

Allergenic Crossreactivities

Pollens and Vegetable Foods

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Introduction

Since the first reported connection between pollinosis and allergy to plant-derived food was made by Tuft et al. in 1942 (1), the clinical observation that patients with type I allergy caused by tree pollen frequently show adverse reactions to fruit and vegetables has been the topic of several studies. The symptoms encountered mostly concern the upper aerodigestive tract and are comprised of itching, inflammation, and angioedema of the afflicted mucosa. This symptomatology has been designated oral allergy syndrome (OAS) (2). Nevertheless, a considerable percentage of pollen allergic patients show systemic reactions (i.e., urticaria, asthma, and anaphylactic shock) upon ingestion of particular vegetables.

These interesting findings of pollen-associated adverse reaction to food led to the assumption of structural similarities between the inhalative and the food allergen. Several clusters of pollen-food allergies have been described so far—among which are the association of allergy to birch pollen and apples/nuts (3), as well as the so-called mugwort-celery syndrome (4)—have been investigated most thoroughly. Although an array of data was published during the years of 1970–1990 concerning the immunological relationship of allergens derived from pollens of trees, grasses, and weeds, little information could be attained regarding the molecules responsible. In the last few years, however, the molecular characterization of allergens and the

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technique of cDNA cloning helped to elucidate the basis for this crossreactivity.

Allergy to Birch Pollen and Associated Foodstuffs

Allergy to birch pollen is very common in Northern and Central Europe as well as in the Northern and Eastern parts of the United States. Taxonomically, the birch (*Betula verrucosa*) belongs to the order *Fagales*, together with hazel (*Corylus avellana*), alder (*Alnus incana*), and hornbeam (*Carpinus betulus*). The pollens of these trees represent an important source of allergens. The major allergens display a high grade of sequence-identity on the nucleic acid as well as on the protein level. This leads to crossreactivities resulting in the occurrence of allergic reactions throughout the whole flowering season of this botanic order (5,6).

Tree Pollen Group I Allergens

The identification and characterization of Bet v 1, the major birch pollen allergen (7-9), played a pivotal role in the quest for a better understanding of tree pollen allergy, as well as the associated allergy to certain fruits and vegetables. The discovery that Bet v 1 and all other tree pollen group I allergens belong to a ubiquitous family of pathogenesis-related proteins that are present at least in all angiosperms (9). This provided an explanation for the coincidence of allergies directed toward plants that show only a distant relationship taxonomically. Bet v 1 is a molecule of 17 kDa and consists of 159 amino acids.

During the last few years, Bet v 1 homologs in apple (*Malus domestica*) and celery (*Apium graveolens*) were characterized and cloned (10,11). Celery harbors a protein of 153 amino acids, and the M_r of 16.2 kDa named Api g 1 displays a 41.6% identity with Bet v 1 in amino acid sequence comparison. The Bet v 1 homolog in apple called Mal d 1 consists of 159 amino acids (M_r of 17.7 kDa) and shows 55.6% identity with Bet v 1. Both proteins are available in recombinant form and crossreactivity has been proven by different molecular, biological, and immunological methods.

Profilin

Profilins are highly conserved proteins present in all eukaryotic cells (12). Members of this family are proteins of the cytoskeleton with a high affinity to actin and are involved in the signal transduction by the interaction with phosphoinositol 4,5 bisphosphate(PIP_2). Profilins could be isolated from tree, grass and weed pollen, as well as from tobacco, several plant-derived food, from human blood platelets, and even from trophozoites of *Entamoeba histolytica*. Sequence homology

between birch and grass pollen profilin can be found in the range of 80%, whereas the comparison of those plant derived with other eukaryotic profilins shows an identity of 30%. Profilins are molecules with a molecular mass in the range of 13–14 kDa. Profilin constitutes a minor allergen in birch pollen (approx 5% of all allergic patients react with profilin. However, these patients are characteristically polysensitized on the basis of crossreactivity).

Tree Pollen-Associated Food Allergy

The crossreactivity between apples and birch pollen is clearly based on the occurrence of genes, mRNA, and gene products that show extensive similarity with Bet v 1, as proved by Southern, Northern, and Western blotting techniques (13).

Recently, we carried out a study that investigated patients with typical birch pollen allergy and hypersensitivity reactions to various fruits and vegetables (14). All of the patients displayed adverse reactions to at least two of the following foods: apple, pear, carrot, celery, and potato. Ninety-five percent of the patients included in this study showed reactions to a protein in the molecular range of 17 kDa that were identified as Bet v 1, the major birch allergen; IgE-binding proteins of a similar M_r could be detected in all the other extracts produced from the above-mentioned plant sources. The majority of the patients reacted with proteins of 15 and 18 kDa in the fruit and vegetable extracts. However, the binding pattern showed individual differences. Investigations with monoclonal antibodies raised against Bet v 1 (Fig. 1) confirmed the view that some epitopes of the Bet v 1-homologs are shared, whereas others showed differences in the epitope composition. Inhibition experiments performed with recombinant Bet v 1 and Bet v 2 revealed that preincubation of the sera with those purified allergens resulted in a blocking of IgE binding to the 15- to 18-kDa molecules of apple, pear, celery, and carrot.

Twenty percent of the sera of this selected patient group displayed reactivity with the birch pollen profilin—Bet v 2—and all these sera detected the respective homologs in all the food extracts investigated. This was proven by crossinhibition studies and by reactivity with a profilin-specific antibody. In all patients with a positive case history, sera reacted with IgE binding proteins indicated in the Western blots of birch, apple, pear, celery, and carrot. However, some of the patients' positive serological results could not be matched with a clinical symptomatology.

It is noteworthy that about half of the patients with birch pollen allergy also showed reactivity to allergens of a high molecular mass (around 60 kDa) in the fruit and vegetable extracts. These allergens have

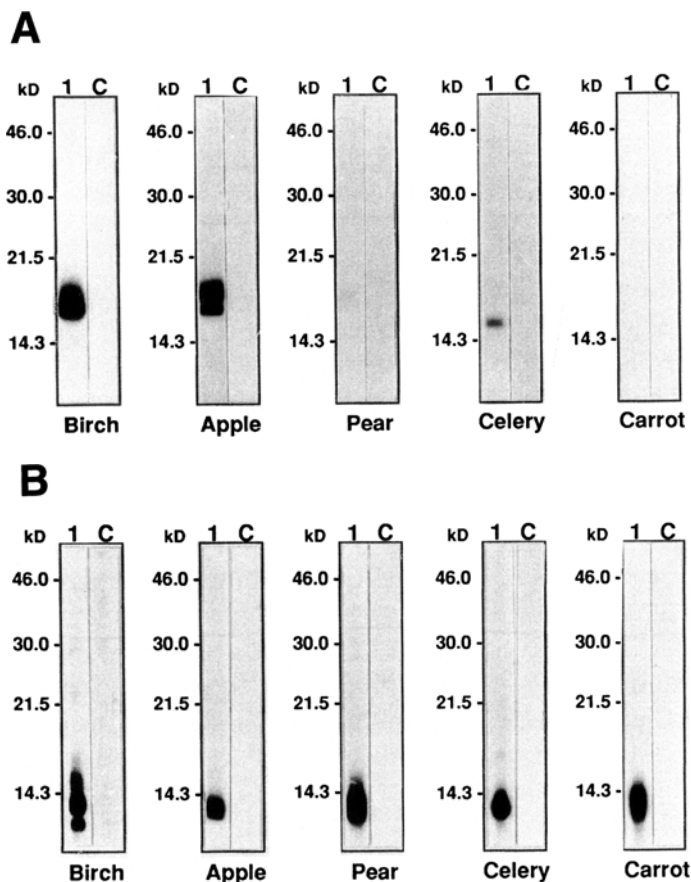


Fig. 1. This depicts the crossreacting proteins in birch, apple, pear, celery, and carrot by immunoblot technique. (A) Lane 1, monoclonal antibody against Bet v 1; lane C, negative control. (B) Lane 1, rabbit antibody against celery profilin; lane C; negative control.

not yet been completely characterized, but seem to play an important role in food allergy, as postulated by Bauer et al. (23), who found a high rate of systemic reactions to celery in patients who recognized this molecular structure exclusively.

The observation that some vegetables can be tolerated by allergic patients when cooked indicates a heat-sensitivity of some allergens involved. In our study, however, 10 min of cooking in sodium-dodecyl-sulfate-buffer did not abolish the IgE binding capacities of our extracts; the explanation for this apparent contradiction could be a quantitative difference of allergens in raw and cooked food, which suggests that cooking may lead to a reduced amount of allergenic material in the vegetables and fruit on the account of a possible enrichment of allergens in the cooking water.

Also, in a different investigation (15), aimed at the identification of the crossreacting allergens in hazel pollen and hazelnuts, the responsible agents were found to be homologous proteins of Bet v 1 and Bet v 2, named Cor a 1 and Cor a 2, as proven by Western blot inhibition techniques, using recombinant allergens.

Calkloven's (16) findings of the crossreacting structures in the *Prunoideae* family (i.e., peach, cherry, apricot, and plum) were confirmed by a study by Pastorello et al. (17). The main allergens seemed to be proteins of 13, 14, and 20 kDa, as characterized by immunoblot and inhibition studies. The 14-kDa protein (obviously being a member of the profilin family) and the 20-kDa protein (presumably representing a Bet v1 homolog) account for the crossreactivity between peach and birch or grass pollen.

Allergy to kiwi fruit constitutes a further birch-pollen associated food allergy (18). This was proven by skin testing and RAST technique in an investigation comprising of 22 kiwi-allergic patients. All of these patients displayed a positive skin reaction and elevated IgE levels when tested with birch pollen. Interestingly, the control group of birch pollen allergic patients without any clinical symptoms of allergy to kiwi fruit also showed positive skin reactions to kiwi. This indicates that birch pollen allergy precedes kiwi allergy.

The Mugwort-Celery Spice Syndrome

Celery tuber constitutes an important food allergen in Central Europe and is known to elicit IgE reactions not only when eaten raw, but also in its cooked form. The association of adverse reactions to celery, a member of the *Apiaceae* family, and allergy to mugwort (*Artemisia vulgaris*) led to the definition of the mugwort-celery syndrome (4,19,20). This term has been extended to the celery-carrot-mugwort-spice syndrome, as cosensitizations to carrot (also a member of the *Apiaceae* family) (21) and spices have been described (22). A recent investigation elaborated the birch-mugwort-celery syndrome by Western blot techniques (23). Immunoblots were performed using birch pollen, celery, and mugwort extracts and revealed three crossreactive IgE binding proteins. One represents the birch pollen major allergen Bet v 1, crossreacting with the homologous celery protein Api g 1. Mugwort, on the contrary, does not harbor any Bet v 1 homologous allergen. Another crossreacting structure contained in birch, celery, and mugwort belongs to the profilin family of panallergens. The third crossreacting allergen could be detected at a molecular range of 60 kDa in all three extracts. All of the three described allergen groups are able to elicit systemic reactions after the ingestion of celery. Again, the phenomenon of serological positivity with no clinical correlate was noticed. Consequently, from a serological point of view, it was suggested to

extend the mugwort-celery syndrome to the birch-mugwort-celery syndrome.

Allergens of a comparable molecular mass that appeared to contain crossreacting carbohydrate determinants have been reported (24). However, differences in the IgE binding potency and pattern in deglycosylated celery extracts could not be observed in our study.

The relationship of allergy to mugwort, birch, and spices was specified in a recent investigation by Jarolim et al. (25). By means of Western blots and inhibition experiments with recombinant Bet v 1 and profilin, the main crossreactive agents in certain spices belonging to the *Apiaceae* family were discovered. The major allergens of anise (*Pimpinella anisum*), fennel (*Foeniculum*), coriander (*Coriandrum sativum*), and cumin (*Carum carvi*) once again represent the homologs of Bet v 1, Bet v 2, and molecules of a higher molecular mass.

Pollen-Related Allergy to Fruit without Connection to Birch Pollen

In areas where birch trees are rare, cosensitization of grass pollen and peach and apples could be observed. In this case, most cross-reactions are accredited to a sensitization to the panallergen profilin, and to a carbohydrate crossreacting determinant of 40 kDa, the biological activity of which has not yet been determined (26).

In 1970, Anderson (27) reported the association of food-induced oropharyngeal symptoms and ragweed pollinosis. In a study published in 1987 by Enberg (28), the authors concentrated on the cosensitization to members of the gourd family (melon, zucchini, and cucumber), banana, and a ragweed pollinosis. The authors found strong cross-reactivities between the closely related members of the botanical family of the *Cucurbitaceae* (gourd) and the botanically distant ragweed. Though specific characterization of the potential crossallergenic structures has not been carried out to the present day, one can assume a further cluster of pollen-associated fruit allergy.

A different survey focused on the association of melon sensitivity with allergy to *Plantago* and grass pollens (29). In a pool of pollen-allergic patients hypersensitivity to certain vegetables and fruit was tested, and predomination of reactions to melon became obvious. In all these melon-allergic patients, cosensitization to *Plantago* and grass was prevalent, and CAP inhibition experiments also indicated the crossreactivity between the member of the gourd family and pollens of grass and *Plantago*.

Conclusion

Cross-sensitization to inhalant pollen allergens and ingested food allergens have been the focus of an increasing number of studies. Dur-

ing the last few years, the molecular characterization of the major allergens of most common pollens and foods has made it possible to identify some potential candidates of crossreactive IgE binding proteins. In this respect, the family of Bet v 1 homologs, the profilin family, and an until recently not completely characterized allergen cluster of approx 60 kDa have been described. These three allergenic structures constitute useful tools in diagnostic procedures, as the majority of vegetable allergy could be detected by this combination of allergens. Further information about crossreacting agents is needed in order to be able to advise pollen allergic patients fully about their risks of a possibly dangerous reaction to food.

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