

Lipid transfer protein allergy: primary food allergy or pollen/food syndrome in some cases

Laurian Zuidmeer and Ronald van Ree

Purpose of review

To summarize recent findings on non-specific lipid transfer proteins in food allergy, with a specific focus on the localization, stability and route of sensitization.

Recent findings

Plant non-specific lipid transfer proteins are major food allergens, especially in the Mediterranean area. They have been identified as allergens in a number of foods and the list grows ever longer. As non-specific lipid transfer proteins are considered to be 'true' food allergens that sensitize directly via the gastrointestinal tract their stability during food processing and gastric digestion has been studied in more detail. In addition, several groups have tried to determine the sensitization patterns of lipid transfer protein-reactive patients, to determine and possibly clarify the observed geographical differences in sensitization. Different sensitization routes (via the respiratory tract or even transdermally) have been suggested.

Summary

As the structure and molecular properties of non-specific lipid transfer proteins are resolved and more purified non-specific lipid transfer proteins become available for diagnostic purposes, detailed studies on the sensitization pattern and route are becoming feasible. Continuing studies on the pattern of lipid transfer protein sensitization will give more insight into the development and possible treatment of lipid transfer protein-related food allergy.

Keywords

allergy, food, non-specific lipid transfer protein, pollen

Introduction

In northern and central Europe, fruit allergy is mostly described as a crossreactive phenomenon resulting from sensitization to homologous allergens from (birch) pollen, and patients usually present with only mild symptoms restricted to the oral cavity (oral allergy syndrome, OAS). In contrast, in the Mediterranean area, patients also suffer from non-pollen-related fruit allergy and frequently have systemic reactions. Approximately 15 years ago, the plant non-specific lipid transfer proteins (nsLTPs) were identified as major allergens in fruits causing these severe symptoms, and since then allergenic lipid transfer proteins (LTPs) have been identified in many more plant foods. Recently published reviews by Breiteneder and Mills [1], Salcedo and co-workers [2] and Mills and co-workers [3] have provided a thorough background on nsLTPs in foods and pollen. NsLTPs are considered to be non-pollen-related 'true' food allergens; however, crossreactivity to pollen LTP has been reported and primary sensitization to pollen LTP leading to fruit allergy still can not be completely ruled out [4–7]. In this review, we focus on new data that have become available on several (purified) nsLTPs with regard to their stability to heat and digestion and new diagnostic tools, and furthermore we describe the results of epidemiological studies that may help to elucidate the possible route of sensitization.

Structure, function and sequence of non-specific lipid-transfer proteins

NsLTPs are members of the prolamin superfamily, which are low-molecular weight proteins (M_r below $\sim 15\,000$), characterized by a conserved pattern of six to eight cysteines, forming three to four disulfide bridges. The LTPs are named after their capacity to transfer lipids between membranes [8]; however, this is in disagreement with their localization in the plant, and all evidence now points against a role in intracellular lipid trafficking [9]. NsLTPs are now mainly implicated in plant defence [8,10]. In this light, it is interesting to note that fungal membrane damage was recently demonstrated for the first time *in vitro* with a sunflower LTP [11]. Another possible role for nsLTPs is in the transport of cutin and suberin monomers to the outer layers of plant organs [12], which is consistent with experimental results showing that nsLTPs are mostly present in the peel of fruits, rather than the pulp [13,14].

Curr Opin Allergy Clin Immunol 7:269–273. © 2007 Lippincott Williams & Wilkins.

Laboratory of Allergy, Department of Experimental Immunology, Academic Medical Center, Amsterdam, the Netherlands

Correspondence to Laurian Zuidmeer, Department of Experimental Immunology, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands
Tel: +31 20 5666819; e-mail: l.zuidmeer@amc.uva.nl

Sponsorship: Laurian Zuidmeer was funded by EuroPrevall (EC 514000).

Current Opinion in Allergy and Clinical Immunology 2007, 7:269–273

Abbreviations

LTP	lipid transfer protein
nsLTP	non-specific lipid transfer protein
OAS	oral allergy syndrome

© 2007 Lippincott Williams & Wilkins
1528-4050

The nsLTPs have been extensively reviewed in the past few years with respect to their structure, function and molecular properties [1,3,15,16[•]]. More than 100 plant nsLTPs have been sequenced, and recent additions to the list of allergenic nsLTPs include that of lemon and orange [17], cabbage [18^{••}], strawberry [19] and rice [20]. In addition, Gao and coworkers [21] reported on the linkage map positions and allelic diversity of two apple LTP (Mal d 3) genes, which will open possibilities for breeders to select for low-allergenic cultivars.

Also at the structural level, the nsLTPs are well characterized with three-dimensional structures available from barley [22], rice [23], maize [24] and wheat [25]. The crystal structure of the first identified allergenic nsLTP, that of peach (Pru p 3) also recently became available [26^{••}]. Interestingly, although the structure showed the well-conserved overall fold, there are significant differences between the structures of barley, rice and wheat on one hand and those of maize and peach on the other. It was previously determined by peptide mapping [27] that Arg39/Thr40 were a relevant epitope of Pru p 3. The determined structure confirmed that these amino acids are well conserved in *Rosaceae* nsLTPs and only partly in cereal nsLTPs, and as such may participate in one of the *Rosaceae*-specific IgE epitopes.

Next to the new structural and sequence data, various groups are also working on detection assays. In addition to previously described assays to quantify Pru p 3 [28], assays have now also been developed for Mal d 3 [29] and the major *Parietaria judaica* nsLTPs, Par j 1 and Par j 2 [30]. The latter assay was reported to correlate well with allergenic potency.

The stability in relation to sensitization and elicitation potential of non-specific lipid-transfer proteins

The nsLTPs have been proposed as a model allergen for true food allergy [31] because of their high resistance to heat treatment and proteolytic digestion. These characteristics are necessary for a food allergen to remain intact during food processing, and to survive the harsh environment of the gastrointestinal tract, respectively. The latter property allows the allergen to reach the intestinal immune system in an immunogenic way, i.e. with the potential to sensitize. Moreover, a conserved IgE-binding conformation is probably the most important factor contributing to the observation that LTP-reactive patients have a higher incidence of severe systemic reactions than classic birch-related food allergic patients [32,33,34^{••}]. It has previously been shown that nsLTPs can retain their IgE-binding activity during (thermal) processing, for example in peach juice or jam [28,35], cooked apple [36], cherry [37], hazelnut [38] or maize [39] and fermented drinks such as wine

[40–42] and beer [43,44]. In addition, Pru p 3 and cherry nsLTP (Pru av 3) have been shown to be resistant to proteolysis by pepsin [28,37]. More groups have recently been working on assessing the resistance of various nsLTPs to processing; resistance to pepsin has been shown for hazelnut LTP (Cor a 8) and Mal d 3 [45], whereas resistance to gastric and duodenal digestion was assessed for grape nsLTP (Vit v 1) in an elegant design by Vassilopoulou and co-workers [46^{••}]. In that study, an in-vitro model of gastric and duodenal digestion was used [47], including an assessment of the effect of the physiological surfactant phosphatidylcholine, which is secreted by the gastric mucosa and occurs in bile. Although Vit v 1 was rapidly degraded into an approximately 6000 M_r fragment, this fragment remained stable during the remainder of digestion. Interestingly, both major IgE epitopes described for Pru p 3 were still intact, whereas a third, located at the clipped C-terminal portion of the protein, remained attached to the main fragment by a disulfide bond. Phosphatidylcholine had a slightly protective effect on the protein and its IgE reactivity during gastric digestion.

Resistance to thermal processing was studied extensively for Mal d 3 [48], with an emphasis on the effect of the Maillard reaction, an important chemical modification that can occur during processing. It was concluded that only severe heat treatment had an effect on the allergenicity of Mal d 3, whereas glycation had a protective effect. Also barley and malt LTPs were shown to be highly stable to heat, and modifications, whether lipid adduction or glycation, had little or no effect on heat stability [49[•]]. Only in the presence of a reducing agent did the nsLTP unfold during heat treatment, and here the lipid-adducted LTPs were more heat stable.

Non-specific lipid transfer protein localization and content: a factor in sensitization potential?

Various groups have shown that nsLTPs are mostly present in the peel of fruits, rather than the pulp [13,14]. This also explains why some LTP-allergic patients can more easily tolerate the fruit after peeling. A recent study showed that peach fuzz, present in fresh fruits sold off the tree and normally removed in all supermarket-sold peaches, contains large amounts of nsLTP, causing a lot of peach-induced contact urticaria, and the authors hypothesized that a transdermal route of sensitization may be an alternative to the proposed oral route [50[•]]. In addition, in a study by Marzban and co-workers [51[•]], the presence of Mal d 1, but more importantly in light of this review, Mal d 3 was detected in apple pollen. Other LTP homologues were also detected in pear, cherry and peach pollen. As the fruit tree pollen exposure documented over the years was measurable (but low in comparison with tree/grass pollen

exposure), the authors hypothesized that these findings may provide a new explanation for the origin of fruit LTP allergy.

Several groups have recently focussed on determining the allergen content of fruit (cultivars) as this may play a role in the sensitization potential of the different allergens. It was shown that nsLTP levels are greatly dependent on maturity, storage conditions and cultivar in apple [52[•]] (A. Sancho, R. van Ree, unpublished data) and peach [53[•],54,55]. For apple, the highest LTP levels are found in mature, freshly picked fruits, whereas LTP levels decrease during storage (with the greatest decrease under controlled atmosphere conditions) [52[•]]. In a study with 88 cultivars from Italy and the Netherlands (A. Sancho, R. van Ree, unpublished data), absolute levels of LTP were determined with three in-vitro assays, and were found to vary up to 100-fold between cultivars. The same cultivars grown at two different locations demonstrated significant differences in LTP content of up to a factor of 10. Allergenicity differences between cultivars were confirmed by in-vivo assays. In contrast, six different cherry cultivars showed no marked difference in nsLTP content [56[•]]. This was, however, only assessed with less accurate, semiquantitative electrophoretic analysis.

As no studies have been performed to determine the threshold level for sensitization or triggering an allergic reaction, clinical studies have to be undertaken to determine whether these in-vitro cultivar differences also translate to the in-vivo situation. This has been done on a small scale in a Spanish study [57^{••}], in which the Mal d 3 content was shown to vary greatly in 10 cultivars, which translated into significantly different wheal sizes when patients were tested with skin-prick tests. This was also shown for three cultivars in a study from Bolhaar and co-workers [58].

Besides the allergen content in fruits, the affinity of IgE might also play a role in the clinical symptoms elicited. A recent study on the role of nsLTPs in strawberry allergy in the Mediterranean area showed that although strawberry LTP (Fra a 3) is present in extracts and is capable of eliciting histamine release in Pru p 3-sensitized patients, the concentrations needed are much higher than for Pru p 3, and these patients do not show clinical strawberry allergy [19].

Geographical distribution of non-specific lipid transfer protein sensitization and allergy

Next to the structural and molecular characteristics that define nsLTPs as important food allergens, there is another important 'feature' of this allergen that makes it so worthwhile to study, and that is its surprising geographical distribution. Until now, nsLTPs have been

described as dominant allergens in the Mediterranean area, whereas allergy to LTP is rarely reported in central and northern Europe. There are isolated reports, for example that of Schad and co-workers [42] on a German patient with IgE-mediated allergy to nsLTP from grapes, that non-pollen-related allergy to nsLTP does occur outside the Mediterranean area, but it is not common.

In the past year, several groups have reported data on larger populations from several countries that have been tested with component-resolved diagnosis with purified allergens. In an article on cherry allergy, 186 patients from central Europe and Spain were tested with three purified cherry allergens, Pru av 1, 3 (nsLTP) and 4 [59^{••}]. Consistent with previous reports, a significantly higher rate of Pru av 3 sensitization, combined with systemic symptoms, was found in the Spanish patients compared with those from central Europe. It is interesting to mention that although they presented with only mild symptoms (OAS), for the first time LTP sensitization in cherry-allergic patients was reported outside the Mediterranean area. To explain these geographical differences, the authors speculated on the exposure to Mediterranean pollen (olive, plane tree, *Parietaria*) as co-factors, possibly fruit-consumption differences and exposure and co-sensitization to nsLTPs from other sources.

In another study, 400 apple-allergic patients from four different countries (Spain, Italy, Austria and the Netherlands) were clinically assessed and tested with Mal d 1–4 [34^{••}]. Also here, the conclusion was that nsLTP (Mal d 3) is the dominant apple allergen in Spain, with more than 35% of systemic reactions, whereas in the other countries apple allergy is mild (OAS, mainly directed to Mal d 1) and is linked to birch pollen. It was found that sensitization to birch pollen was associated with a decreased risk of Mal d 3 sensitization by a factor of 3.5, whereas sensitization to mugwort and plane tree increased the risk by a factor of 2.3–2.8, respectively. It was therefore hypothesized that exposure to peach and mugwort or plane pollen at a young age, combined with a lack of exposure to birch pollen could facilitate the development of LTP allergy in Spain.

Sensitization route

Under previous headings we have already discussed some of the possible routes of sensitization that are suggested for nsLTP allergic patients. Until now, no non-pollinosis patients were described that recognize non-*Rosaceae* LTP without IgE antibodies against peach LTP, which suggested that LTP from peach is probably the starting point for sensitization in those patients. Egger and co-workers [60^{••}] wrote a very comprehensive review on the pollen-food syndromes associated with weed pollinosis; especially the role of mugwort pollen LTP (Art v 3), which cross-reacts with Pru p 3, and as such is

involved in the mugwort–peach association [6], deserves attention. Although Lombardero *et al.* [6] stated that Art v 3 behaves as a primary sensitizer in some patients with IgE to both Pru p 3 and Art v 3, Pastorello and co-workers [7] claimed that this Art v 3 reactivity is only a consequence of peach sensitization. A mugwort–mustard allergy syndrome was recently suggested by Figueroa and co-workers [61], and they also hypothesized on the possible role of Art v 3 as a causative cross-reacting allergen. In a follow-up study a new nsLTP was isolated from cabbage, Bra o 3, which is a highly clinically significant cross-reactive allergen to Pru p 3 and Art v 3 [18[•]]. Interestingly, in the latter study it was suggested that an Art v 3-positive skin-prick test could be used as a clinical tool to detect those mugwort allergic patients at risk of severe food allergy. It is also interesting to mention that in chestnut allergy without associated latex allergy, chestnut nsLTP, Cas a 8, plays a significant clinical role and all patients tested were also sensitized to Art v 3 and Pru p 3 [62[•]]. As mentioned previously in this review, we may still have to take into account the sensitization via the respiratory tract of fruit tree pollen itself, as it also contains LTP [51[•]]. In addition, sensitization via the transdermal route was suggested by Asero and co-workers [50[•]], although this is not likely to be the primary route of sensitization, as most patients probably buy supermarket-sold peaches. Finally, Garcia and co-workers [63] suggested the possibility of occupational allergy to peach as a result of primary sensitization to the inhalation of Pru p 3 from peach leaves.

Conclusion

As the list of plant-derived nsLTPs causing food allergy grows longer, the need for information on the sensitization pathway increases. Peach consumption alone does not seem to be the only explanation, as several studies also point to a direct or indirect involvement of pollen. There is possibly more than one LTP-syndrome: (1) primary sensitization to a food LTP without concomitant pollen allergy; (2) primary sensitization to a food allergen against a background of existing pollen allergy; (3) primary sensitization to a pollen allergen. In all three cases, the patient may suffer from food allergy (either from primary sensitization or as a result of cross-sensitization) but the symptoms may be quite different, also dependent on the avidity of the IgE antibodies involved. Animal models on nsLTP allergy should give valuable information on the route of sensitization.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 292).

- 1 Breiteneder H, Mills C. Nonspecific lipid-transfer proteins in plant foods and pollens: an important allergen class. *Curr Opin Allergy Clin Immunol* 2005; 5:275–279.
- 2 Salcedo G, Sanchez-Monge R, Diaz-Perales A, *et al.* Plant nonspecific lipid transfer proteins as food and pollen allergens. *Clin Exp Allergy* 2004; 34:1336–1341.
- 3 Mills ENC, Jenkins JA, Alcocer MJC, Shewry PR. Structural, biological, and evolutionary relationships of plant food allergens sensitizing via the gastrointestinal tract. *Crit Rev Food Sci Nutr* 2004; 44:379–407.
- 4 Enrique E, Cistero-Bahima A, Bartolome B, *et al.* *Platanus acerifolia* pollinosis and food allergy. *Allergy* 2002; 57:351–356.
- 5 Florido Lopez JF, Quiralte EJ, Arias de Saavedra Alias JM, *et al.* An allergen from *Olea europaea* pollen (Ole e 7) is associated with plant-derived food anaphylaxis. *Allergy* 2002; 57 (Suppl 71):53–59.
- 6 Lombardero M, Garcia-Selles FJ, Polo F, *et al.* Prevalence of sensitization to *Artemisia allergens* Art v 1, Art v 3 and Art v 60 kDa. Cross-reactivity among Art v 3 and other relevant lipid-transfer protein allergens. *Clin Exp Allergy* 2004; 34:1415–1421.
- 7 Pastorello EA, Pravettoni V, Farioli L, *et al.* Hypersensitivity to mugwort (*Artemisia vulgaris*) in patients with peach allergy is due to a common lipid transfer protein allergen and is often without clinical expression. *J Allergy Clin Immunol* 2002; 110:310–317.
- 8 Kader JC. Lipid-transfer proteins in plants. *Annu Rev Plant Physiol Plant Mol Biol* 1996; 47:627–654.
- 9 Blein JP, Coutos-Thevenot P, Marion D, Ponchet M. From elicitors to lipid-transfer proteins: a new insight in cell signalling involved in plant defence mechanisms. *Trends Plant Sci* 2002; 7:293–296.
- 10 Garcia-Olmedo F, Molina A, Segura A, Moreno M. The defensive role of nonspecific lipid-transfer proteins in plants. *Trends Microbiol* 1995; 3:72–74.
- 11 Regente MC, Giudici AM, Villalain J, De la Canal L. The cytotoxic properties of a plant lipid transfer protein involve membrane permeabilization of target cells. *Lett Appl Microbiol* 2005; 40:183–189.
- 12 Douliez JP, Michon T, Elmorjani K, Marion D. Structure, biological and technological functions of lipid transfer proteins and indolines, the major lipid binding proteins from cereal kernels. *J Cereal Sci* 2000; 32:1–20.
- 13 Fernandez-Rivas M, Cuevas M. Peels of Rosaceae fruits have a higher allergenicity than pulps. *Clin Exp Allergy* 1999; 29:1239–1247.
- 14 Marzban G, Puehringer H, Dey R, *et al.* Localisation and distribution of the major allergens in apple fruits. *Plant Sci* 2005; 169:387–394.
- 15 Breiteneder H, Mills EN. Molecular properties of food allergens. *J Allergy Clin Immunol* 2005; 115:14–23.
- 16 Breiteneder H, Mills C. Structural bioinformatic approaches to understand cross-reactivity. *Mol Nutr Food Res* 2006; 50:628–632.
- Review focussing on the feasibility and added value of using structural bioinformatics approaches to predict IgE cross-reactivity of allergens.
- 17 Ahrazem O, Ibanez MD, Lopez-Torreson G, *et al.* Lipid transfer proteins and allergy to oranges. *Int Arch Allergy Immunol* 2005; 137:201–210.
- 18 Palacin A, Cumplido J, Figueroa J, *et al.* Cabbage lipid transfer protein Bra o 3 is a major allergen responsible for cross-reactivity between plant foods and pollens. *J Allergy Clin Immunol* 2006; 117:1423–1429.
- A report on the isolation of a new plant food nsLTP that may play an important role in food–pollen cross-reactivity.
- 19 Zuidmeer L, Salentijn E, Rivas MF, *et al.* The role of profilin and lipid transfer protein in strawberry allergy in the Mediterranean area. *Clin Exp Allergy* 2006; 36:666–675.
- 20 Asero R, Amato S, Alfieri B, *et al.* Rice: another potential cause of food allergy in patients sensitized to lipid transfer protein. *Int Arch Allergy Immunol* 2006; 143:69–74.
- 21 Gao ZS, Van de Weg WE, Schaart JG, *et al.* Linkage map positions and allelic diversity of two Mal d 3 (nonspecific lipid transfer protein) genes in cultivated apple (*Malus domestica*). *Theor Appl Genet* 2005; 110:479–491.
- 22 Lerche MH, Poulsen FM. Solution structure of barley lipid transfer protein complexed with palmitate. Two different binding modes of palmitate in the homologous maize and barley nonspecific lipid transfer proteins. *Protein Sci* 1998; 7:2490–2498.
- 23 Poznanski J, Sodano P, Suh SW, *et al.* Solution structure of a lipid transfer protein extracted from rice seeds. Comparison with homologous proteins. *Eur J Biochem* 1999; 259:692–708.
- 24 Shin DH, Lee JY, Hwang KY, *et al.* High-resolution crystal structure of the nonspecific lipid-transfer protein from maize seedlings. *Structure* 1995; 3:189–199.
- 25 Tassin-Moindrot S, Caille A, Douliez JP, *et al.* The wide binding properties of a wheat nonspecific lipid transfer protein. Solution structure of a complex with prostaglandin B2. *Eur J Biochem* 2000; 267:1117–1124.

- 26 Pasquato N, Berni R, Folli C, *et al.* Crystal structure of peach Pru p 3, the prototypic member of the family of plant nonspecific lipid transfer protein pan-allergens. *J Mol Biol* 2006; 356:684–694.
- The crystal structure of Pru p 3, the nsLTP that is still considered to be the primary sensitizer in most cases.
- 27 Garcia-Casado G, Pacios LF, Diaz-Perales A, *et al.* Identification of IgE-binding epitopes of the major peach allergen Pru p 3. *J Allergy Clin Immunol* 2003; 112:599–605.
- 28 Duffort OA, Polo F, Lombardero M, *et al.* Immunoassay to quantify the major peach allergen Pru p 3 in foodstuffs. Differential allergen release and stability under physiological conditions. *J Agric Food Chem* 2002; 50:7738–7741.
- 29 Zuidmeer L, Van Leeuwen WA, Kleine BI, *et al.* Lipid transfer proteins from fruit: cloning, expression and quantification. *Int Arch Allergy Immunol* 2005; 137:273–281.
- 30 Arilla MC, Gonzalez-Rioja R, Ibarrola I, *et al.* A sensitive monoclonal antibody-based enzyme-linked immunosorbent assay to quantify *Parietaria judaica* major allergens, Par j 1 and Par j 2. *Clin Exp Allergy* 2006; 36:87–93.
- 31 Van Ree R. Clinical importance of nonspecific lipid transfer proteins as food allergens. *Biochem Soc Trans* 2002; 30:910–913.
- 32 Pastorello EA, Vieths S, Pravettoni V, *et al.* Identification of hazelnut major allergens in sensitive patients with positive double-blind, placebo-controlled food challenge results. *J Allergy Clin Immunol* 2002; 109:563–570.
- 33 Schocker F, Luttkopf D, Scheurer S, *et al.* Recombinant lipid transfer protein Cor a 8 from hazelnut: a new tool for in vitro diagnosis of potentially severe hazelnut allergy. *J Allergy Clin Immunol* 2004; 113:141–147.
- 34 Fernandez-Rivas M, Bolhaar S, Gonzalez-Mancebo E, *et al.* Apple allergy across Europe: how allergen sensitization profiles determine the clinical expression of allergies to plant foods. *J Allergy Clin Immunol* 2006; 118:481–488.
- An important analysis of the sensitization to individual allergens linked to clinical data. This information may give an insight into the possible sensitization pathways and help evaluate the potential of allergens to induce mild or severe symptoms.
- 35 Brenna O, Pompei C, Ortolani C, *et al.* Technological processes to decrease the allergenicity of peach juice and nectar. *J Agric Food Chem* 2000; 48:493–497.
- 36 Asero R, Mistrello G, Roncarolo D, *et al.* Analysis of the heat stability of lipid transfer protein from apple. *J Allergy Clin Immunol* 2003; 112:1009–1011.
- 37 Scheurer S, Lauer I, Foetisch K, *et al.* Strong allergenicity of Pru av 3, the lipid transfer protein from cherry, is related to high stability against thermal processing and digestion. *J Allergy Clin Immunol* 2004; 114:900–907.
- 38 Schocker F, Luttkopf D, Muller U, *et al.* IgE binding to unique hazelnut allergens: identification of non pollen-related and heat-stable hazelnut allergens eliciting severe allergic reactions. *Eur J Nutr* 2000; 39:172–180.
- 39 Pastorello EA, Pompei C, Pravettoni V, *et al.* Lipid-transfer protein is the major maize allergen maintaining IgE-binding activity after cooking at 100 degrees C, as demonstrated in anaphylactic patients and patients with positive double-blind, placebo-controlled food challenge results. *J Allergy Clin Immunol* 2003; 112:775–783.
- 40 Pastorello EA, Farioli L, Pravettoni V, *et al.* Identification of grape and wine allergens as an endochitinase 4, a lipid-transfer protein, and a thaumatin. *J Allergy Clin Immunol* 2003; 111:350–359.
- 41 Garcia-Robaina JC, De la Torre-Morin F, Sanchez-Machin I, *et al.* Anaphylaxis induced by exercise and wine. *Allergy* 2001; 56:357–358.
- 42 Schad SG, Trcka J, Vieths S, *et al.* Wine anaphylaxis in a German patient: IgE-mediated allergy against a lipid transfer protein of grapes. *Int Arch Allergy Immunol* 2005; 136:159–164.
- 43 Asero R, Mistrello G, Roncarolo D, *et al.* A case of allergy to beer showing cross-reactivity between lipid transfer proteins. *Ann Allergy Asthma Immunol* 2001; 87:65–67.
- 44 Garcia-Casado G, Crespo JF, Rodriguez J, Salcedo G. Isolation and characterization of barley lipid transfer protein and protein Z as beer allergens. *J Allergy Clin Immunol* 2001; 108:647–649.
- 45 Akkerdaas JH, Wensing M, Asero R, *et al.* IgE binding to pepsin-digested food extracts. *Int Arch Allergy Immunol* 2005; 138:203–208.
- 46 Vassilopoulou E, Rigby N, Moreno FJ, *et al.* Effect of in vitro gastric and duodenal digestion on the allergenicity of grape lipid transfer protein. *J Allergy Clin Immunol* 2006; 118:473–480.
- In this study the stability of an allergen, grape LTP, was assessed for the first time in an in-vitro model of gastric and duodenal digestion, including the effect of a physiological surfactant, phosphatidylcholine.
- 47 Moreno FJ, Jenkins JA, Mellon FA, *et al.* Mass spectrometry and structural characterization of 2S albumin isoforms from Brazil nuts (*Bertholletia excelsa*). *Biochim Biophys Acta* 2004; 1698:175–186.
- 48 Sancho AI, Rigby NM, Zuidmeer L, *et al.* The effect of thermal processing on the IgE reactivity of the nonspecific lipid transfer protein from apple, Mal d 3. *Allergy* 2005; 60:1262–1268.
- 49 Perrocheau L, Bakan B, Boivin P, Marion D. Stability of barley and malt lipid transfer protein 1 (LTP1) toward heating and reducing agents: relationships with the brewing process. *J Agric Food Chem* 2006; 54:3108–3113.
- A paper assessing the influence of modifications and adducts on the stability of nsLTPs from barley and malt during brewing.
- 50 Asero R, Mistrello G, Amato S, *et al.* Peach fuzz contains large amounts of lipid transfer protein: is this the cause of the high prevalence of sensitization to LTP in Mediterranean countries? *Allerg Immunol (Paris)* 2006; 38:118–121.
- In this paper an alternative sensitization route for some peach allergic patients is suggested.
- 51 Marzban G, Mansfeld A, Herndl A, *et al.* Direct evidence for the presence of allergens in Rosaceae fruit tree pollen. *Aerobiologia* 2006; 22:237–245.
- These authors for the first time assessed the presence of allergens in fruit tree pollen, looking at a possible other source for sensitization to nsLTPs.
- 52 Sancho AI, Foxall R, Rigby NM, *et al.* Maturity and storage influence on the apple (*Malus domestica*) allergen Mal d 3, a nonspecific lipid transfer protein. *J Agric Food Chem* 2006; 54:5098–5104.
- In this study the influence of storage on nsLTP levels in fruit was determined.
- 53 Botton A, Vegro M, De Franceschi F, *et al.* Different expression of Pp-LTP1 and accumulation of Pru p 3 in fruits of two *Prunus persica* L. Batsch genotypes. *Plant Sci* 2006; 171:106–113.
- A comparison of the nsLTP content in two different peach fruit genotypes.
- 54 Brenna OV, Pastorello EA, Farioli L, *et al.* Presence of allergenic proteins in different peach (*Prunus persica*) cultivars and dependence of their content on fruit ripening. *J Agric Food Chem* 2004; 52:7997–8000.
- 55 Carnes J, Fernandez-Caldas E, Gallego MT, *et al.* Pru p 3 (LTP) content in peach extracts. *Allergy* 2002; 57:1071–1075.
- 56 Primavesi L, Brenna OV, Pompei C, *et al.* Influence of cultivar and processing on cherry (*Prunus avium*) allergenicity. *J Agric Food Chem* 2006; 54:9930–9935.
- A paper indicating the influence of processing on cherry nsLTP allergenicity, suggesting that chemical peeling may be a solution for some patients.
- 57 Carnes J, Ferrer A, Fernandez-Caldas E. Allergenicity of 10 different apple varieties. *Ann Allergy Asthma Immunol* 2006; 96:564–570.
- An interesting paper for the first time comparing different apple varieties using an in-vivo method.
- 58 Bolhaar ST, Van de Weg WE, van Ree R, *et al.* In vivo assessment with prick-to-prick testing and double-blind, placebo-controlled food challenge of allergenicity of apple cultivars. *J Allergy Clin Immunol* 2005; 116:1080–1086.
- 59 Reuter A, Lidholm J, Andersson K, *et al.* A critical assessment of allergen component-based in vitro diagnosis in cherry allergy across Europe. *Clin Exp Allergy* 2006; 36:815–823.
- In this paper the authors show for the first time that there are also cherry nsLTP-allergic patients outside the Mediterranean.
- 60 Egger M, Mutschlechner S, Wopfner N, *et al.* Pollen–food syndromes associated with weed pollinosis: an update from the molecular point of view. *Allergy* 2006; 61:461–476.
- An excellent review on weed–pollen allergens and associated crossreactive allergens from food, indicating the interesting new links between certain foods and pollen that may explain different nsLTP sensitization routes.
- 61 Figueroa J, Blanco C, Dumpierrez AG, *et al.* Mustard allergy confirmed by double-blind placebo-controlled food challenges: clinical features and cross-reactivity with mugwort pollen and plant-derived foods. *Allergy* 2005; 60:48–55.
- 62 Sanchez-Monge R, Blanco C, Lopez-Torrejon G, *et al.* Differential allergen sensitization patterns in chestnut allergy with or without associated latex–fruit syndrome. *J Allergy Clin Immunol* 2006; 118:705–710.
- A paper outlining the clinical differences in chestnut nsLTP allergic patients with or without associated latex allergy.
- 63 Garcia BE, Lombardero M, Echechipia S, *et al.* Respiratory allergy to peach leaves and lipid-transfer proteins. *Clin Exp Allergy* 2004; 34:291–295.