for patients that were mainly suspected of allergy related to inhalation allergens. In a RAST for *Lol p* profilin >30% ($n = 24$) was positive. In a panel of sera that was selected on ground of IgE against potato and celery, in addition to grass pollen ($n = 73$), >65% had IgE against profilin ($n = 48$). This finding confirms similar results reported for a smaller panel of sera (35) and suggests involvement of anti-profilin IgE in the IgE reactivity with potato and celery. To investigate this hypothesis, a series of depletion experiments was performed. Sera were depleted for their anti-profilin reactivity by a 4-hr incubation with an excess of Sepharose-coupled *Lol p* profilin. Supernatants were tested in a RAST for vegetable foods. As control, sera were depleted with the same amount of glycine-inactivated Sepharose. In addition, a RAST was performed for *Lol p I* to check whether the depletion was specific for profilin. The effect of depletion on the RAST for celery and potato is illustrated for two sera in Fig. 1. The IgE reactivity of serum 1 with potato and celery was inhibited for 93% and 94%, respectively. Serum 2 was much less affected: 49% for potato and 44% for celery. A similar experiment was performed for 19 sera with IgE against *Lol p* profilin, and against potato and celery. Fig. 2 summarizes the results. All

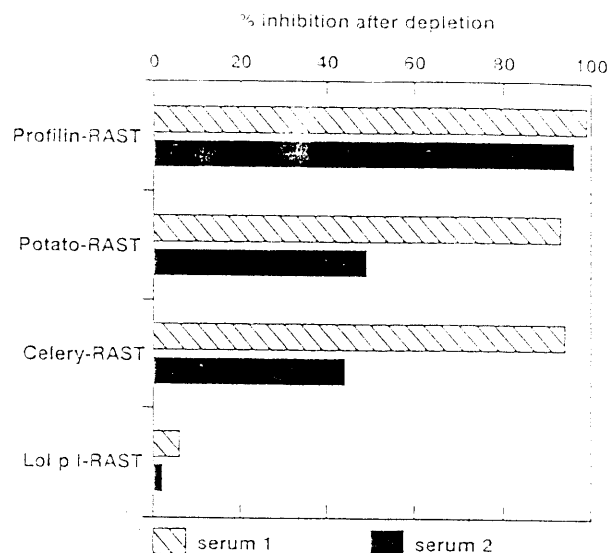


Fig. 1 Effect of anti-profilin depletion on potato and celery RAST. Depletion of sera was performed with Sepharose-coupled *Lol p* profilin. For both sera <5% of anti-profilin IgE was left in the supernatant. IgE against *Lol p* I was practically unaffected: >95% was retained. The anti-profilin depletion resulted in a complete inhibition of the potato and celery RAST for serum 1. Serum 2 was less affected.

sera were affected by the depletion with respect to their anti-potato and anti-celery reactivity (>20% inhibition). Eleven of the sera demonstrated an 80 to 100% inhibition of both potato and celery RAST. These results indicate that crossreactive anti-profilin IgE is of major importance for the IgE reactivity of pollen-positive patients with vegetables like potato and celery. Although allergy to fruits from the family of the *Rosaceae* is predominantly connected to the

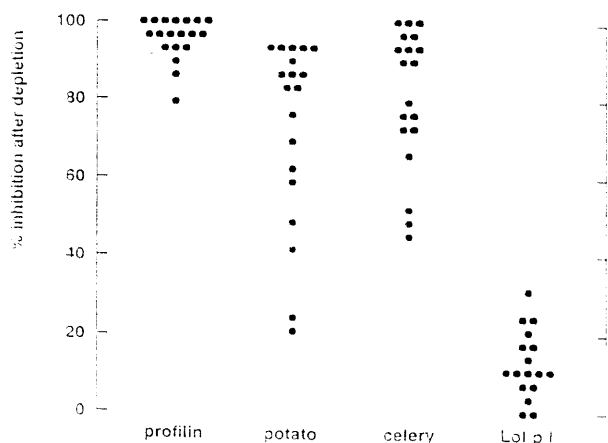


Fig. 2 Importance of profilin for pollen-potato-celery crossreactivity. Depletion of anti-profilin IgE was performed for 19 sera. For more than half of the sera, this resulted in a 80–100% loss of anti-potato and anti-celery IgE. This proves the major role of profilin in pollen-potato/celery crossreactivity.

major birch pollen allergen *Bet v* I, sera exist that are pollen-fruit crossreactive mainly due to anti-profilin IgE. In Fig. 3 the effect of anti-*Lol p* profilin depletion on the reactivity with birch pollen and apple is shown for such a serum. The birch pollen RAST is inhibited for 71%, the apple RAST for 81%. This is comparable to the inhibition observed for celery (75%) and potato (84%).

What is the clinical relevance of crossreactive IgE against profilin? The above mentioned panel of 66 grass-pollen-positive sera was tested in a RAST for potato and celery. Of these sera, 49 had a positive RAST for potato, and 30 for celery. The depletion experiments with Sepharose-coupled *Lol p* profilin had shown that anti-profilin IgE of grass-pollen-positive sera is of major importance for their IgE reactivity with potato and celery. It is unlikely that so many subjects with IgE against grass pollen suffer from vegetable food allergy. Possibly profilin is a heat-labile allergen. This would imply that only raw vegetables are causing symptoms. Probably, there is also a threshold level for anti-profilin IgE to become of clinical relevance. There are indications that profilin plays a role in food-related symptoms. The patient whose serum was used for the depletion experiment shown in Fig. 3 suffers from OAS-like symptoms after ingestion of raw vegetables like celery and carrot. Peeling uncooked potatoes or grating carrots causes symptoms such as conjunctivitis and rhinitis. Recently this patient suffered from an asthmatic attack following the consumption of an apple. As demonstrated in Fig. 3, the positive RAST to vegetable foods is mainly due to

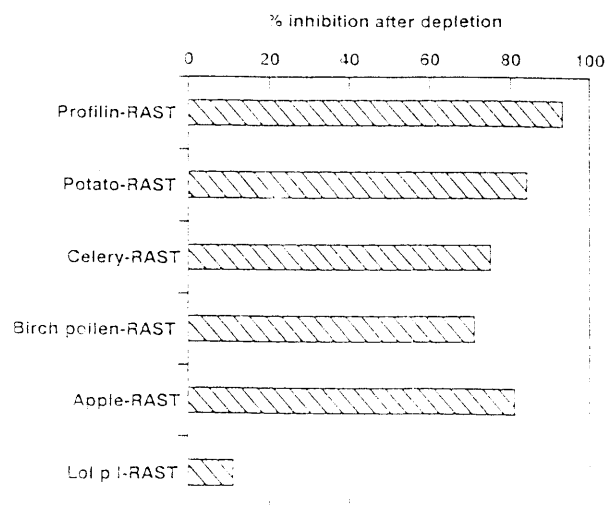


Fig. 3 Anti-profilin IgE responsible for IgE-reactivity with birch pollen/apple. Depletion of this serum for its anti-profilin IgE resulted in a 70–80% inhibition of the RAST for birch pollen and apple. This shows that birch pollen–apple crossreactivity is not always caused by *Bet v* I.

crossreactive anti-profilin IgE. It is therefore plausible that the complaints are related to profilin. The fact that only raw vegetables induced symptoms seems to confirm that profilin is a heat-labile allergen. There are other examples of patients whose food-related complaints are most likely caused by anti-profilin IgE. One patient (pf40) whose IgE responses to vegetable foods were shown to be highly dependent on anti-profilin IgE had food-related dermatitis. Depletion of anti-profilin IgE resulted in an inhibition of anti-potato and anti-celery reactivity of >80%. IgE of patient pf40 was shown to recognize profilin from a large variety of vegetable foods, including fruits, nuts, legumes, cereals, herbs, and other vegetables (35). Although the exact role of profilin as a food allergen still needs to be determined, these preliminary results indicate that it is a clinically relevant allergen for selected patients. Both patients described above (serum of Fig. 3 and serum pf40) had high levels of crossreactive IgE against profilin (RAST class 4+/5+), indicating that the titers of anti-profilin IgE could be decisive in determining its clinical relevance.

Crossreactive carbohydrate determinants (CCD)

Allergen extracts are complex mixtures of proteins, of which some are glycosylated. Especially in vegetable extracts some of these glycoproteins are known to be lectins (36). These lectins can bind IgE through the carbohydrate side chains on the Fc-part of IgE. This is the main reason that the existence of IgE specific for carbohydrate structures on glycoproteins was doubted and thought to be lectin binding (3). Other experiments, however, demonstrated that IgE with anti-carbohydrate specificity does exist (37-39). The IgE binding capacity of various allergen extracts was shown to be (partly) periodate-sensitive, pointing to involvement of carbohydrate determinants in the IgE binding. A strong argument against lectin binding was the fact that several sera with elevated IgE titers were shown to be unreactive. Sera with CCD-reactive IgE are usually crossreactive beyond the borders of plant-derived allergens. The carbohydrate structures responsible for eliciting IgE responses are found not only in plants but also in allergenic extracts from invertebrates like molluscs and arthropods. An example of an allergen with an IgE-binding carbohydrate structure is PLA₂ (40). The antigenic structure was found to be an N-linked oligosaccharide, in which an α 1,3-linked fucose seems to be involved in its immunogenicity (41-43). In plant-derived N-linked oligosaccharides a β 1,2-linked xylose is thought to have a role in antibody binding (44,45), in addition to the α 1,3-linked fucose. Both the fucose and the xylose linked in this way are unknown in mammalian carbohydrate structures and are therefore highly immunogenic. Anti-CCD IgE plays a role in crossreactivity between veg-

etable food allergens, allergens from invertebrate animals (foods and venoms), and pollen. This can be demonstrated by performing RAST inhibition. Fig. 4 illustrates that the CCD-type crossreactivity is not restricted to vegetable allergens. RAST inhibition was performed for serum VW with vegetable food extracts and mussel extract. Mussel extract inhibited the RAST for vegetable foods. The mussel RAST was inhibited by various vegetable extracts. The IgE reactivity of this patient with food extracts was completely inhibited after periodate treatment of the extracts. This is shown for a few food extracts in Fig. 5. This points in the direction of carbohydrate epitopes. Not all sera with IgE against carbohydrate structures on vegetable allergens are crossreactive with invertebrate allergens. Even among vegetable allergens, crossreactivity patterns can be different. Fig. 5 shows the influence of periodate treatment of allergen extracts on vegetable RASTs for three sera. Serum VW is unreactive with all five periodate-treated vegetable extracts. For serum 3325 only the wheat RAST is inhibited significantly. Serum 4429 again has a different pattern. The RAST for potato, peanut, wheat, and green pea is clearly affected by periodate treatment, although not as strong as observed for serum VW. The RAST for hazelnut, however, is totally unaffected. These differences could imply that IgE of some patients distinguishes carbohydrate groups with small structural differences.

Although periodate treatment is generally accepted as a method to destroy carbohydrate structures, it cannot be ruled out completely that the pep-

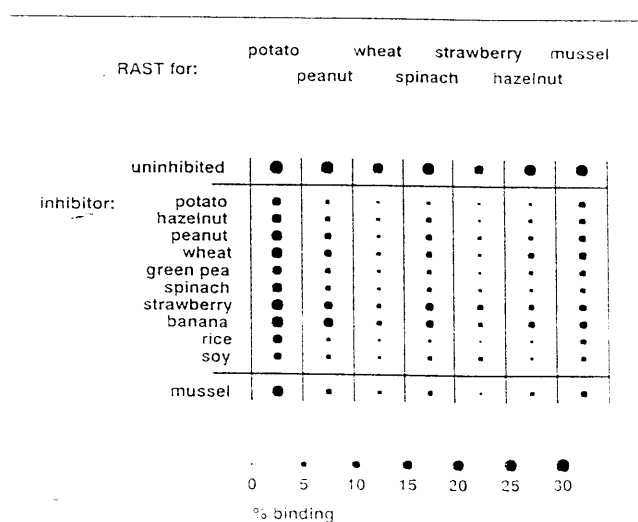


Fig. 4 CCD-type crossreactivity is not restricted to vegetable foods. RAST inhibition was performed with serum VW. This serum was shown to have a high titer of anti-CCD IgE. Mussel extract inhibited the RAST of a wide variety of vegetable foods. The mussel RAST was inhibited by all vegetable extracts used as inhibitor. This illustrates the broad spectrum of CCD crossreactivity.

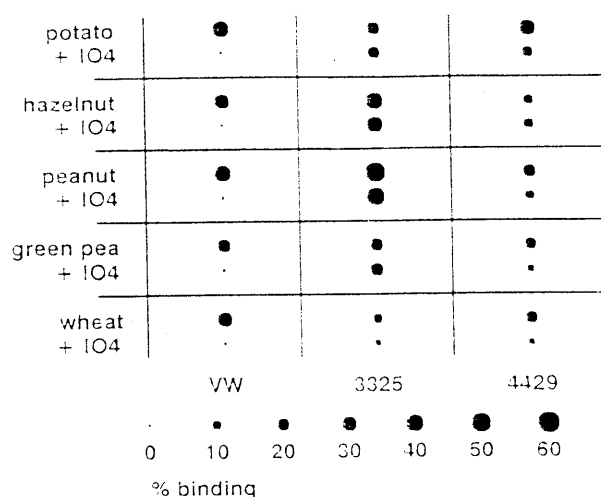


Fig. 5 Anti-CCD IgE is unreactive with periodate-treated allergen extracts. Periodate treatment of allergen extracts destroys the carbohydrate determinants on glycoproteins. This results in a complete inhibition of the IgE reactivity of serum VW. Sera 3325 and 4429, which both have anti-CCD reactivity, are less affected. This indicates that carbohydrate determinants on glycoproteins from different vegetable foods demonstrate enough variety to be distinguished by anti-CCD IgE.

tide backbone is changed as well. Some amino acids, such as cysteine, methionine, and tryptophan, are sensitive to oxidation, indicating that they could be periodate-sensitive. In addition, destruction of carbohydrate determinants can have an indirect effect on the conformation of the protein, since it is known that glycosylation can be necessary for proper folding of glycoproteins. It is therefore not sufficient to show that glycoproteins lose their IgE-binding capacity after chemical modification of their sugar groups. Demonstration of IgE binding to carbohydrate structures separated from the peptide backbone is a more direct proof of IgE with anti-CCD reactivity. This can be achieved by digesting the peptide backbone of glycoproteins enzymatically, for example with pronase or proteinase K, resulting in sugar groups linked to small peptide fragments, or even a single asparagine. For example, pronase digestion was applied in order to demonstrate that the sugar groups on PLA₂ from bee venom are recognized by IgE antibodies (40). When coupled to Sepharose, the carbohydrate groups can be used for screening sera on anti-CCD activity.

Is anti-CCD IgE of clinical relevance? From a group of seven patients with high anti-CCD titers, reported in the study by Aalberse et al. no one had food-allergic symptoms after consumption of vegetable foods, indicating that the role of carbohydrate epitopes in food allergy is negligible (38). There are, however, recent reports claiming that sugar groups can be clinically relevant (46,47). This was demonstrated for a

glycoprotein from sea squirt. A single sugar group with a MW of 1 kD isolated from the glycoprotein was shown to elicit a skin reaction in sea-squirt-allergic patients. This means that such a small structure would contain at least two epitopes. The relevance of this observation is of course highly dependent on the purity of the isolated material. Whether other carbohydrate structures, in vegetable extracts for example, can elicit skin reactions or induce in vitro basophil histamine release still has to be determined. Although single sugar groups were claimed to be capable of eliciting skin reactions (46,47), multiple copies linked to a nonallergenic protein from human origin would be a more potent tool. This could be achieved by expressing cDNA, encoding a human protein with multiple glycosylation sites, in a plant expression system. The aim would be to obtain a human protein, glycosylated like a plant glycoprotein. By application of such a recombinant allergen in skin tests or histamine-release assays, the clinical role of carbohydrate groups could be determined.

Concluding remarks

As pointed out above, at least three crossreactive structures are involved in pollen-vegetable food cross-reactivity: (1) *Bet v I* and the related proteins in other allergen sources, (2) profilin, and (3) CCD. In addition, the carbohydrate determinants are involved in cross-reactivity between allergen sources of both plant and invertebrate animal origin. The analysis of sera with a broad spectrum of IgE reactivity is therefore very complex. A serum can be crossreactive to pollen and vegetable foods because it contains crossreactive IgE directed to one of these structures or to any combination of the three. The availability of the crossreactive allergens in purified form creates the opportunity to investigate their importance for the (cross)reactivity of individual sera with pollen and vegetable foods. Profilin and *Bet v I* can both easily be obtained in purified form by affinity purification with Sepharose-coupled poly(L-proline) and Sepharose-coupled anti-*Bet v I* monoclonals, respectively. For the carbohydrate determinants, the most important issue is to separate anti-CCD activity from anti-peptide activity. Combining anti-CCD titers with anti-*Bet v I* and anti-profilin titers will provide insight into the crossreactivity patterns of individual sera. In some cases, sera turned out to be uniquely reactive with only one of the crossreactive structures. Such sera can give an indication on its clinical relevance, as was already discussed for two patients with mainly anti-profilin IgE. For *Bet v I* and profilin, it seems clear that they are involved in clinical manifestations. For CCD this is still debatable. It is important to clarify this issue. If clinically irrelevant, this would mean that anti-CCD IgE would preferably go undetected in allergy diagnostics.

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