

The role of plant panallergens in sensitization to natural rubber latex

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Latex allergy represents an increasing occupational problem, mainly among healthcare workers. An association between latex allergy and hypersensitivity to some plant foods, particularly fruits (the latex–fruit syndrome), has been established. Class I chitinases with an N-terminal hevein-like domain from avocado, chestnut, banana and other foods, and latex hevein seem to be the allergens responsible for the cross-reactions involved in the latex–fruit syndrome. The potential role of other latex allergens, such as profilin, *Hev b 5*, *Hev b 7* and β -1,3-glucanases, in the co-sensitization to latex and plant foods is also discussed. *Curr Opin Allergy Clin Immunol* 1:177–183. © 2001 Lippincott Williams & Wilkins.

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Abbreviations

ELISA	enzyme-linked immunosorbent assay
RAST	radioallergosorbent test
SPT	skin prick test

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Introduction

IgE-mediated (type I reaction) allergic diseases provoked by contact with latex-made products have become an increasing occupational and public health problem. The prevalence of latex sensitization in the general population has been estimated to be approximately 4–7% by analysing specific anti-latex IgE [1•]. These values significantly increased in risk groups, such as patients with spina bifida (24–60%), healthcare workers (5–15%), and rubber and greenhouse workers [2•]. However, taking into account the actual diagnostic value of the samples and methods used, the prevalence levels of latex hypersensitivity must be carefully considered [3,4].

Several allergenic proteins have been purified and characterized from the latex of the *Hevea brasiliensis* tree [5,6,7••]. Eleven have so far been assigned official numbers by the International Nomenclature Committee of Allergens. Current evidence indicates that different proteins behave as major allergens in different risk groups. *Hev b 1* (rubber elongation factor) and its homologous *Hev b 3* (small rubber particle protein) are thus mainly associated with spina bifida patients, whereas *Hev b 5* (acidic protein), *Hev b 6* (prohevein/hevein) and *Hev b 7* (patatin-like protein) are linked more to adult latex-allergic patients [5,6]. A recent work [8•] confirmed this pattern of reactivity in adults, testing recombinant allergens using the skin prick test (SPT). Other authors have shown a high response to *Hev b 2* (β -1,3-glucanase) when determining specific IgE levels and peripheral blood mononuclear cell responses to purified latex allergens [9•,10]. Intercentre studies using similar standardized preparations of all natural and recombinant allergens will clarify the relevance of each in different risk groups, as well as their actual role in latex cross-reactions with plant foods and pollens.

Cross-sensitization to latex and plant foods: the latex–fruit syndrome

It is now well established that an unexpectedly high rate of latex-allergic patients also show hypersensitivity to some plant foods, particularly fruits [11,12,13•]. This association has led to the proposal of the so called ‘latex–fruit syndrome’. Approximately 50% of associated food allergies have been found among latex-allergic patients, although wide variations have been reported, probably caused by differences in diagnostic criteria [13•]. Systemic anaphylaxis has been shown as a reaction to food ingestion in many of these patients, indicating the clinical relevance of co-sensitization.

The plant foods and their degrees of association with latex allergy also vary among different studies, possibly as a reflection of nutritional habits [13•]. However, banana, avocado, chestnut and kiwi emerge as the foods more closely implicated in the syndrome, potato also being prominent in certain cases [14,15].

Besides the sensitization of latex-allergic patients to specific plant foods, the opposite condition has also been described [13•]. Determined food hypersensitivities seem to enhance the risk of latex allergy, and a significant number of fruit allergic patients became sensitized to latex. Banana, avocado and chestnut are among the fruits most frequently detected in these cases. Moreover, hypersensitivity to fruits linked to the latex–fruit syndrome has been claimed as a possible explanation for the lack of a decrease in SPT reactivity and specific IgE levels to latex in a follow-up study of allergic children [16].

Sensitization to pollens is another factor that can determine both the rate and type of associated food allergy in latex-allergic patients [17•]. Those who are not sensitized to pollens seem to show significantly less positive SPT reactions to foods. Furthermore, a joint allergy to latex and pollens led to sensitization mainly to banana, avocado, papaya, chestnut, kiwi and mango, whereas celery, peach and apple are foods more frequently associated with pollen non-latex allergy.

The above-mentioned cross-reactions among plant foods and latex reflects the presence of homologous allergens sharing common IgE epitopes in both allergen sources. This theory was supported by radioallergosorbent test (RAST) and immunoblot inhibition data using crude extracts from latex and fruits [13•]. Homology has been shown between several plant foods and latex allergens (see Table 1). Latex hevein and profilin should be emphasized in the context of this review as members of

plant panallergen families potentially involved in the latex–fruit syndrome. *Hev b 6.02* (hevein) is a 43 amino acid residue polypeptide processed from the N-terminal domain of its precursor, prohevein (*Hev b 6.01*), which is a major latex allergen [18]. The main IgE-binding epitopes of prohevein have been located in its hevein N-terminal fragment [19,20]. Several plant proteins, such as some lectins (i.e. wheat germ agglutinin) and class I chitinases, include hevein-like domains in their primary structure [21•]. Latex profilin (131 amino acid residues; 14 100 M_r) belongs to a family of plant allergens extensively identified in plant foods and pollens [22,23,24•].

Fruit class I chitinases as major panallergens involved in the latex–fruit syndrome

Different research groups have identified class I chitinases from avocado [25–27,28•], banana [29,30•] and chestnut [25,31], three of the plant foods more closely associated with latex allergy, as the major allergens responsible for latex–fruit co-sensitization.

The strongest IgE-binding components of approximately 32 000 M_r, recognized by sera from latex- and fruit-allergic patients, have been isolated and characterized in these three foods. A major allergen, named *Prs a 1* and *Cas s 5*, is thus now well defined in avocado pear (*Persea americana*) and chestnut (*Castanea sativa*) seed, respectively. Two main isoallergens, *Mus a 1.1* and *Mus a 1.2* (proposed designations) are also located in banana fruit (*Musa acuminata*). All the purified proteins showed chitinase activity, and reacted with monospecific polyclonal antibodies against plant chitinases and with specific IgE of sera from latex–fruit-allergic subjects. Furthermore, all fully inhibited IgE binding by 32 000 M_r components of the corresponding crude fruit extract separated by sodium dodecylsulfate–polyacrylamide gel electrophoresis in the immunoblot inhibition test, and reached blocking values higher than 85% in RAST inhibition assays, when the same food commercial preparations were used in solid phase [25,30•,31]. In-vivo reactivity of the four isolated allergens (proved by SPT) rendered positive results in 72–53% of the latex–fruit-allergic patients tested [30•,31]. On the other hand, cross-reactions with latex components were supported by both immunoblot and RAST inhibition assays [26,27,28•,29,30•,31].

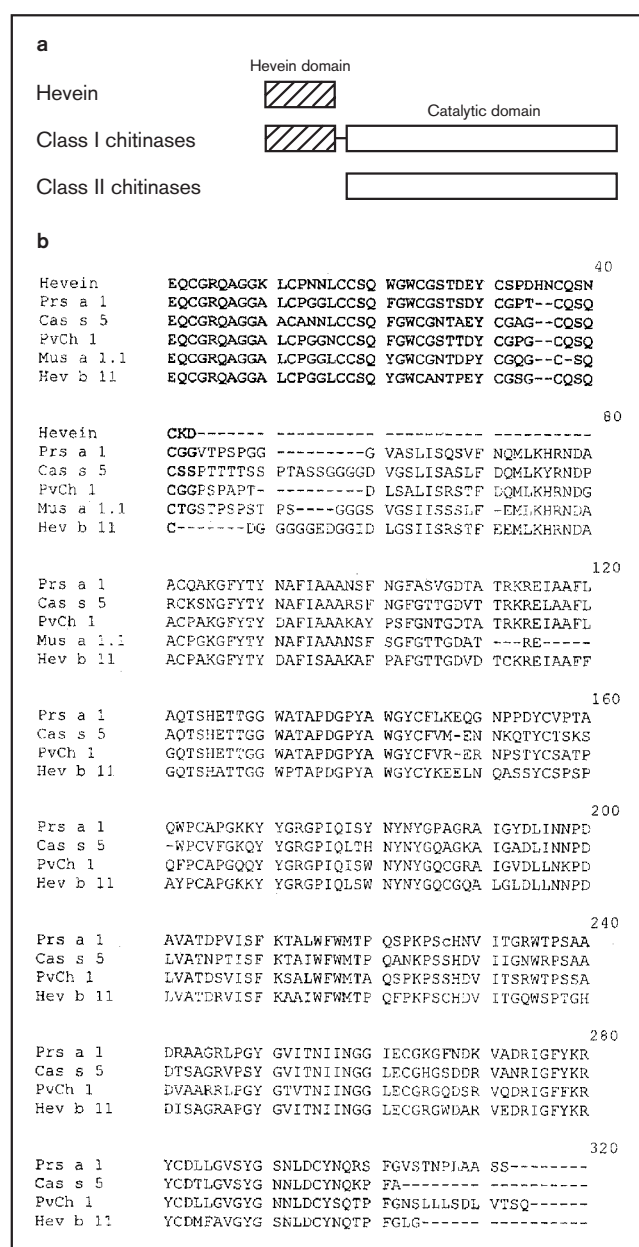
The four identified fruit allergens have an N-terminal hevein-like domain of 40–41 amino acid residues, which share 65–70% sequence identity with latex hevein [21•], suggesting that these protein domains play a key role in latex–fruit co-sensitization (see below). The high level of primary structure conservation (66–72% of identical residues) found among the complete amino acid sequences of *Prs a 1*, *Cas s 5* and *Mus a 1.1* (see Fig.

Table 1. Potential cross-reactive allergens from plant foods and latex

Plant food proteins	Latex allergens
Class I chitinases (chestnut ^a , avocado ^a , banana ^a , green bean ^a)	Prohevein (<i>Hev b 6.01</i>)/ Hevein (<i>Hev b 6.02</i>)
Proheveins (turnip ^a)	
Profilins (<i>Bet v 2</i> , ^a etc.)	Profilin (<i>Hev b 8</i>)
Acidic protein (kiwi)	Acidic protein (<i>Hev b 5</i>)
Patatin-homologous (potato ^a , tomato)	Patatin-like (<i>Hev b 7</i>)
β-1,3-glucanases (plant food glucanases)	β-1,3-glucanase (<i>Hev b 2</i>)
Chitinases/lysozyme (plant food class III chitinases?)	Hevamine

^aSpecific IgE-binding and potential cross-reactivity have been proved with purified allergens.

Figure 1.



(a) Structural scheme of latex hevein, and the hevein-like and catalytic domains of class I and II plant chitinases. (b) Amino acid sequences comparison of latex hevein and plant food class I chitinases, whose potential allergenic activity has been proved. The chitinases have been purified from avocado pear (*Prs a 1*; clone accession number Z78202), chestnut seed (*Cas s 5*; U48687), banana fruit (*Mus a 1.1*; partial clone, AF 001524), green bean pods (*PvCh*; P36361), and rubber latex (*Hev b 11*; O'Riordan G *et al.*, unpublished; AJ238579).

1) indicated the close relationship of this family of plant food panallergens.

The relevance of class I chitinases at latex–food cross-reactions has been highlighted by analysing the specific

IgE-binding capacity of proteins from 20 plant foods differentially associated with the latex–fruit syndrome [32•]. On the basis of common sodium dodecylsulfate–polyacrylamide gel electrophoresis band recognition patterns obtained by immunodetection with monospecific polyclonal anti-chitinase antibodies and a serum pool from allergic patients to latex and fruits, together with immunoblot inhibition assays using the purified avocado allergen *Prs a 1* and a latex crude extract as inhibitors, potentially allergenic class I chitinases were detected. These putative allergens were found in most fruits previously linked with latex allergy (kiwi, papaya, mango, passion fruit, cherimoya and tomato), and in wheat flour, a plant source very rarely associated with the latex–fruit syndrome [13•]. By contrast, the lack of reactivity of class I chitinases when sera from latex but not fruit allergic patients are used, suggests that these plant panallergens do not play a relevant role in latex allergy non-associated with food co-sensitization [32•]. On the other hand, the data by Diaz-Perales *et al.* [32•] also indicated that complex asparagine-linked glycans of plant glycoproteins are not important antigenic determinants in the context of latex–fruit cross-sensitization.

Ethylene induction and potential heat and digestive inactivation of allergenic class I chitinases

Allergenic class I chitinases, as well as latex prohevein/hevein, are members of plant defence protein families whose expression can be induced by hormone treatment and biotic or abiotic stress [7••]. Potential implications of this fact on latex–food allergy have recently been suggested by evidence indicating that a class I chitinase from green beans (*Phaseolus vulgaris*) [33•], highly related to avocado allergen *Prs a 1*, and a prohevein-like protein from turnip (*Brassica rapa*) [34••], are induced by ethylene treatment. In-vitro and in-vivo reactivity has been demonstrated in a group of latex-allergic patients for both proteins. Ethylene is a plant hormone widely used to hasten fruit ripening of climacteric fruits (banana, avocado, tomato, etc.), and can thus substantially change allergen levels in the consumed fruits.

The presence of an allergenic class I chitinase in green bean pods is very surprising, because this widely eaten legume has not been associated with the latex–fruit syndrome. The evidence provided by Sanchez-Monge *et al.* [33•] on the heat inactivation of *Prs a 1* and the allergenic chitinase from the green bean, both by in-vitro and in-vivo assays, can explain this apparent contradiction. If class I chitinases are actually inactivated by heating, it can explain why those foods that are usually consumed after cooking (such as green beans), even containing potentially allergenic members of this enzyme family, are not associated with latex allergy.

Besides heat inactivation, digestive proteolytic resistance has been cited as an allergen property. Very recent data based on protein staining and IgE immunodetection [35•] suggested that putative class I chitinases from avocado and banana, as well as kiwi and potato proteins that could cross-react with latex components, are very quickly hydrolysed when simulated gastric fluid is used for digestibility tests. Interestingly, latex hevein was stable in the same conditions. Unfortunately, no evaluation of the allergenic properties exerted by digested products was performed in that report. This test was carried out with the avocado allergen *Prs a 1* [28•]. Although the avocado endochitinase exhibited no digestive stability, its 4000–6000 M_r permanent accumulating fragments (evoking its hevein-like domain size) held the IgE-binding capacity.

What is the role of the hevein-like domain of fruit class I chitinases in their allergenic capacity?

By contrast with *Prs a 1* and *Cas s 5* allergens, the homologous avocado and chestnut class II chitinases without an N-terminal hevein-like domain (see Fig. 1) did not show any allergenic activity when tested either *in vitro* or *in vivo* [25,31]. These data, together with the strong inhibition exerted by latex hevein on the IgE-binding capacity of banana and avocado class I chitinases [27,28•,29], point to an essential role of the hevein-like domain harboured to plant chitinases in their allergenic properties. Further in-vitro evidence in support of this theory was obtained by expressing recombinant *Cas s 5*, as well as its catalytic domain, in the yeast *Pichia pastoris*, and by testing the IgE-binding capacity of both recombinant products [36]. Immunoblot and immunoinhibition assays showed that *rCas s 5* is as reactive as the natural allergen (*Cas s 5*), whereas the recombinant catalytic domain is not recognized by sera from latex–fruit-allergic patients (see Fig. 2). Moreover, latex hevein exerted a strong inhibition of the IgE-binding to both *rCas s 5* and natural *Cas s 5*.

The above-mentioned evidence strongly indicates that the major IgE epitopes of allergenic class I chitinases are located in their N-terminal hevein-like domains, and that

hevein is the main latex cross-reacting allergen involved in the latex–fruit syndrome. However, at least three lines of evidence shaded this clear-cut scenario. First, other hevein-like domains of plant proteins, such as those present in wheat germ agglutinin, are not recognized by sera from patients with allergy to latex and fruit who are reactive to class I chitinases [25]. Second, very low inhibition levels (10–40%) of IgE binding to hevein are reached when avocado and banana extracts, or banana class I chitinases are used as inhibitors [27,28•,29]. Third, chestnut and avocado class II chitinases inhibit approximately 150% of the IgE binding to the corresponding commercial extracts in RAST inhibition assays ([31] and Diaz-Perales *et al.*, unpublished data). This evidence suggests that latex hevein, besides possessing common IgE epitopes with class I chitinases, also presents other IgE antigenic determinants not shared with these plant enzymes. Furthermore, the catalytic domain of class I chitinases (or, at least, its homologous class II enzymes) seems to include IgE-binding epitopes not detected by immunoblotting, but reactive by using the RAST inhibition test.

Recently, a latex class I chitinase (O’Riordain *et al.*, unpublished), named *Hev b 11*, has been located and produced as a fusion-recombinant protein. Its amino acid sequence has 70% identity with that of *Prs a 1*. Only 19% of latex- or fruit-allergic patients show specific IgE to *Hev b 11* by immunodetection. High inhibition values (up to 85%) of IgE-binding were obtained by using latex hevein as an inhibitor against *Hev b 11*, whereas only levels of approximately 15% were achieved in the opposite assay.

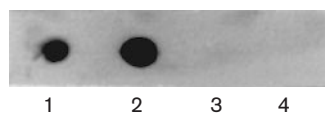
Other latex allergens potentially involved in cross-reactions with plant foods

Besides food class I chitinases and latex hevein, the similarity of other latex and plant food allergens (see Table 1) could also explain the latex-food co-sensitization.

Profilin (*Hev b 8*)

A member belonging to the panallergen profilin family has been identified in latex [22,24•]. The deduced amino acid sequence from its corresponding complementary DNA clone shows over 80% identity with homologous proteins from several plant foods and pollens, and 75% with *Bet v 2*, the birch pollen profilin. *Hev b 8* seems to be a minor allergen according to the low number (10–20%) of latex-allergic patients who present detectable specific IgE antibodies to *Hev b 8* [22,24•]. Homology among latex and plant profilins can explain the positive RAST test to latex in a high rate of subjects sensitized to pollens, fruits or vegetables [37]. However, no clinical symptoms to latex associated with these positive RAST results were found in most cases. The

Figure 2



Specific IgE immunodetection by dot-blot of a chestnut protein preparation enriched in chitinases (1), purified recombinant chestnut allergen *rCas s 5* (2) and its recombinant catalytic domain (3), and bovine serum albumin (4; negative control).

relevance of *Hev b 8* in latex–plant food cross-reactivity, if any, has still not been demonstrated.

***Hev b 5* and latex-kiwi co-sensitization**

Hev b 5 is an acidic (pI 3.5) and proline-rich protein with a predicted molecular weight of 17 000 M_r, which reacts with IgE from more than 50% of latex-allergic patients [38,39]. Its high sequence identity (46%) with a kiwi acidic protein [38,39] suggests a molecular basis for the observed clinical co-sensitization to latex and this fruit. Unfortunately, the recent mapping of human B and T cell epitopes of *Hev b 5* does not support this hypothesis [40,41]. The main IgE and T cell epitopes in the latex allergen (except T cell immunodominant fragment from residues 109–128) were located in protein regions showing low similarity with the kiwi protein. The anomalous behaviour of *Hev b 5* regarding its staining, electrophoretic mobility and transfer to membranes [38–40] has probably delayed to establish the actual role of this allergen in latex and plant food cross-reactions.

Patatin and *Hev b 7*

Homologous (39–42% sequence identity) allergens of 43 000 M_r have been characterized in latex (*Hev b 7*) and potato (*Sol t 1*; patatin) [42–44]. The relationship between both proteins could explain the association of latex and potato allergy reported by some authors [14]. The evidence now available regarding this point seems to be contradictory. Seppälä *et al.* [45] found concomitant IgE binding to *Hev b 7* and *Sol t 1* in approximately 30% of latex-allergic adults, as well as a partial degree of cross-reactivity between both allergens by in-vitro inhibition methods. However, the pattern of co-sensitization was not confirmed in latex-allergic children, who frequently show specific IgE to *Sol t 1* but not to *Hev b 7* [45]. By contrast with the above-mentioned evidence, Sowka *et al.* [46] described a full lack of cross-reactivity between patatin and *Hev b 7*, on the basis of their immunoblot and enzyme-linked immunosorbent assay (ELISA) inhibition results using sera from patients with both latex and potato allergy. Therefore, further studies are needed to elucidate the role of patatin-related allergens in the association of latex and plant food allergies.

β -1,3-Glucanase (*Hev b 2*) and hevamine

A 35 000 M_r basic β -1,3-glucanase (*Hev b 2*), and a 29 500 M_r class III chitinase lacking an hevein-like domain and displaying lysozyme activity (hevamine), have also been identified as latex allergens [5,7,47]. The evidence of *Hev b 2* in latex allergy is still controversial (see above), whereas hevamine seems to be a minor allergen [5,47]. Both proteins are plant defence-related enzymes showing a significant degree of sequence identity with their counterparts from other plant sources [47,48]. These characteristics have led

them to be claimed as potential components implicated in latex, fruit and vegetable cross-reactions. However, their actual involvement in these associations remains unclear.

Conclusion

The interactions between latex and plant food allergies is an emerging clinical problem. To elucidate the molecular basis of this cross-reactivity is crucial for improving the tools for diagnosing and treating this condition. Fruit class I chitinases and their relationship with latex hevein seem to be the main clue to explain the latex–fruit syndrome. However, future efforts to clarify the actual role of the N-terminal hevein-like domain of these enzymes in latex–plant food co-sensitization are uneludible. The expectations of other latex allergens that could potentially support wide (profilins, β -1,3-glucanase) and narrow (*Hev b 5*, *Hev b 7*) patterns of cross-reactivity with plant foods remain to be clearly confirmed.

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