

EDITORIAL

Fruit allergy: plant defence proteins as novel potential panallergens

Introduction

Fruit allergy is on the rise, often associated with birch- and grass-pollinosis, as well as with the latex–fruit syndrome [1,2]. However, the commercial extracts used for diagnosis (skin prick tests [SPT], specific IgE determinations, etc.) are usually non-standardized, and their main allergen components are poorly characterized (e.g. see [3] for peach). Recently, novel families of fruit allergens have been uncovered. Their biochemical characterization, together with the knowledge of their expression patterns throughout fruit development, as well as in the different fruit tissues, can help to improve the tools for diagnosis and treatment of fruit allergy in the near future. Several of these new potential panallergens are briefly discussed in the following lines.

Bet v 1-homologues and lipid transfer proteins (LTPs): two different patterns of sensitization to Rosaceae fruits

It is well established the link between allergy to Rosaceae fruits and birch pollinosis in areas where trees of the Fagales order are common (Central and Northern Europe, Northern America, etc.) [1,4]. The identification and characterization in fruits, such as apple and cherry [5,6], of 16–18 kDa allergens homologous to Bet v 1, the major birch pollen allergen [7], have accounted for most pollen–fruit cross-reactions involving Fagales. Besides the Bet v 1 family, profilins [8], and a group of 35-kDa proteins (isoflavone reductases) [9] are thought to also be implicated in birch pollen–fruit cross-sensitizations.

By contrast with this pollen–fruit allergic pattern, patients sensitized to Rosaceae fruits living in areas basically free of birch and other Fagales trees (such as the Mediterranean countries), usually show a negative SPT response and undetectable specific IgE levels to birch pollen [10–12]. Major 10–13-kDa allergens not inhibited by birch pollen extract in immunoblot inhibition assays, have been detected in apple, plum, peach, apricot and cherry [10,12]. These low molecular weight allergens have been isolated and characterized from apple and peach, and identified as LTPs by amino acid sequence comparisons [12,13]. Their close similarity with other plant LTPs, particularly those from almond and grapevine, as well as

their weak but significant sequence identity with Par j 1.0102 from *Parietaria judaica* [12], indicate a potential role for this protein family as panallergens not only in fruits, but also in some pollens.

Plant LTPs constitute a family of 9-kDa polypeptides (90–95 amino acid residues) that is widely distributed throughout the plant kingdom. Most of them conserve eight cysteines forming four disulphide bridges, which seem to be essential to maintain their three-dimensional structure [14]. These polypeptides often accumulate in the exposed surfaces of plant organs (outer epidermal layers) [15]. The report by Fernandez-Rivas and Cuevas [16] in this issue of *Clinical and Experimental Allergy* can be better understood when the tissue accumulation patterns of many LTPs are considered. A higher increase of LTPs (allergens) amount in peels than in pulps of apples, peach and pears during fruit ripening, can account for the stronger allergenicity of the peel extract from Rosaceae fruits. Furthermore, the fact that up to 40% of patients belonging to the fruit-allergic population under study, tolerated the ingestion of the pulp but not the whole fruit, can be also explained in the same line.

Chitinases with a N-terminal hevein-like domain as panallergens associated to the latex–fruit syndrome

In the past years, associations between latex allergy and reactions to fruits, primarily avocado, chestnut, banana and kiwi, have been reported, leading to the proposal of a 'latex–fruit syndrome' [2,17]. Evidence for 30–35-kDa allergens with common epitopes present in latex and in several fruits has also been obtained [18,19]. The recent purification, characterization and cDNA cloning of these allergens from avocado, chestnut and banana, have allowed their identification as class I chitinases harbouring an N-terminal hevein-like domain [20–22]. The allergenic capacity of the purified enzymes has been proven, both *in vitro* and *in vivo* (more than 50% positive SPTs when tested in latex fruit-allergic patients) [21,23]. The IgE-binding reactivity of these fruit allergens can be fully inhibited by latex extracts, as well as by isolated hevein, which together with its precursor (pro-hevein), is a major latex allergen [23–25]. Interestingly, another type of plant chitinase, named class II, highly similar to class I in the catalytic domain (above 60% of sequence identity), but lacking the N-terminal hevein-like domain [26], has been also purified from chestnut and

Correspondence: G. Salcedo, Unidad de Bioquímica, Departamento de Biotecnología, E.T.S. Ingenieros Agrónomos, Ciudad Universitaria, 28040 Madrid, Spain.

avocado extracts. These class II chitinases show neither *in vitro* nor *in vivo* allergenic capacity [20,23].

Joint consideration of the above-mentioned data strongly indicates that class I, but not class II, chitinases are major panallergens involved in the latex–fruit syndrome. Their presence in a large number of fruits, seeds and vegetables [26], can help explain, at least in part, the link between several plant foods and latex allergy. Furthermore, their hevein-like domains seem to be essential for allergenicity, as they probably share cross-reacting epitopes with latex hevein [20,23,24]. By contrast, the lack of reaction of class I enzymes in subjects allergic to latex but not to plant foods suggests that in these patients chitinases are not relevant allergens [23].

Novel fruit panallergens are plant defence proteins

LTPs and class I chitinases, as well as Bet v 1-homologues, belong to protein families involved in the plant defence mechanisms against pathogens (bacteria, fungi, etc.) [27]. A fourth group of potential fruit panallergens, the thaumatin-like proteins [28,29], has also been implicated in plant protection (see Table 1). Many members of the four protein families show antifungal and/or antibacterial properties. The fact that several types of biotic and abiotic stress

induce the synthesis of defence proteins must be kept in mind. Stress may thus lead to the accumulation of varying amounts of the potential panallergens in plant foods, depending on treatments, as well as environmental and storage conditions. Finally, the potential allergenicity of these proteins must be taken into account in biotechnological projects aimed at obtaining transgenic plants with enhanced pathogen resistance.

Note added in proof

In the time since this editorial has been in press, three closely related papers have been published [30–32].

References

- 1 Fritsch R, Ebner C, Kraft D. Allergenic cross reactivities. Pollens and vegetable foods. *Clin Rev Allergy Immunol* 1997; 15:397–404.
- 2 Raulf-Heimsoth M, Posch A, Chen Z, Baur X. Cross-reactivity between natural rubber latex and food allergens. *Env Toxicol Pharmacol* 1997; 4:169–73.
- 3 Cuesta-Herranz J, Lazaro M, Martinez A et al. A method for quantitation of food biologic activity: results with peach allergen extracts. *J Allergy Clin Immunol* 1998; 102:275–80.
- 4 Vieths S. Allergenic cross-reactivity, food allergy and pollen. *Env Toxicol Pharmacol* 1997; 4:61–70.
- 5 Scheurer S, Metzner K, Hanstein D, Vieths S. Molecular cloning, expression and characterization of Pru a 1, the major cherry allergen. *Mol Immunol* 1997; 34:619–29.
- 6 Vieths S, Schonning B. Characterization of Mal d 1, the 18 kD major apple allergen, at the molecular level. In: Wuethrich B, Ortolani C, eds. *Highlights in Food Allergy*, Basel: Karger, 1996:63–72.
- 7 Breiteneder H, Pettenburger K, Bito A et al. The gene coding for the major birch pollen allergen Bet v 1, is highly homologous to a pea disease resistance response gene. *EMBO J* 1989; 8:1935–8.
- 8 Valenta R, Duchene M, Ebner C et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992; 175:377–85.
- 9 Vieths S, Frank E, Scheurer S, Meyer HE, Hrazdina G, Hanstein D. Characterization of a new IgE-binding 35 kDa protein from birch pollen with cross-reacting homologues in various plant foods. *Scand J Immunol* 1998; 47:263–72.
- 10 Pastorello EA, Ortolani C, Farioli L et al. Allergenic cross-reactivity among peach, apricot, plum and cherry in patients with oral allergy syndrome: an *in vivo* and *in vitro* study. *J Allergy Clin Immunol* 1994; 94:699–707.
- 11 Fernandez-Rivas M, van Ree R, Cuevas M. Allergy to Rosaceae fruits without related pollinosis. *J Allergy Clin Immunol* 1997; 100:728–33.
- 12 Sanchez-Monge R, Lombardero M, Garcia-Selles FJ, Barber D, Salcedo G. Lipid-transfer proteins are relevant allergens in fruit allergy. *J Allergy Clin Immunol* 1999; 103:514–9.
- 13 Pastorello EA, Incorvaia C, Pravettoni V et al. New allergens in fruits and vegetables. *Allergy* 1998; 53 (Suppl. 46):48–51.

Table 1. Fruit potential panallergens and plant protein defence families

Protein family/ species	Proposed designation	Reference(s)
<i>Bet v 1-homologues</i>		
Apple	Mal d 1	6
Cherry	Pru a 1	5
<i>Lipid-transfer proteins*†</i>		
Apple	Mal d 3	12
Peach	Pru p 3	12,13
<i>Class I chitinases†</i>		
Avocado	Prs a 1	20,22
Chestnut	Cas s 1	20
Banana	Mus p 1.1	21
	Mus p 1.2	21
<i>Thaumatococcus-like proteins†</i>		
Apple	Mal d 2 (?)	29
Cherry	Pru a 2	28

*Additional members of this allergen family have been partially characterized in pear, apricot, plum, cherry and maize (E. A. Pastorello, oral communication, 7th International Symposium on Immunological, Chemical and Clinical Problems of Food Allergy, Giardini Naxos-Taormina, Italy, 1998). †Final designations are being arbitrated by the appropriate IUIS Subcommittee.

- 14 Kader JC. Lipid-transfer proteins in plants. *Annu Rev Plant Physiol Plant Mol Biol* 1996; 47:627–54.
- 15 Garcia-Olmedo F, Molina A, Segura A, Moreno M. The defensive role of non-specific lipid-transfer proteins in plants. *Trends Microbiol* 1995; 3:72–4.
- 16 Fernandez-Rivas M, Cuevas M. Peels of Rosaceae fruits have a higher allergenicity than pulps. *Clin Exp Allergy* 1999; 29:1239–47.
- 17 Blanco C, Carrillo T, Castillo R, Quiralte J, Cuevas M. Latex allergy: clinical features and cross reactivity with fruits. *Ann Allergy* 1994; 73:309–14.
- 18 Lavaud F, Prevost A, Cossart C, Guerin L, Bernard J, Kochman S. Allergy to latex, avocado pear and banana: evidence for a 30 kD antigen in immunoblotting. *J Allergy Clin Immunol* 1995; 95:557–64.
- 19 Delbourg MF, Guilloux L, Moneret-Vautrin DA, Ville G. Hypersensitivity to banana in latex-allergic patients. Identification of two major banana allergens of 33 and 37 kD. *Ann Allergy Asthma Immunol* 1996; 76:321–6.
- 20 Diaz-Perales A, Collada C, Blanco C et al. Class I chitinases with hevein-like domain, but not class II enzymes, are relevant chestnut and avocado allergens. *J Allergy Clin Immunol* 1998; 102:127–33.
- 21 Sanchez-Monge R, Blanco C, Diaz-Perales A et al. Isolation and characterization of major banana allergens. Identification as fruit class I chitinases. *Clin Exp Allergy* 1999; 29:673–80.
- 22 Sowka S, Hsieh L-S, Krebitz M et al. Identification and cloning of Prs a 1, a 32 kDa endochitinase and major allergen of avocado, and its expression in the yeast *Pichia pastoris*. *J Biol Chem* 1998; 273:28091–7.
- 23 Blanco C, Diaz-Perales A, Collada C et al. Class I chitinases as potential panallergens involved in the latex–fruit syndrome. *J Allergy Clin Immunol* 1999; 103:507–13.
- 24 Chen Z, Posch A, Cremer R, Raulf-Heimsoth M, Baur X. Identification of hevein (Hev b 6.02) in Hevea latex as a major cross-reacting allergen with avocado fruit in patients with latex allergy. *J Allergy Clin Immunol* 1998; 102:476–81.
- 25 Alenius H, Kalkkinen N, Reunala T, Turjanmaa K, Palouso T. The main IgE-binding epitope of a major latex allergen, prohevein, is present in its N-terminal 43-amino acid fragment, hevein. *J Immunol* 1996; 156:1618–25.
- 26 Collinge DB, Kragh KM, Mikkelsen JD, Nielsen KK, Rasmussen U, Vad K. Plant chitinases. *Plant J* 1993; 3:31–40.
- 27 Shewry PR, Lucas JA. Plant proteins that confer resistance to pests and pathogens. *Adv Bot Res* 1997; 26:135–92.
- 28 Inschlag C, Hoffmann-Sommergruber K, O’Riordain G et al. Biochemical characterization of Pru a 2, a 23 kD thaumatin-like protein representing a potential major allergen in cherry (*Prunus avium*). *Int Arch Allergy Immunol* 1998; 116:22–8.
- 29 Hsieh LS, Moos M, Lin Y. Characterization of apple 18 and 31 kD allergens by microsequencing and evaluation of their content during storage and ripening. *J Allergy Clin Immunol* 1995; 96:960–70.
- 30 Mikkola JH, Alenius H, Kalkkinen N, Turjanmaa K, Palosuo T, Reunala T. Hevein-like protein domains as a possible cause for allergen cross-reactivity between latex and banana. *J Allergy Clin Immunol* 1998; 102:1005–12.
- 31 Posch A, Wheeler CH, Chen Z et al. Class I endochitinase containing a hevein domain is the causative allergen in latex-associated avocado allergy. *Clin Exp Allergy* 1999; 29:667–72.
- 32 Pastorello EA, Farioli L, Pravettoni V et al. The major allergen of peach (*Prunus persica*) is a lipid transfer protein. *J Allergy Clin Immunol* 1999; 103:520–6.

G. SALCEDO
A. DÍAZ-PERALES
R. SÁNCHEZ-MONGE
*Unidad de Bioquímica,
Departamento de Biotecnología,
E.T.S. Ingenieros Agrónomos,
Madrid,
Spain*