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Can cross-reactivity studies enable generic allergy prevention?

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Abstract

The occurrence of homologous proteins in foods, pollen and latex is the molecular basis of the plant sources' allergenic cross-reactivity. However, there is not a generic cross-reactivity. Each allergic patient is sensitized to particular allergens and even to particular IgE-binding epitopes. Molecular studies on the main panallergens may explain the major plant-food, pollen and latex syndromes. Some examples of the best-characterized plant panallergens are presented. Novel technologies on recombinant proteins and microarrayed allergens will provide allergen-sensitization profiles, associated to food allergies and cross-reactivity, that will enable the diagnosis and prediction of cross-reactions as well as individual treatment of these pathologies.
Keywords: cross-reactivity; panallergen; allergy prevention; hevein-like domains; lipid-transfer proteins; vicilins

Introduction

Allergenic cross-reactivity is not generic. As it is shown in Figure 1, patterns of cross-reactions between pollen and foods (panel A), latex and fruits (panel B), as well as plant-related foods (panels C and D), are complex and variable. Prevalence variations can be the result of geographic, genetic and dietary differences among allergic populations.

In view of this scenario, avoidance of potential cross-reactive sources seems to be a very restrictive way of allergy prevention. The knowledge of allergens and IgE-binding epitopes involved in allergic reactions will allow a more accurate diagnosis and treatment of each individual patient. The novel recombinant and microchip allergen technologies can help to reach this aim.

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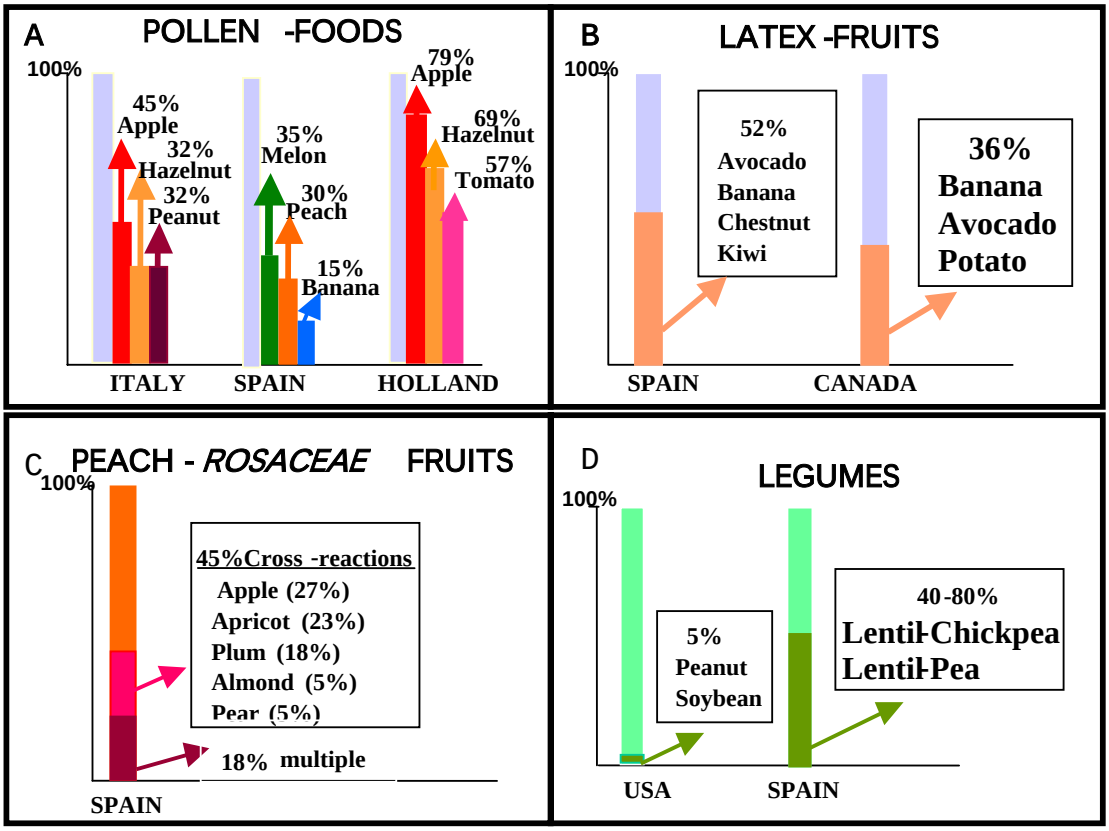

Figure 1. Prevalence of plant foods, pollen and latex cross-reactions (Based on: Bernhisel-Broadbent and Sampson 1989; Ortolani et al. 1989; Blanco et al. 1994; Beezhold et al. 1996; Pascual et al. 1999; Rodriguez et al. 2000; Crespo et al. 2002; Wensing et al. 2002; Ibáñez et al. 2003)

The molecular basis of cross-reactivity

The molecular basis of allergenic cross-reactivity is the occurrence of homologous proteins (or protein domains) in both the primary sensitizer and the cross-reactive allergenic sources. Homologous proteins share different degrees of sequence identity, similar 3D-structures and common epitopes recognized by IgE antibodies (Aalberse, Akkerdaas and Van Ree 2001). However, a patient who has developed allergy to a particular source does not necessarily become allergic to all other allergenic sources with potential cross-reactive allergens. Allergenic sources frequently have several proteins acting as allergens. Each particular patient is sensitized to a different set of them and the relevance of these allergens on cross-reactions with clinical consequences is in many cases unknown. Moreover, specific IgE for a cross-reactive protein does not imply clinical symptoms. It seems that valency (number of cross-reactive epitopes) and affinity to the IgE receptors of mast and basophil cells may determine the release of clinical symptoms mediators (Van Ree 1999; Pierson-Mullany et al. 2002). Finally, IgE epitopes are patient-specific and, therefore, different patients sensitized to the same allergen may develop allergy to different sets of secondary cross-reactive sources.

Molecular studies on the main plant panallergens can explain the major plant-food and pollen-food syndromes. These studies will lead to the development of IgE-binding profiles associated to particular allergies and cross-reactions.

LTP syndrome

Plant non-specific lipid-transfer proteins (LTPs) are basic polypeptides of around 9 kDa with a defence function against plant pathogens. Thus, they are classified into group 14 of the PR (pathogenesis-related) proteins. Plant LTPs are a good example of true food allergens. Their resistance to proteolytic digestion explains their role as primary sensitizers by the oral route and the induction of severe systemic reactions (Van Ree 2002). The thermal stability of LTPs explains their behaviour as allergens in processed drinks and foods such as peach juice, beer, wine or roasted hazelnuts.

In North and Central Europe allergy to *Rosaceae* fruits (particularly apple), is mainly caused by cross-sensitization with birch pollen. Bet v 1 and its plants homologues, as well as profilins, are the main allergens implicated. In the Mediterranean area, where the incidence of birch-pollen allergy is very low, allergy to *Rosaceae* (mainly peach), is in many cases not associated to pollinosis. Systemic severe symptoms are frequently observed and LTPs are major allergens associated (Fernández-Rivas, Van Ree and Cuevas 1997; Sánchez-Monge et al. 1999b; Pastorello et al. 1999).

Food allergens belonging to the LTP family have been identified in several *Rosaceae* fruits like peach, apple, apricot, cherry and plum, as well as in other fruits such as grape, nuts such as chestnut and hazelnut, cereals like maize and barley, and in vegetables such as asparagus, lettuce and carrot. Additionally, LTPs have been described as allergens of *Artemisia*, *Parietaria* and *Olea* pollen. Even more, one of the latex allergens is an LTP (Marion et al. 2004)**.** The degree of sequence identity among these allergenic LTPs is highly variable, ranging from 28% (*Parietaria* pollen Par j 1 and peach Pru p 3) to 95% (plum Pru d 3 and Pru p 3). Thus, LTPs are plant panallergens potentially responsible of cross-reactions among fruit, nuts, cereals, latex and pollen.

Cross-reactivity among purified LTPs from foods and pollen has been demonstrated both *in vitro* and *in vivo*. In ELISA inhibition assays, using sera from peach-allergic patients, peach LTP inhibits the IgE-binding of apple, mugwort and chestnut LTPs by more than 90%, whereas the inhibition of Pru p 3 IgE binding by the other LTPs was 83% for apple, 46% for mugwort and 41% for chestnut (Díaz-Perales et al. 2000). These data suggest that mugwort and chestnut proteins share some IgE epitopes with the apple and peach allergens, but lack other main ones, only present in the fruit LTPs. Skin-prick tests (García-Sellés et al. 2002) have shown different patterns of cross-reactivity. Thus, in a group of peach-allergic patients 10% were sensitized only to peach LTP, 45% to peach and apple LTPs and 21% recognized peach, apple, chestnut and mugwort LTPs. These results suggest a higher allergenicity for peach LTP and also that it probably acts as the primary sensitizer allergen in this group of patients.

The clinical significance of these cross-reactivities is unknown in many cases. Most of the allergic patients included in a Spanish study (Crespo et al. 2002) showed positive skin-prick test responses and specific IgE by capsulated hydrophilic carrier polymer (CAP) assays to several *Rosaceae* fruits. However, the clinical reactivity ranged from less than 10% for pear to 90% for peach. Moreover, 54% of the patients had positive clinical responses to only one fruit, always peach, and 27% showed

clinical reactivity to two fruits, one of them peach and the other apple, apricot or almond. A small number of patients had clinical reactivity to four or even six fruits. In summary, the patterns of cross-reactivity between LTP allergens from foods and pollen are complex and the clinical relevance is not clear.

The isolation of cDNA clones encoding several allergenic LTPs has allowed deducing their full amino-acid sequences and their expression in heterologous systems, such as prokaryotic *E. coli* or eukaryotic *P. pastoris*, to produce the recombinant allergens (Díaz-Perales et al. 2002a; Beezhold et al. 2003; Schocker et al. 2004). It has been demonstrated that the recombinant peach LTP has equivalent immunologic activity to its native counterpart and can be a useful tool for the diagnosis and potential immunotherapy of fruit allergy (Díaz-Perales et al. 2003b).

The identification of IgE-binding epitopes in Pru p 3 may help to explain the molecular basis of its cross-reactivity with other allergenic LTPs. In this sense, the IgE immunodetection of overlapping synthetic peptides covering the full amino-acid sequence of Pru p 3 has led to the identification of three major sequential epitopes on regions 11 to 25, 31 to 45 and 71 to 80. Interestingly, similar epitope regions have been identified in the latex and Parietaria LTPs, although with great amino-acid sequence changes in the latter case, which can result in lack of cross-reactivity. Furthermore, a hypoallergenic mutant was obtained by site-directed mutagenesis of three of the amino acids predicted as potential antibody recognition sites in a 3D modelling. This mutant, R39A/T40A/R44A, showed approximately 5 times less IgE binding than rPru p 3 and can serve as a starting point to produce hypoallergenic forms of Pru p 3 for future immunotherapy (García-Casado et al. 2003).

Plant class I chitinases and the latex-fruit syndrome

Less than 1% of the general population is allergic to latex. However, the prevalence of latex allergy is higher in people who wear latex gloves at work as sanitary workers (17%) and in people who have suffered many surgical operations, like *spina bifida* children (50%). Between 21 and 58% of latex-allergic patients show associated food allergy and a latex-fruit syndrome was proposed in 1994 (Blanco 2003). In the Spanish population the foods more frequently associated to the syndrome are banana, avocado, chestnut and kiwi. Other foods related to the syndrome are potato, tomato, papaya, pineapple, passion fruit, mango and fig. In the Spanish population, this syndrome causes systemic anaphylaxis in more than 40% of the patients

Thirteen latex allergens have been described. Most of them are defence proteins (Salcedo, Díaz-Perales and Sánchez-Monge 2001; Wagner and Breiteneder 2002). Among them, hevein is a major allergen on healthcare workers. It is a small protein of 4.7 kDa, implicated in the coagulation of latex, that binds chitin and has a defence function.

Class I chitinases from avocado, chestnut and banana were proposed as major food allergens implicated on the latex-fruit syndrome (Díaz-Perales et al. 1998; Mikkola et al. 1998; Blanco et al. 1999; Sánchez-Monge et al. 1999a). Class I chitinases are defence enzymes that hydrolyse chitin, a major structural polysaccharide of invertebrates exoskeleton and fungal cell walls. Their N-terminal domain shows around 70% sequence identity with latex hevein and could explain, at least partially, the latex-fruit cross-reactivity. In fact, the main IgE-binding epitopes of class I chitinases are located in the hevein-like domain, although the C-terminal catalytic domain harbours conformational ones (Díaz-Perales et al. 2002b).

Class I chitinases are widely distributed in plant foods, and putative class-I chitinases recognized by sera from latex-fruit-allergic patients were found in other foods associated with this syndrome, like kiwi, papaya, mango, cherimoya, passion fruit and tomato, as well as in foods not related to the syndrome, like wheat. The IgE-binding of these proteins is fully inhibited by purified avocado allergenic class-I chitinase and by a latex extract. These proteins were not recognized by a pool of sera from patients allergic to latex but not to fruits, suggesting different patterns of sensitization for the two types of latex-allergic patients (Díaz-Perales et al. 1999).

As defence proteins, class-I chitinases are induced by hormones and stress. This fact may have clinical relevance because the amount of these allergens can be increased with ethylene treatment, a hormone used to accelerate fruit ripening after storage of several climacteric fruits, such as apple, banana, avocado and tomato. Interestingly, the induction by ethylene of a fully cross-reactive green-bean class-I chitinase, with near 75% of sequence identity to the avocado allergen, has been demonstrated (Sánchez-Monge et al. 2000).

Purified class-I chitinases lose their allergenic properties both *in vitro* and *in vivo* after a heat treatment of 15 minutes at 100ºC (Sánchez-Monge et al. 2000). This fact could explain why only raw consumed plant foods, like fruits, are mainly associated to the latex-fruit syndrome. Many plant foods containing these potential allergens do not provoke allergenic symptoms because they undergo industrial thermal treatment or home cooking before being consumed.

Stability to digestion by the gastrointestinal tract has been proposed as a property of plant food allergens that cause systemic symptoms (Astwood, Leach and Fuchs 1996). However, the digestion by simulated gastric fluid (pepsin at acidic pH) of avocado and banana extracts indicate that class-I chitinases are readily hydrolysed (Yagami et al. 2000), although the mixture of the resulting peptides retains most of its IgE-binding capacity (Posch et al. 1999; Díaz-Perales et al. 2003a). The purification of these reactive peptides has allowed showing the digestive stability of the hevein domain, fully active both *in vivo* an *in vitro*. Other stable reactive peptides were located in the catalytic domain (Díaz-Perales et al. 2003a).

Vicilins and legume cross-reactions

In North America, the United Kingdom and Japan, peanuts and soybean are the main allergenic legumes. Despite a high rate of cross-sensitization to several legumes, shown by IgE binding and even positive skin-prick test responses (Barnett, Bonham and Howden 1987; Bock and Atkins 1989), their clinical relevance is low. Only 1-5% of legume-allergic patients have clinical reactions with more than one legume (Bernhisel-Broadbent and Sampson 1989; Burks et al. 1998). In Mediterranean countries and India, the main legumes involved in IgE-mediated hypersensitivity are lentil, chickpea and pea. In Spain, with an early introduction of these legumes in the diet, legume allergy is the fifth pediatric allergy, and clinical relevant cross-reactions are frequent, the main associations being lentil with pea and lentil with chickpea (Pascual et al. 1999; Ibáñez et al. 2003).

Allergenic vicilins, the 7S storage proteins of legumes, have been found in peanut, soybean, cashew, lentil and pea (Astwood, Silvanovich and Bannon 2002; Mills, Jenkins and Bannon 2004). They are trimeric proteins with subunits of around 50kDa. Common IgE-binding epitopes of legume vicilins could explain the cross-sensitivity to several legumes found in legume-allergic patients. However, only some of their epitopes may have clinical relevance, and their prevalence in the two types of legume

allergic populations seems to be different. In this sense, some patients with clinical pea–peanut cross-reactivity have been recently found in the United States. Pea seems to be the primary sensitizer and vicilins of both legumes are implicated. It was shown by cross-immunoinhibition that Ara h 1 lacks some of the IgE epitopes shared by the possible sensitizer pea vicilin (Wensing et al. 2003).

Bet v 1 and the pollen-food syndrome

In North and Central Europe, the most important pollen sensitizing agent is that of birch trees. Between 50 and 90% of birch-pollen-allergic patients show allergic reactions to plant foods. These foods are, in order of importance (with regional differences): hazelnut, apple, celery, carrot, peach, cherry, peanut, potato, pear, plum, almond, walnut, brazil nut and soybean (Vieths, Scheurer and Ballmer-Weber 2002). Allergic symptoms are mostly mild and restricted to mouth and pharynx, although systemic reactions to celery, carrot and soybean have been reported.

Bet v 1, to which 90% of birch-pollen-allergic patients are sensitized, seems to be the main pollen allergen implicated in the pollen-food cross-reactivity. Bet v 1 is a protein with no clear function included in group 10 of the PR proteins (Breiteneder and Ebner 2000; Hoffmann-Sommergruber 2002). Proteins with 38-70% of sequence identity with Bet v 1 and recognized by IgE antibodies of pollen-food-allergic patients have been identified in many foods implicated in the syndrome, such as hazelnut, apple, celery, carrot, pear, cherry, potato and soybean. IgE-binding inhibition with recombinant Bet v 1 and plant homologues confirms that Bet v 1 behaves as the primary allergen sensitizer (Kazemi-Shirazi et al. 2000). However, some cases of carrot allergy without birch-pollen sensitization have been described suggesting the carrot Bet v 1 homologous Dau c 1 to act as a potential true food allergen (Moneo et al. 1999).

The 3D structure of Bet v 1 and some of its food homologues has been solved by NMR and X-ray crystallography, and a dominant conformational epitope has been proposed (Gajhede et al. 1996; Schweimer et al. 1999). Site-directed mutagenesis demonstrated its implication in cross-reactions and the highly patient-specific sensitivity to amino-acid variations that can even enhance the IgE reactivity for some of the patients (Neudecker et al. 2003)

The highly conformation-dependent IgE-binding reactivity of food Bet v 1 homologues (Son et al. 1999), along with their thermal and digestive instability, may explain the lack of systemic symptoms in birch pollen-food cross-reactions. However, as has been mentioned, there are some cases of severe symptoms which may be due to a greater stability of carrot, celery and soybean allergens or to sequential epitopes recognized by some patients.

Are profilins and CCDs pollen sensitizers with low clinical relevance?

Profilins are proteins of 12-15 kDa present in almost all eukaryotic cells. They bind actin, the protein component of cytoskeleton filaments, and may regulate its organization. Profilin allergens have been found in pollen (birch and other trees, grasses and weeds), plant foods (hazelnut, celery, carrot, apple, peach, cherry, pear, melon, lychee, tomato, bell pepper, peanut, soybean, …) and latex. Although profilin sensitization occurs in only 10-30% of pollen-allergic patients, profilins confer a broad spectrum of cross-sensitization with plant foods because of their high degree (70-80%) of sequence identity. However, the relevance of profiling-specific IgE on

clinical symptoms may be low. Low affinity, conformational epitopes, thermal and digestive inactivation and proteolysis by mast-cell chymase can limit the allergic responses in sensitized individuals (Rihs et al. 1999; Scheurer et al. 2001; Wensing et al. 2002; Mellon, Frank and Fang 2002; Rodriguez-Perez et al. 2003).

Some carbohydrate structures (complex asparagines-linked N-glycans) of plant glycoproteins behave as IgE-binding epitopes and have been termed 'cross-reactive carbohydrate determinants' (CCDs). Due to their wide distribution in plants and invertebrates, CCDs are implicated in a broad spectrum of IgE cross-reactivities between pollen, plant foods and even insect venoms. However, there is a controversy about their clinical relevance (Aalberse 1998). Glycoproteins with only one IgE-binding glycan are unable to cross-link IgE bound to the receptors of mast cells and basophils, but a tomato β-fructofuranosidase, with 3 N-linked glycans, elicits the basophils' histamine release (Fötisch et al. 2003).

Novel technologies for the diagnosis and prediction of allergenic cross-reactivy

Purified natural or recombinant allergens have become powerful tools in food-allergy diagnosis. It is easier to obtain large amounts of recombinant allergens than to purify native allergens from their natural sources. However, it must be verified, for each recombinant allergen, that its immunoreactivity is the same as that of its natural counterpart. It has been shown that recombinant birch profilin (r Bet v 2) has significantly lower IgE-binding capacity than natural Bet v 2. It could thus be a poor marker for profilin sensitization (Wensing et al. 2002). Probably, its expression in a prokaryotic system such as *E. coli* leads to an improper folding. Obtaining recombinant allergens from eukaryotic plant or yeast expression systems might solve this problem, as in the case of the recombinant peach LTP allergen rPru p 3 (Díaz-Perales et al. 2003b), although this may lead to spurious glycosylation (Díaz-Perales et al. 2002b).

The increasing number of available recombinant allergens allows their use in IgE-reactivity studies of allergic patients. This component-resolved allergy diagnosis enables the precise identification of disease-eliciting allergens, and thus the sensitization profile of each patient (Valenta et al. 1999). A careful selection of purified allergen panels will make it possible to undertake with great reliability the *in vitro* diagnosis of food allergies, and to predict the severity of clinical symptoms, as well as cross-reactivity. To achieve this, more studies about the relationships of different allergens and even IgE epitopes with clinical symptoms and cross-reactivity profiles are needed. This kind of studies have allowed to associate LTP allergens to severe clinical manifestations of peach, cherry and hazelnut allergy in Mediterranean patients, whereas sensitization to Bet v 1 homologues is associated with mild allergy symptoms to apple, peach and hazelnut in patients from North and Central Europe (Wensing et al. 2002; Ballmer-Weber et al. 2002; Fernández-Rivas et al. 2003; Schocker et al. 2004).

The association of sensitization profiles to food allergies and cross-reactivities for diagnosis and prediction of allergies that will enable individual treatment, may have an exponential development due to microchip technologies. Based on genomic and proteomic technologies, microarrayed allergens will permit the analysis of the IgE-binding capacity of sera from allergic patients to hundreds or even thousands of allergens. On preactivated glass slices, picograms of allergens are immobilized using spotting robotic techniques. The incubation of these microchips with microliters of

allergic patients' sera, detection with fluorescently labelled anti-IgE antibody, scanning with a conventional microarray laser scanner and software analysis of results, leads in a few hours to the quantification of several thousands of specific IgE-binding epitopes (Hiller et al. 2002; Harwanegg et al. 2003).

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