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Review

Clinical role of lipid transfer proteins in food allergy

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Lipid transfer proteins are widespread plant food allergens, highly resistant to food processing and to the gastrointestinal environment, which have recently been described as true food allergens in the Mediterranean area, where they have been associated with severe allergic reactions to foods in patients without pollen allergy. In this review we analyze their molecular structure, biological function, and clinical relevance in food allergy.

Keywords: Food allergy / Lipid transfer protein / Oral allergy syndrome / Panallergen / Review / Rosaceae fruits

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Contents

1 Introduction

Lipid transfer proteins (LTPs) and their biological role in plants have been known since 1975, when they were first described by Kader [1]. The importance of LTPs as food allergens was only recently understood: the first allergenic LTPs were described in Rosaceae fruits, particularly in those belonging to the Prunoideae subfamily (peach, apricot, cherry, and plum). In 1992, Lleonart *et al.* [2] identified a peach-specific low-molecular-mass allergen (around 10 kDa), preferentially located in the peel of the fruit, which was not sequenced. In 1994, Pastorello *et al.* [3] described a low-molecular-mass allergen (around 13 kDa) of peach, which bound the sera of 90% of the peach-allergic patients and was the sole allergenic protein recognized by those patients not sensitized to birch pollen; this allergen was cross-reactive with homologous proteins in other Prunoideae fruits (apricot, cherry, and plum) but not with birch or grass pollen allergens. The identification of a nonpollen-related allergen was a relevant finding, as up to that date it was commonly believed that allergy to Rosaceae was closely associated with birch pollinosis, maybe because the majority of the studies came from countries with significant prevalence of birch pollen allergy [4, 5]. Subsequent studies by Spanish authors confirmed the existence of a subgroup of patients allergic to Rosaceae fruits showing distinct clinical characteristics: in 1997, Fernandez-Rivas *et al.* [6] described a population of fruit-allergic patients from Central Spain who did not suffer from pollinosis, the majority of whom reported more severe symptoms than oral allergy syndrome (OAS); in this study, however, the responsible allergens were not sequenced. It was not until 1999 that Pastorello *et al.* [7] demonstrated that the previously described low-molecular-mass major allergen of peach was an LTP, responsible for severe systemic reactions in peach allergic patients both with concomitant birch pollinosis and without pollen allergy. This result was confirmed by Sanchez-Monge *et al.* [8], who demonstrated that LTP was the major allergen of peach and apple in a population of ten patients with clinical history of systemic reactions to both fruits. Subsequently Asero and co-workers [9 – 11], who demonstrated *in vitro* cross-reactivity between taxonomically unrelated plant-derived foods, hypothesized that LTP could be a relevant panallergen.

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Abbreviations: DBPCFC, double-blind placebo-controlled food challenge; **LTP**, lipid transfer protein; **OAS**, oral allergy syndrome; **SPT**, skin prick test

2 Structure and function of LTPs

Plant LTPs are small molecules containing 91 – 95 amino acid residues, with a molecular mass around 9 kDa and high isoelectric point. These highly homologous proteins belong to the so-called α-class proteins, which share a common three-dimensional structure, with four α-helices kept together by four disulfide bridges between eight cysteine residues at conserved positions [12]. The particular structure of LTPs – a bundle of four α-helices linked by flexible loops – has a functional role, as it provides a hydrophobic cavity which can accommodate a fatty acyl chain, allowing the binding of phospholipids and their transferring across membranes. Moreover, this three-dimensional structure is probably responsible for the extreme resistance of LTPs to physical and chemical treatments.

LTPs play a major role in the formation of the cellular membrane, by conveying phospholipids (particularly waxes and cutin) to the membrane; they also take part in plant defence, as they have potent antifungal and antibacterial properties [13, 14]. The first LTPs were described by an *in vitro* bioassay measuring the transfer of phospholipids from microsomes to mitochondria, which explains their name [1]. As LTPs have been found to be expressed in a variety of plant organs, with higher expression levels in the epidermal or peripheral cell layers of cutinized organs, it was hypothesized that they might be involved in the deposition of cutin monomers or other highly lipophilic substances. The preferred location of LTPs in outer cell layers may also be a clue to their role in repulsion or suppression of pathogenic attack from outside. This defensive function is confirmed by the fact that LTPs are induced by abscisic acid, a pleiotropic endogenous stress hormone produced by plants after wounding and in response to drought stress, low temperature, and osmotic stress. LTPs also show antimicrobial properties: their expression in plants is induced by fungal infection, and they are able to inhibit the growth of bacterial pathogens and fungi, maybe acting as membrane permeabilizing agents because of their basic character [15]. For this reason, LTPs are classified into the group of the so-called "pathogenesis-related proteins" (PRPs), a big family of inducible proteins that are produced by plants upon different stimuli, including pathogens (viruses, bacteria, fungi) and chemicals such as ethylene and salicylic acid that mimic the effects of pathogen infection. LTPs belong to the PRP group 14: all these proteins share common biochemical characteristics, like low molecular mass, basic character, stability at low pH, and resistance to proteolysis [16]. These features make LTPs "ideal" food allergens, capable of resistance to gastric pH and peptic digestion.

3 Route of sensitization

Allergic reactions to plant-derived foods in patients with OAS are usually due to sensitization to pollens which contain allergens cross-reacting with homologous molecules in foods. This cross-reactivity explains some clinical syndromes, like the birch-apple, mugwort-celery-spice, and latex-fruit syndrome [17 – 20]. In such cases, the primary sensitization takes place towards the inhalant allergens, and food allergy develops only secondarily as a result of a molecular cross-reactivity. In the birch-fruit syndrome, the patients are mostly sensitized to the major birch pollen allergen Bet v 1, and react to the Bet v 1 homologous proteins that are found in apple (Mal d 1), celery (Api g 1), carrot (Dau c 1), cherry (Pru av 1), and many other fruits or vegetables [21]. These homologues, however, are labile allergens, as they are rapidly destroyed by pepsin digestion, heat and also by the oxidative processes mediated by polyphenolic compounds that are released upon disruption of the plant tissues [10, 22, 23]; such allergens are only able to elicit symptoms in patients already sensitized by pollens, but are not able to act as primary sensitizers through ingestion in nonpollen-allergic subjects. More recently, studies from the Mediterranean area, where birch pollinosis is not as frequent as in Northern Europe, reported allergic reactions to plant-derived foods in nonpollen-allergic patients: in these patients, the most frequently involved allergens were LTPs, which behave as "true" food allergens, capable of sensitizing through the gastrointestinal tract [3, 6, 7, 9].

The lack of inhibition of immunoglobulin E (IgE) binding to peach, cherry, and hazelnut LTPs by birch pollen extract demonstrates that sensitization to LTP is not related to a previous sensitization to birch pollen [3, 24, 25]. The association of allergy to Rosaceae fruits with grass pollen sensitization is due to cross-reacting allergens other than LTPs, particularly profilin and carbohydrate cross-reactive determinants (CCDs) [26]; however, no cross-reactivity was demonstrated between grass pollen allergens and LTPs from plant foods like peach, maize or lettuce [3, 27, 28].

Although sensitization to LTPs is commonly believed to take place primarily by the oral route, the peculiar geographical distribution of LTP sensitization, which is frequent in the Mediterranean area and rare in Central and Northern Europe, led to the hypothesis of an environmental factor that favors LTP sensitization. Some studies considered the potential role of a primary sensitization to pollens predominantly grown in the Mediterranean area: wall pellitory, ragweed, mugwort, olive, and plane tree pollen were found to contain allergens homologous to LTPs [10, 29, 30], but only partial cross-reactivity was demonstrated between mugwort pollen LTP and LTPs from lettuce, peach, apple, and chestnut [27, 29, 31, 32], and between plane tree or Parietaria pollen LTP and lettuce LTP [27]. This low degree of cross-reactivity is probably due to a low sequence identity between pollen and food LTPs, with only few IgE-binding epitopes being shared. Recently, Pastorello *et al.* [31] demonstrated that allergy to LTP in food is not dependent on sensitization to LTP-containing pollens: no cross-reac-

tivity was found between peach and ragweed or peach and wall pellitory, while only partial cross-reactivity was demonstrated between peach and mugwort or peach and olive, with immunoblotting cross-inhibition experiments showing that the primary sensitizer was peach and not pollen. The authors thus concluded that monosensitization to mugwort LTP is only an epiphenomenon of sensitization to peach, which is responsible for the frequent RAST (radioallergosorbent test positivity to mugwort at low titer in peach-allergic patients, but never gives symptoms of pollinosis. More recently, latex has also been proposed as a potential cause of sensitization to LTP by the inhalative route [33].

4 Clinical relevance of LTP sensitization

LTPs have been found to be relevant allergens in many plant-related foods, particularly in those belonging to the Rosaceae family (Prunoideae, Pomoideae), but also in many others (Table 1): peach (Pru p 3) [7, 8], cherry (Pru av 3) [24], plum (Pru d 3) [34], apricot (Pru ar 3) [35], apple (Mal d 3) [8, 36], hazelnut (Cor a 8) [25], walnut (Jug r 3) [37], chestnut (Cas s 8) [29], grape (Vit v 1) [38], maize (Zea m 14) [28], asparagus (Aspa o 1) [39], and lettuce (Lac s 1) [27]. Cross-reactivity among LTPs from different plant foods has been widely documented: *in vivo* and *in vitro* cross-reactions have been demonstrated between the major peach allergen Pru p 3 and other allergenic LTPs in foods like cherry, apricot, plum [3], apple [8, 10], grape [38], chestnut [29], maize [10, 28], rice, walnut, hazelnut, and peanut [10, 11, 25]. This extensive cross-reactivity is probably due to the ubiquitous distribution of LTPs throughout the plant kingdom which makes sensitization to multiple foods very common in LTP-allergic patients, giving rise to the so-called "LTP syndrome". Moreover, LTPs have a highly conserved tertiary structure, even though the amino acid sequence identity of LTPs varies considerably among different plant genus. It is interesting to notice that IgE cross-reactivity between different LTPs is found when the amino acid sequence identity is quite high, as demonstrated for the Prunoideae fruits (about 90% sequence homology) [3], while no *in vitro* and *in vivo* cross-reactivity is found when considering LTPs with low sequence identity, like the major allergens of Parietaria pollen and peach (less than 30% sequence identity) [31].

The peculiar molecular characteristics of LTPs are responsible for the particular clinical picture of patients sensitized to them. Allergy to foods of plant origin is usually caused by cross-reactivity between pollen and vegetables or fruits: a typical model is the birch-fruit syndrome. This syndrome is characterized by symptomatic primary sensitization to birch pollen, with respiratory symptoms beginning months or years before the food-related symptoms, which are usually mild and limited to the oral mucosa (the so-called "oral allergy syndrome"). Both the route of sensitization (through inhalation) and the characteristic symptoms (local and mild) are presumably due to the lability of the allergens to digestion in the gastrointestinal tract. Moreover, the heat-sensitivity of pollen-related food allergens explains the lack of reactivity to cooked or processed foods.

Table 1. LTP allergens in inhalants and foods

Allergen name[a)]	Taxonomic name of allergenic source	Common name of allergenic source
Pollens		
Amb a 6	*Ambrosia artemisiifolia*	Short ragweed
Art v 3	*Artemisia vulgaris*	Mugwort
Par j 1	*Parietaria judaica*	Wall pellitory
Par j 2	*Parietaria judaica*	Wall pellitory
Par o 1	*Parietaria officinalis*	Wall pellitory
Cas s 8	*Castanea sativa*	Chestnut
Cor a 8	*Corylus avellana*	Hazel
Pla a 3	*Platanus acerifolia*	London plane tree
Ole e 7	*Olea europea*	Olive
Latex		
Hev b 12	*Hevea brasiliensis*	Rubber (latex)
Foods		
Zea m 14	*Zea mays*	Maize
Cor a 8	*Corylus avellana*	Hazelnut
Mal d 3	*Malus domestica*	Apple
Pru ar 3	*Prunus armeniaca*	Apricot
Pru av 3	*Prunus avium*	Sweet cherry
Pru d 3	*Prunus domestica*	European plum
Pru p 3	*Prunus persica*	Peach
Aspa o 1	*Asparagus officinalis*	Asparagus
Lac s 1	*Lactuca sativa*	Lettuce
Vit v 1	*Vitis vinifera*	Grape
Jug r 3	*Juglans regia*	English walnut

a) Based on IUIS nomenclature (www.allergen.org)

Reactivity to LTP in plant-derived foods gives rise to a completely different clinical picture: first of all, subjects sensitized to LTP are frequently nonpollen-allergic, thus suggesting that primary sensitization to pollens is not needed, as LTPs contained in foods pass intact through the gastrointestinal tract and are able to come into contact with the immune system and to elicit an IgE-mediated response [3, 6].

Another characteristic of LTP allergy is the high frequency of severe systemic reactions, which is in contrast with the predominance of local oral symptoms found in plant-allergic patients: the first authors who described this particular clinical feature were Fernandez-Rivas *et al.* [6]. Subsequently, several studies confirmed this observation, particularly those including double-blind placebo-controlled food challenge (DBPCFC), which is the gold standard for the diagnosis of food allergy. In a study on 76 Spanish patients with peach allergy confirmed by DBPCFC, 28 subjects reported systemic reactions [40]. An Italian study described

7 patients with severe anaphylactic reactions or positive DBPCFC results to maize, all sensitized to maize LTP [41]. In hazelnut allergy, almost all the patients with birch-pollen allergy develop exclusively an OAS after DBPCFC [42], while the majority of the patients sensitized to LTP without birch pollinosis experience systemic symptoms up to anaphylaxis [25, 43]. In cherry-allergic patients, monosensitization to the Bet v 1 homologous allergen Pru av 1 leads to mild symptoms, which in 55% of patients are localized to the oral cavity (OAS), while monosensitization to the cherry LTP Pru av 3 causes in three-quarters of the patients severe systemic reactions like urticaria/angioedema [44]. Asero *et al.* [9] demonstrated that, in a population of patients with OAS to Rosaceae fruits and nuts, the subgroup of patients sensitized to stable allergens like LTP had a 10 times higher incidence of systemic symptoms. Conversely, when considering among plant food-allergic patients the subgroup with more severe symptoms, the percentage of sensitization to LTP in this subgroup is very high: Sanchez-Monge *et al.* [8] demonstrated that of ten patients with urticaria/angioedema, asthma or glottis oedema caused by peach and apple, ten were sensitized to peach LTP and 9 to apple LTP. San Miguel-Moncin *et al.* [27] noted that five out of seven patients with anaphylaxis to lettuce were sensitized to LTP. Pastorello *et al.* reported that 86% of 22 patients with systemic symptoms to maize were sensitized to LTP [28], as well as 71% of 14 patients with severe systemic reactions to grape or wine [38]; moreover, they described seven Italian patients with severe anaphylactic reactions to hazelnut showing IgE reactivity to hazelnut LTP [25].

Sensitization to LTPs shows a peculiar geographical distribution, being more common in people from the Mediterranean area and less frequent in Northern Europe, where sensitization to plant-derived foods is secondary to tree pollen allergy. Ballmer-Weber *et al.* [44] analyzed two different populations of cherry-allergic patients: Swiss patients had all pollinosis symptoms for birch, and 92% of them were sensitized to the Bet v 1-homologous cherry allergen Pru av 1, while only one patient was sensitized to cherry LTP; Spanish patients were not sensitized to birch pollen but to plane or mugwort pollen, and showed reactivity to cherry LTP (89%) but not to Pru av 1. Scheurer *et al.* [24] found that LTP was recognized by only 3 out of 101 German patients with birch pollinosis and OAS to cherry, but was recognized by all the 7 Italian cherry-allergic patients. Pastorello *et al.* [25] in a multicenter study on hazelnut allergy, involving Northern and Southern European countries, demonstrated that only Italian patients without birch pollen allergy showed IgE reactivity to hazelnut LTP, while none of the birch-allergic patients from Copenhagen and Zürich was LTP-positive. In a Spanish population of 26 subjects allergic to hazelnuts and plane pollen without birch pollen allergy, LTP was the major allergen, recognized by 77% of patients, while only one patient recognized the Bet v 1-related hazelnut allergen Cor a 1 [43]. By contrast, a group of 53 German patients with allergy to both birch pollen and hazelnuts, recognized Cor a 1 as the major hazelnut allergen; only one patient, a young woman with severe allergic reactions after ingestion of hazelnuts without any association to tree pollen allergy, reacted to low-molecular-mass proteins below 10 kDa, which were not cross-reactive with birch pollen [45].

5 Resistance to treatments

Many studies demonstrated the resistance of LTPs to proteolysis, heat, chemicals, and oxidative processes. LTPs are stable even upon heating at very high temperatures: it has been demonstrated that peach LTP maintains its IgE binding capacity after heating at 121°C for 30 min [46] and that maize LTP maintains its lipid-transfer activity after a 5-min incubation at 90°C [12] and is still IgE-reactive after heating at 100°C for 160 min [41]; this explains why patients sensitized to LTP do not tolerate fruit juices and jams and experience very severe reactions when eating maize-based cooked products like tacos or the Italian dish polenta. Barley LTP is extremely heat-stable and resistant to processing [47]: it is able to survive malting and brewing processes and thus to become a beer allergen [48]. Apple LTP also shows great heat-stability: in LTP-sensitized apple-allergic patients, oral challenge with baked or boiled apple induces the same symptoms as oral challenge with raw apple; the skin prick tests (SPTs) are positive with both raw and cooked apple peel; IgE immunoblotting shows a band around 10 kDa in both raw and heat-processed apple extracts [49]. Pastorello *et al.* [25] demonstrated that sera from patients monosensitized to hazelnut LTP were IgE-reactive to both raw and roasted hazelnut, thus indicating that LTP is a heat-stable component in hazelnut. In a previous study, Schocker *et al.* [45] described a 10 kDa hazelnut allergen, probably corresponding to hazelnut LTP, which was demonstrated to be heat-stable by performing immunoblot experiments with extracts from raw and heated hazelnuts and EAST inhibition tests.

LTPs are resistant to the acidic and proteolytic conditions of the stomach: Asero *et al.* [10] demonstrated that after pepsin digestion peach LTP maintained its IgE binding capacity, while the peach homologue of Bet v 1 did not; Brenna *et al.* [46] found that peach LTP was still detectable by SDS-PAGE after treatment with proteolytic enzymes for 60 min. This allows LTP to reach the gastrointestinal tract in an almost unmodified form, being thus able both to sensitize through the oral route and to elicit symptoms that are not restricted to the oral cavity, but systemic.

Due to their role in plant defence, LTPs are found particularly in the outer layers of plant organs and fruits: it is

known that in peaches LTP concentration is about seven times greater in the skin than in the flesh, accounting for 25% of the total protein content of skin [50], and that peach skins are more allergenic than the flesh [51], so that chemical peeling of peach is up to now the only effective treatment to reduce peach allergenicity in industrial products [46].

6 New tools for diagnosis

In food allergy research the concept is gaining ground that different clinical pictures – severity of reactions, cross-reactivity patterns, and natural history of food allergy – are closely related to the sensitizing allergen. It has been demonstrated that LTPs have unique molecular and immunological characteristics, which make them able to sensitize nonpollen-allergic patients by ingestion, to cause extensive cross-reactions among botanically nonrelated foods, and to resist food treatments. Therefore, it is important to establish the allergenic recognition pattern of a patient sensitized to plant-derived foods. Unfortunately, the SPTs with commercially available extracts are often affected by false-positive results, due to clinically insignificant cross-reactions, and by false-negative results, due to lack of standardization of allergenic content. Some important allergens like LTPs may be completely absent in whole food extracts: Diaz-Perales *et al.* [39] demonstrated that IgE immunoblotting did not detect LTP in the crude asparagus extract because of its low concentration, but detected it in an LTP-enriched fraction from cation-exchange. Akkerdaas *et al.* [52] evaluated nine commercial hazelnut extracts used for SPT, and found that the total protein concentration and the concentrations of the single allergens (Cor a 1, lipid transfer protein, profilin, and thaumatin-like protein) differed by up to a factor of 100 among the analyzed extracts; LTP was not detected on immunoblot in three out of nine extracts.

The most recent studies use purified or recombinant allergens for the *in vitro* or *in vivo* diagnosis of food allergy: it is felt that this approach is far more sensitive and specific than the use of the whole food extracts, because the single pure allergens can be standardized in concentration and biological activity, and are much more stable than the antigens present in the food extract, because they are not in contact with other components of the plant matrix responsible for degradation. LTPs purified from peach and apple were used for skin prick testing of 47 Spanish patients with peach allergy: 91% of them had positive SPT to Pru p 3 and 77% to Mal d 3, and strong correlation was found between the skin responses to the fruit extract and the purified allergen [53]. Similarly, 20 out of 24 (83%) peach-allergic patients with positive SPT to peach skin extract had positive SPT results to the purified LTP [50]. Fernandez-Rivas *et al.* [40] also found that a positive result to SPT with purified Pru p 3 was related to the clinical expression of peach allergy, because it was found in 62% of patients with peach allergy confirmed by DBPCFC and in only 31% of patients whose history of reactions to peach was not confirmed by oral challenge.

Recombinant LTPs were used as diagnostic material for peach [54], cherry [24, 44], and hazelnut allergy [43]. Recombinant Pru p 3 shows immunological activity equivalent to its natural counterpart when considering IgE binding capacity, basophil activation, histamine release and sulphidoleukotriene production [54]. Ballmer-Weber *et al.* [44] found that in patients with cherry allergy confirmed by DBPCFC, the sensitivity of a positive SPT response to a panel of three recombinant allergens (two pollen-related allergens, Pru av 1 and the profilin Pru av 4, and one pollen-unrelated allergen, the LTP Pru av 3) was equal to that of a prick-by-prick test with cherry (96%), while the sensitivity of the SPT with commercial cherry extract was very low (20%). Similar results were reported *in vitro* by Scheurer *et al.* [24], who evaluated by enzyme-allergosorbent test (EAST) the IgE reactivity to the same three cherry recombinant allergens in 101 patients with a clinical history of OAS to cherry: the EAST was positive in 97% of patients, including three patients who had false-negative results to the fresh cherry extract, probably because of a low relative content of the relevant allergens in the whole protein extract.

7 Conclusions

The review of the literature highlights that LTPs are among the most important allergens in plant-derived foods. These low-molecular-mass basic proteins show a highly conserved three-dimensional structure, which is responsible for their resistance to gastric pH and peptic digestion and for their stability upon heating and processing of foods, making them ideal food allergens. LTPs from different allergenic sources show extensive IgE cross-reactivity, which is at the basis of multiple sensitizations to foods, the so-called "LTP syndrome". Some clinical features are characteristic of LTP allergy: compared to other patients allergic to the same fruits or vegetables, LTP-sensitized subjects show a higher prevalence of systemic symptoms up to severe anaphylaxis. In these subjects, sensitization to plant-derived foods is not dependent on sensitization to the LTP-containing pollens that typically cause hay fever (mugwort, ragweed, wall pellitory, olive, and plane tree); an open problem is whether other less common pollens might play a role, like the pollen of the same allergenic source (*e.g.*, maize pollen for patients allergic to maize grains). Finally, LTP allergy has a peculiar geographical distribution with predominance in the Mediterranean area, but the reason for this is still unknown: the role of genetic factors was not studied, while

the importance of dietary habits was suggested. In fact, LTP allergy is most frequently reported in countries like Italy or Spain, where consumption of LTP-containing foods is traditional: these countries are among the most important growers of peaches, which are consumed in high quantities for a long period of the year; moreover, maize-based products, containing an LTP highly resistant to treatments [41], are typical dishes in some Italian regions.

All the above mentioned characteristics make LTP allergy a typical and unique kind of food allergy, which needs to be appropriately diagnosed and studied. Currently new methods for the diagnosis of LTP sensitization are under development, but further studies are needed to evaluate their applicability in everyday practice.

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