

Diagnostic problems due to cross-reactions in food allergy

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The immunochemical cross-reactivity of allergens is a common problem of diagnostic procedures, especially in food allergy (1). Cross-reactivity can elicit positive *in vitro* or *in vivo* allergy tests in subjects who do not display any clinical symptom, thus giving rise to the so-called asymptomatic sensitization. Cross-reactivity is an *in vitro* phenomenon caused by IgE antibodies directed against epitopes expressed in molecular structures from different allergenic sources, and does not always produce clinical symptoms. Cross-reactions in food allergy mainly affect specificity. Nonetheless, the positive tests in subjects who do not have symptoms cannot be simply classified as "false-positive results", since they do not occur in nonatopic control subjects (2); therefore, their significance has yet to be fully understood. Cross-reactive allergens can be divided into three groups on the basis of the appearance/absence of resulting clinical symptoms. Group I comprises allergens which provoke clear clinical symptoms (Table 1), most of which are taxonomically related, and clinical syndromes in which respiratory and food allergies are combined, such as the "birch-fruit" or "bird-egg" syndromes (3). Group II comprises cross-reactive allergens that do not always produce symptoms. Examples of these are profilins in the pollen-vegetable foods allergy association; cysteine proteases contained in kiwi fruit, papaya, pineapple, and mites; and the cross-reacting allergens in the "latex-fruit" syndrome. Group III comprises those cross-reactive allergens that do not produce symptoms, and thus

ent sources of cross-reacting structures that share a common carbohydrate determinant (CCD).

Group 1 – symptom-provoking cross-reactions

Fish

The dominant allergen in fish allergy was first identified, purified, and characterized in cod and named allergen M (4) by Aas in 1967; it was subsequently classified as Gad c 1 (5) by the WHO-IUIS. It is a sequential allergen of 12 kDa identified as a parvalbumin, whose epitope is formed by a repetitive group of a few amino acids (6), a fact which explains its capacity to resist cooking and digestion. The high sensitivity and specificity of RAST with allergen M achieved in patients allergic to cod was the first demonstration of the utility of purified allergens in the diagnosis of food allergy (7).

However, it is well known that a number of subjects, despite positive tests to several types of fish, may eat considerable quantities and still not experience any symptoms. In skin tests with 10 different fish species in fish-allergic patients, positive reactions to all species were observed in 73% of cases, but a limited number of subjects had a positive oral challenge to more than one species; namely, 18% to three species and 10% to two species (8). Immunoblotting showed that the cross-reacting allergen was in the MW range of Gad c 1,

suggests that allergens other than Gad c 1, as demonstrated by the lack of resistance to lyophilization, may be involved (9).

Crustaceans

Crustaceans are frequently involved in allergic reactions and cause both systemic and local symptoms, the latter in the form of oral allergy syndrome (OAS) (10). The most investigated crustacean is shrimp, the major allergen of which, Pen a 1, was identified as a 36-kDa protein, recognized by sera from more than 90% of shrimp-allergic patients (11). RAST-inhibition experiments in subjects with no exposure to crustaceans other than shrimp (12) proved that positivity of IgE tests for other crustaceans, such as lobster, crab, and crawfish, in shrimp-allergic patients can be caused by cross-reactions. Cross-reactivity also includes other seafood, such as oyster, scallop, and squid, and even insects and mites. The explanation of this broad cross-reactivity was provided by the identification of the responsible molecule, corresponding to Pen a 1, as tropomyosin, a protein ubiquitous in arthropods due to its role in muscle contraction (13). It must be remembered that tropomyosin in mites is only a minor allergen; thus, most mite-sensitive patients can eat crustaceans without problems. However, when tropomyosin is the sensitizing agent, concomitant respiratory and food allergies may occur (14).

The birch-fruit and mugwort-vegetables syndromes

The association between pollinosis and OAS is well known (15). Birch pollen and fruits of the Rosaceae family are the most significant sources, while grass and mugwort pollen and other fruits and vegetables may also be involved in these clinical associations (16–18). The immunochemical basis of the cross-reactions depends on the sharing of common epitopes in molecules present in pollen and foods. The cross-reaction between birch and apple was demonstrated to rely upon a 17-kDa apple allergen highly homologous to the major birch allergen, Bet v 1 (19). The major apple allergen, Mal d 1, was then purified and characterized, and it showed an amino-acid sequence identical to Bet v 1 by 63% (20). These allergens were identified as members of a family, the pathogenesis-related proteins (PRP), which are ubiquitous in the vegetable kingdom owing to their protective effect against bacteria, viruses, and fungi (21). The major allergen of celery, Api g 1, 60% homologous with Bet v 1, is also a PRP (22), a fact

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celery as a culprit food in subjects with birch pollinosis (16, 17). Due to the importance of this allergen, the availability of recombinant Bet v 1 (rBet v 1) suggested its introduction in diagnostic tests (23), and positivity to rBet 1 in *in vitro* (24) and skin tests (25) appeared in some studies to be capable of identifying subjects with birch-apple syndrome. However, a recent study by Wüthrich & Straumann, which carefully considered the patients' history data, failed to confirm such a diagnostic role, and concluded that "the routine determination of IgE to rBet v 1 seems to be of little diagnostic value" (26). Another major allergen to be considered in cross-reactions is Art v 1 from mugwort, which is a pectate lyase homologous to a number of molecules occurring in other pollens, such as Amb e 1 from ragweed and Cry j 1 from Japanese cedar, and in foods such as celery and tomato (27).

In most cases, the birch-fruit or mugwort-vegetables syndromes can be diagnosed by history and the results of skin tests – using the prick + prick technique for foods (28) – with eventual challenges with fresh foods if the data are not clear-cut.

The bird-egg syndrome

This syndrome derives its denomination from the first report of the clinical association of allergic reactions to egg ingestion and respiratory symptoms caused by inhalation of bird antigens (29). The bird-egg syndrome mainly affects adults, unlike egg allergy, which is prevalent in children, and this is explained by the fact that the major egg allergens (of egg white) are not involved, the causative allergen being α -livetin, a 70-kDa protein which cross-reacts with bird serum albumin (30).

Group 2 – cross-reactions that do not always provoke clinical symptoms

The latex-fruits syndrome

Latex is an allergen source of sharply increasing importance, and recently its hypersensitivity was reported to be associated with allergic reactions to fruits or nuts such as banana, pear, avocado, chestnut, kiwi, and papaya (31) as well as to vegetables such as potato and tomato (32). The cross-reactions do not seem to be caused by the major latex allergen Hev b 1 – identified as prohevein (33) – but rather by allergens of about 30 and 36 kDa in fruits, namely, banana (34), and of 46 kDa in vegetables (32). The lack of identification and recognition of the biologic function of these allergens still prevents explanation of this cross-

Table 1. Cross-reacting food allergens with demonstrated clinical importance

Food	Allergen	Characterization	Cross-reacting sources
Codfish (<i>Gadus callarias</i>)	Gad c 1	Parvalbumin	Other fishes
Shrimp (<i>Penaeus aztecus</i> , <i>P. indicus</i> , <i>Metapenaeus</i> <i>ensis</i>)	Pen a 1 Pen i 1 Met e 1	Tropomyosin	Crustaceans, mollusks, mites, insects
Apple (<i>Malus domestica</i>)	Mal d 1	Pathogenesis-related protein	Birch pollen, other vegetables or fruits of Rosaceae and Umbelliferae families
Celery (<i>Apium graveolens</i>)	Api g 1	Pathogenesis-related protein	As above
Hazelnut (<i>Corylus avellana</i>)	Cor a 1	Pathogenesis-related protein	As above
Egg yolk	Not yet named	α -Livetin (bird serum albumin)	Bird feathers, avian derivatives

Cross-reactions from sensitization to profilins

As reported above, the association between pollinosis and vegetable food allergy is commonly observed. In all studies dealing with this issue, profilins were reported to be cross-reacting allergens (35). Profilins are molecules of about 15 kDa, the ubiquitous presence of which is due to their role in plant fertilization (36). The observation that animals immunized with recombinant profilins have positive skin tests to birch and grass pollens and to celery (37) demonstrates that profilins are strong sensitizers, but the weak correlation obtained between positive skin tests with rBet v 2 (profilin) and allergic reactions to birch pollen and to vegetables, namely, carrot and celery (25), suggests that they do not so strongly cause clinical symptoms.

Cross-reactions among cereals

Cereals can cause respiratory symptoms through flour inhalation, in the form of baker's asthma, and skin symptoms from ingestion. A number of allergens of 12–69 kDa were found by sera from asthmatic patients (38). In comparison of the IgE-binding pattern in patients with asthma or skin symptoms, the causative allergens were found to belong for the former to albumins and for the latter to globulins (39). A major wheat allergen was identified as a 15-kDa α -amylase inhibitor, belonging to a family of inhibitors acting on mammalian and insect α -amylases (40), but possible cross-reactions with different sources have yet to be investigated.

Group 3 – Cross-reactions as an *in vitro* phenomenon

Sensitization to legumes

Multiple sensitization to legumes was early described as very common (41) and was later confirmed by immunoblot studies (42). A study conducted by double-blind, placebo-controlled

the cross-reactivity has no clinical importance, since only 5% of patients reacted to more than one legume, this requiring a careful consideration of positive results of IgE tests (43). The allergens involved in legume cross-reactivity are still unidentified.

Sensitization to CCD

Cross-reactive carbohydrate determinants (CCD) are formed by the sugar side-chains of vegetable (and invertebrate) glycoproteins and thus are hugely diffuse in nature (44). IgE to CCD are extremely common, but the role of these molecules in inducing symptoms seems to be negligible, as they have a monovalent structure which is probably unable to cross-link IgE antibodies and cause mast-cell or basophil degranulation (45).

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