Lipid transfer proteins and 2S albumins as allergens

Plant lipid transfer proteins, a widespread family of proteins, have been recently identified as important food allergens. Their common structural features, such as eight conserved cysteines forming disulfide bridges, basic isoelectric point and high similarity in amino acid sequence, are the basis of allergic clinical cross-reactivity. This has been demonstrated for the LTP allergens of the Prunoideae subfamily, whose similarity is about 95% as demonstrated for the purified allergens of peach, apricot, plum and apple. A relevant aspect is the existence of sequence homology of LTPs of botanically unrelated foods, as demonstrated for LTPs of maize and peach. A class of food allergens of well recognized clinical importance is that of seed storage 2S albumins. They have been identified in the most diffused edible seeds and nuts, such as mustard, sesame, Brazil nut, walnut and peanut. In particular, a strong correlation between IgE-binding to these proteins and food-induced anaphylaxis has been demonstrated for Brazil nut and sesame seeds.

Lipid transfer proteins

Lipid transfer proteins (LTP) form a broad family of proteins very widespread in Nature. The most studied are of plant origin, characterized by the typical features of this class of proteins: eight conserved cysteines forming disulfide bridges, basic isoelectric point and common crystallographic structure (1). Their function was initially associated with their in vitro properties of facilitating inter-membrane nonspecific transfer of lipids, but further studies have highlighted their important in vivo action in the defense of plants from different kinds of pathogens and environmental stress (2). Thus, lipid transfer proteins were recently included in the “Pathogenesis Related Proteins” classification with the name of PR-14 (3).

The first LTPs identified as allergens were proteins of the pollinic tissue of Parietaria judaica (4), a plant with a very important clinical role in the Mediterranean area, where it is the main cause of pollinosis. These allergens, named Par j 1 and Par j 2, as recently reviewed by Colombo (5), have been cloned and totally sequenced, showing a significant homology of the N-terminal region. However, the study of the immunological properties of the two recombinant allergens did not show a relevant cross-reactivity, thus giving an idea of the complexity of the relationship between allergenicity and amino acid structure.

Until 1998, no allergenic activity had been shown for other members of this family, but in 1999 we published a study demonstrating that the major allergen of peach is a 9 kDa LTP (6). Our study, conducted in the context of a EU FAIR project, has been the first demonstration of the role of LTPs in food allergy. These studies were mainly focused on the fruits of the Prunoideae subfamily, whose LTPs were shown to be highly homologous to each other. In fact, we demonstrated in 1994 (7) that the major allergen of peach is a low molecular weight protein to which all patients allergic to this fruit react. By oral food challenge we found a high rate of clinical cross-reactivity between allergic reactions to peach and the other Prunoideae fruits such as plum, cherry and apricot. The immunoblotting of these fruits showed the presence of a similar major allergen, which was recognized by all patients allergic to these fruits. Thus we started by analyzing the peach major allergen, which, after purification and characterization, turned out to be a 9 kDa LTP (6). The purified protein was
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tested in vivo and demonstrated to be highly allergenic since all clinically sensitive patients showed positive skin-prick tests up to very high dilutions.

The N-terminal sequence of the purified plum and apricot 9 kDa allergens demonstrated that they are LTPs, too, with a 94% similarity to peach LTP sequence (8, 9). In the same chromatographic fraction of apricot allergen, a molecule of 7 kDa was also detected and identified as a second LTP. But although very similar, this LTP does not show allergenicity, thus suggesting the importance of studying molecular epitopes to identify which ones are really allergenic (8).

Investigations were then extended to apple, the main fruit of the Rosaceae family to which Prunoideae belong.

Immunoblotting of apple (10) showed that a low molecular weight allergen was the only one recognized by patients allergic only to apple and not to birch, while it was recognized by only 28% of patients allergic to both birch pollen and apple. We purified this protein and its N-terminal sequence and confirmed that it corresponds to a lipid transfer protein.

The reported studies clearly show that LTPs are important allergens in the Rosaceae family. As said above, all the patients allergic to these fruits but not to pollens reacted only to this low molecular weight band and not to other proteins. This suggests that this protein is able to sensitize by a general route in a different way from other allergens such as Bet v 1, which only sensitzes through an inhalant route.

We found a very high cross-reactivity among the Prunoideae LTPs. This was due to the high degree of homology of their N-terminal sequences. This, however, was expected because they belong to the same genus. What was unexpected was to find, upon research in the Swiss Prot Data Bank, that peach LTP is highly homologous to LTPs from botanically unrelated plants, such as sunflower seed, French bean and maize (11). It was immediately clear from analysis of several histories from patients allergic only to peach but not to pollen that patients not allergic to other cereal grains or grass pollen had severe reactions to maize.

We thus performed a study (12) to evaluate the IgE pattern reactivity to maize. We were able to show a relevant IgE binding to the 9 kDa protein. Purified by HPLC, the N-terminal sequence showed it to be an LTP, corresponding to the one already described in 1988 (13). We then studied the cross-reactivity of maize with other foods and found that, among cereals, there was no cross-reactivity involving the 9 kDa allergens, whereas 15 kDa proteins, corresponding to the family of alpha-amylase/trypsins inhibitors, which also have allergenic activity, highly cross-reacted. Thus an LTP is the major allergen of maize and seems to condition reactions to maize in patients allergic to peach but with no reactions to other cereals (12).

As LTPs show a significant clinical role in allergy to fruits, it could be useful for allergic patients to have hypoallergenic foods available with a reduced content of such allergenic molecules. To meet such a requirement, a recent food-science study (14) was published giving useful guidelines about industrial processing of peaches to obtain probably non allergenic or undoubtedly hypoallergenic limpid juices and nectars, which could then be used as intermediates to obtain various products after the addition of further ingredients.

In conclusion, plant LTPs seem to represent a new class of ubiquitous allergens specific for foods such as fresh fruit and cereals (15). Sensitization to LTPs seems clinically important as they are stable and resistant with several, well-conserved domains which may condition a high degree of cross-reactivity among botanically unrelated foods.

2S albumin

Besides pathogenesis-related proteins there is another class of proteins which shows allergenic activity, that of seed storage proteins (16). Albumins and globulins belong to this class and are identified on the basis of sedimentation coefficient as 2S albumins and 7S and 11S globulins (17).

The 2S albumin family includes several important food allergens which have been identified in the last few years; first of all, allergens such as Sin a 1 (16) and Bra j 1 (18), which have similar amino acid compositions and share common epitopes. A strong correlation also emerged between IgE-binding to these proteins and clinical symptoms of food allergy, as later assessed as a typical feature of the 2S albumin class.

Studies were then extended to other edible seeds. In castor bean the allergenic 2S albumin storage proteins proved to be two different heterodimeric polypeptides, as expected for their class (19).

We recently demonstrated the real correlation of the sensitization to the major allergen with the clinical expression of allergy in Brazil nut (20). The purified allergenic molecule, identified as the large chain of the 2S albumin of the nut (21), had already been extensively studied for its notable nutritional value in animals due to a high cysteine and methionine content. It had also been used for genetic engineering of soybean to improve its nutritional value. However, this approach was completely unsuccessful because of the IgE-binding properties of the 2S protein of Brazil nut expressed in the transgenic soybean (22). Recently, sunflower albumin is thought to be a suitable alternative for such an application, but allergenic properties have also been found for this protein, even though they have not yet been evaluated in a population of selected patients (23).

The IgE-specific reactivity to the 2S albumin of walnut has been recently found in the group of nuts, and the major allergen named Jug r1 (24). Its precursor
has been cloned and showed a 46% sequence identity with the precursor of the Brazil nut 2S albumin and even with the other allergenic albums, thus suggesting this class of proteins is inherently allergenic.

The peanut allergens Ara h 6 and Ara h 7 also belong to the class of 2S albums. These allergens were identified by Crameri’s group using selective cloning by phage display technology (25). They proved to be homologous to the conglutin family of seed storage proteins and also showed significant similarity to the 2S albumins of Cucurbita maxima and Ricinus communis.

By studying patients with anaphylaxis caused by sesame seeds, we have recently found that the clinically most important major allergen of sesame is a protein belonging to the 2S albumin family, which had never been described before either as a component or as an allergen (26).

In conclusion, the 2S albumin family is an important class of common allergenic proteins in seeds. Their presence in almost all edible seeds must be taken into account because of the high incidence of possible clinical reactions occurring in sensitized people and because of the real possibility of cross-reactivity among different proteins of the same class. These could be due to differences in molecular structure and still remain to be evaluated in detail.

References
26. PASTORELLO EA, VARIN E, FAROLI L, et al. The major allergen of sesame seeds (Sesamum indicum) is a 2S albumin. J Chromatography, in press.