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The Role of Lipid Transfer Proteins in Allergic Diseases

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Abstract Nonspecific lipid transfer proteins (LTPs) are important allergens in fruits, vegetables, nuts, pollen, and latex. Despite their wide distribution throughout the plant kingdom, their clinical relevance is largely confined to the Mediterranean area. As they can sensitize via the gastrointestinal tract, LPTs are considered true food allergens, and IgE reactivity to LTPs is often associated with severe systemic symptoms. Although Pru p 3 represents the predominant LTP in terms of patients' IgE recognition, the contribution of pollen LTPs in primary sensitization cannot be ruled out. Due to structural homology, LTPs from different allergen sources are generally IgE cross-reactive. However, sensitization profiles among allergic patients are extremely heterogeneous, and individual cross-reactivity patterns can be restricted to a single LTP or encompass many different LTPs. Molecule-based approaches in allergy research and diagnosis are important for better understanding of LTP allergy and could assist clinicians with providing adequate patient-tailored advice.

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Introduction

Based on structural and evolutionary relationships, seed storage proteins, inhibitors of α-amylase, and trypsin, as well as nonspecific lipid transfer proteins (LTPs) can be integrated into the huge protein superfamily of prolamins. Currently, this superfamily contains more than 3,000 proteins originating from more than 250 species mainly found in the plant kingdom [1]. Although individual members of this huge protein family display little sequence similarity, they share common structural features based on a conserved scaffold of eight cysteine residues. Given that almost 10% of currently known allergens feature this motif, the prolamin superfamily comprises the highest number of allergens [2]. Typically, members of the prolamin superfamily are important allergens in plant seeds. LTPs represent an exception, as they also have been isolated from nonseed plant tissues, such as fruits, leaves, roots, and pollen. Therefore, their impact on allergic disease goes far beyond plant food allergy [3]. Gene expression of LTPs is stimulated by biotic and abiotic plant stress, a typical trait of the diverse group of pathogenesis-related (PR) proteins. Interestingly, 50% of fourteen defined PR protein families contain allergens, among them nonspecific LTPs, also known as the PR-14 protein family [4].

To date, the International Union of Immunological Societies Allergen Nomenclature Subcommittee lists 39 allergenic LTPs originating from fruits (n=18), pollen of trees and weeds (n=9), vegetables (n=7), nuts and seeds (n=4), as well as latex (n=1) (Table 1). The present article

Table 1 Distribution of allergenic plant LTPs throughout the plant kingdom[a]

Botanical order	Allergen	Plant species	IUIS
Ingestants			
Fruits			
Caryophyllales	Hyl un LTP	*Hylocereus undatus* (dragonfruit)	–
Ericales	Act c 10	*Actinidia chinensis* (gold kiwi)	+
	Act d 10	*Actinidia deliciosa* (green kiwi)	+
	Vac m 3	*Vaccinium myrtillus* (whortleberry)	–
Myrtales	Pun g 3	*Punica granatum* (pomegranate)	–
Rosales	Fra a 3	*Fragaria ananassa* (strawberry)	+
	Mal d 3	*Malus domestica* (apple)	+
	Mor a 3	*Morus alba* (white mulberry)	–
	Mor n 3	*Morus nigra* (black mulberry)	+
	Pru ar 3	*Prunus armeniaca* (apricot)	+
	Pru av 3	*Prunus avium* (cherry)	+
	Pru d 3	*Prunus domestica* (plum)	+
	Pru p 3	*Prunus pérsica* (peach)	+
	Pyr c 3	*Pyrus communis* (pear)	+
	Ros r 3	*Rosa rugosa* (rose)	–
	Rub i 3	*Rubus idaeus* (raspberry)	+
Sapindales	Cit l 3	*Citrus limon* (lemon)	+
	Cit r 3	*Citrus reticulata* (tangerine)	+
	Cit s 3	*Citrus sinensis* (orange)	+
Solanales	Lyc e 3	*Lycopersicon esculentum* (tomato)	+
Vitales	Vit v 1	*Vitis vinifera* (grape)	–
Zingiberales	Mus a 3	*Musa acuminata* (banana)	+
Vegetables			
Apiales	Api g 2	*Apium graveolens* (celery)	+
	Dau c 3	*Daucus carota* (carrot)	–
	Pet c 3	*Petroselinum crispum* (parsley)	–
Asparagales	All c 3	*Allium cepa* (onion)	–
	Aspa o 1	*Asparagus officinalis* (asparagus)	+
Asterales	Lac s 1	*Lactuca sativa* (garden lettuce)	+
Brassicales	Bra o 3	*Brassica oleracea* (cauliflower)	+
Seeds			
Asterales	Hel a 3	*Helianthus annuus* (sunflower)	+
Brassicales	Sin a 3	*Sinapis alba* (white mustard)	+
Fabales	Ara h 9	*Arachis hypogaea* (peanut)	+
	Pha v 3	*Phaseolus vulgaris* (kidney bean)	+
Fagales	Cas s 8	*Castanea sativa* (chestnut)	+
	Cor a 8	*Corylus avellana* (hazel)	+
	Jug r 3	*Juglans regia* (walnut)	+
Lamiales	Ses i LTP	*Sesamum indicum* (sesame)	–
Poales	Hor v 14	*Hordeum vulgare* (barley)	–
	Ory s 14	*Oryza sativa* (rice)	–
	Tri a 14	*Triticum aestivum* (wheat)	+
	Tri s 14	*Triticum spelta* (spelt)	–
	Tri td 14	*Triticum turgidum* (durum)	–
	Zea m 14	*Zea mays* (maize)	+
Rosales	Pru du 3	*Prunus dulcis* (almond)	+

Table 1 (continued)

Botanical order	Allergen	Plant species	IUIS
Inhalants			
Pollen			
Asterales	Amb a 6	*Ambrosia artemisiifolia* (short ragweed)	+
	Art v 3	*Artemisia vulgaris* (English mugwort)	+
Brassicales	Ara t 3	*Arabidopsis thaliana* (Thale cress)	–
	Bra r 3	*Brassica rapa* (field mustard)	–
Lamiales	Ole e 7	*Olea europea* (olive tree)	+
Proteales	Pla a 3	*Platanus acerifolia* (plane tree)	+
	Pla or 3	*Platanus orientalis* (oriental plane tree)	+
Rosales	Par j 1	*Parietaria judaica* (pellitory)	+
	Par j 2	*Parietaria judaica* (pellitory)	+
	Par m 1	*Parietaria mauritanica* (pellitory)	–
	Par o 1	*Parietaria officinalis* (pellitory)	+
Leaves			
Urticales	Can s 3	*Cannabis sativa* (hemp)	–
Contactants			
Malpighiales	Hev b 12	*Hevea brasiliensis* (latex)	+
Rosales	Cot l 3	*Cotoneaster lacteus* (clusterberry)	–

[a] All currently known plant nonspecific LTPs entered in the Allergome database (http://www.allergome.org) are listed (n=58). LTPs can be found in 19 taxonomical plant orders and 57 different species. Allergens included in the IUIS Allergen Nomenclature Sub-Committee (http://www.allergen.org) are marked with a (+) (n=39); others are marked with a (–) (n=19)

IUIS International Union of Immunological Societies; *LTP* lipid transfer protein

provides an overview and update on the physicochemical and immunologic properties of these molecules and discusses their impact on the allergic patient.

Structure and Biological Function

Plant LTPs were found to be evolutionary distinct from those identified in animals and can be further divided into those that are specific for certain classes of phospholipids and those that can bind several classes of lipids (termed *nonspecific LTPs*). Only the latter class was shown to contain allergens.

Structure and Lipid-binding Capacity

According to their molecular masses, nonspecific LTPs can be classified into the 9-kDa LTP1 and the 7-kDa LTP2 subfamilies. To date, several three-dimensional structures from members of both subfamilies have been solved by nuclear magnetic resonance and x-ray crystallography. Although they differ in their amino acid sequence, they display a similar secondary structure composed of four α-helices and share a basic isoelectric point as well as the typical prolamin cysteine signature (C-X_n-C-X_n-CC-X_n-CXC-X_n-C-X_n-C) (Fig. 1) [5•]. The large internal, tunnel-like cavity of both LTP subfamilies can accommodate a variety of lipids through interaction with their hydrophobic parts. It was further demonstrated that lipid-binding cavities exhibit high plasticity and flexibility and vary among individual members of the LTP family. Small hydrophobic amino acids (eg, Ile, Val, Leu, and Ala) appear throughout the LTP1 sequences. Some of these residues play an important role in defining the hydrophobic tunnel, showing affinity for a variety of lipids (eg, phospholipids, glycolipids, fatty acids, and acyl coenzyme A). Interestingly, three-dimensional structures of family 1 LTPs from rice and wheat revealed simultaneous interactions with two lipid molecules bound in a "tail-to-tail" conformation. Although smaller, the cavity of family 2 LTPs has been reported to be more flexible, resulting in binding of a wider variety of lipids, including sterols [6]. A recent study showed that lipid binding to Par j 1 and Par j 2 did not cause structural alterations but rather protected the disulfide bond-stabilized structure from reduction by dithiothreitol [7]. Thus, binding of naturally occurring ligands may stabilize the fold and therefore contribute to the allergenic potential of LTPs.

Biological Function

LTPs mainly localize in epidermal plant tissues, and corresponding gene products are secreted and accumulate

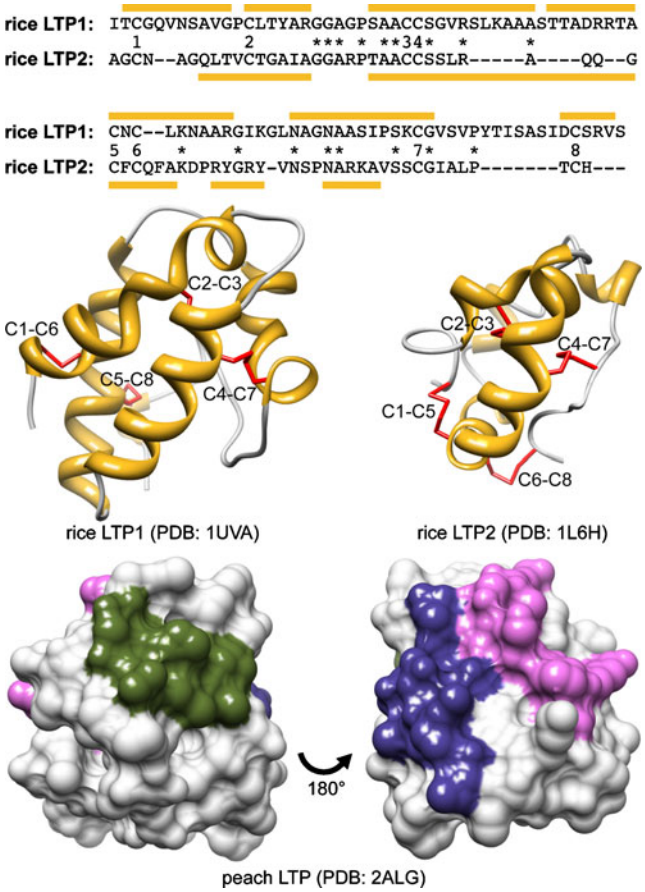

Fig. 1 Structural features of plant lipid transfer proteins (LTPs). Comparison of primary and tertiary structures of rice LTP1 and LTP2 reveals that LTP subfamilies display less than 30% sequence homology but share a similar α-helical fold (highlighted in yellow) that is stabilized by four disulfide bonds (depicted in red). Identical amino acids are indicated by asterisks, and cysteine residues showing the typical prolamin distribution pattern (C-X_n-C-X_n-CC-X_n-CXC-X_n-C-X_n-C) are numbered consecutively from 1 to 8. Linear IgE-binding epitopes of peach LTP are exposed to the allergen's surface and illustrated in green (Pru p $3_{11–20}$), blue (Pru p $3_{31–40}$), and purple (Pru p $3_{71–80}$), respectively. All models were obtained from the Protein Structure Database (http://www.pdb.org) and visualized using the program chimera (http://www.cgl.ucsf.edu/chimera)

outside the cell walls of aerial organs (LTP1) or roots (LTP2). Because of their lipid-binding capacities, LTPs have been suggested to be involved in the transport of hydrophobic monomers that compose the waxy and polymeric cutin and suberin layers of plant tissues found above and below the ground, respectively [8]. However, their extracellular localization contradicts the originally proposed role in intracellular trafficking of lipids. Thus, the actual in vivo function of this plant protein family remains unclear. Based on the finding of their ability to inhibit microbial growth in vitro, LTPs are reported to be involved in the defense against bacterial and fungal pathogens. This function is supported by the fact that LTP expression is increased upon pathogen challenge. Although exact mechanisms have not been revealed, it was speculated that LTP toxicity results from interaction with biological membranes, possibly leading to permeabilization and loss of membrane integrity. For example, sunflower LTP was demonstrated to induce weak leakage of model membranes (liposomes) and is able to provoke permeabilization of intact spores of the pathogenic fungus *Fusarium solani* [9]. In addition to biotic factors, abiotic stressors such as drought, cold, and high salinity have been reported to upregulate LTP production in some plant species [5•]. Due to their role in plant defense, LTPs have been classified as PR-14 protein members [4].

Allergological Relevance and Distribution

Although the biological function of plant LTPs is not completely elucidated, their conservation during evolution and expansion throughout the plant kingdom indicates an involvement in vital processes. Allergens displaying such properties are frequently responsible for IgE cross-reactions, even between unrelated pollen and plant food allergen sources, and therefore were classified as "eurallergens," emphasizing their wide distribution [3]. However, IgE sensitization to LTPs is strongly influenced by geographic aspects and may largely depend on differences in eating habits or patterns of pollen exposure.

Geographic Distribution

Plant Food Lipid Transfer Proteins

In the Mediterranean area, LTPs are major allergens from *Rosaceae* spp fruits, as well as different vegetables and nuts. Among food-related LTPs (n=36), Pru p 3 represents the most studied in terms of epidemiology. Pru p 3-positive tests range between 4.6% and 100%, depending on selection criteria, geographic location, and number of participants (Table 2). Barber et al. [10] reported 13% positive Pru p 3 test results among 785 Spanish study participants. An epidemiologic study ranked Pru p 3 16th among 75 tested allergens on a microarray chip and first among all food-related allergens, with a sensitization prevalence of 9.8% [11]. Large screenings of *Rosaceae* fruit allergic patients from different countries showed that in Northern and Central Europe, where birch trees are abundant, allergy to apple and cherry seems to be linked to food homologues of Bet v 1, the birch pollen major allergen. By contrast, in the Mediterranean area, allergy to *Rosaceae* fruits is related to peach allergy and sensitization to LTPs [12]. Notably, LTPs essentially concentrate in the pericarp of fruits, whereas the pulp contains lower amounts [13]. This explains why some patients can more easily tolerate fruits after peeling. Protein levels of LTPs vary

Table 2 Epidemiologic data on LTP sensitization

Allergen	Prevalence[a]				Studies[b]					
	Lowest		Highest		Barber et al. [10]		Ciardiello et al. [21]		Scala et al. [11]	
	Patients, *n*	%	Patients, *n*	%	Patients, *n*	%	Patients, *n*	%	Patients, *n*	%
Ingestants										
Ara h 9	12	16.7	31	90	NA		NA		NA	
Aspa o 1	18	62.5	16	50	NA		NA		NA	
Bra o 3	34	52.9	17	94.1	NA		NA		NA	
Cas s 8	20	15	47	23.4	NA		NA		NA	
Cit l 3	NA		27	51.8	NA		NA		NA	
Cit s 3	28	25	27	48.1	NA		NA		NA	
Cor a 8	65	0	17	71	NA		4,297	5.7	NA	
Dau c 3	NA		34	61.8	NA		NA		NA	
Fra a 3	NA		17	29.4	NA		NA		NA	
Hor v 14	NA		4	100	NA		NA		NA	
Jug r 3	NA		46	78.3	NA		NA		NA	
Lac s 1	NA		14	71.4	NA		NA		NA	
Mal d 3	20	5	47	76.6	NA		NA		NA	
Mor n 3	NA		26	88.5	NA		NA		NA	
Pru ar 3	NA		30	100	NA		NA		NA	
Pru av 3	108	3	7	100	NA		NA		NA	
Pru d 3	NA		23	82.6	NA		NA		NA	
Pru p 3	22	4.6	31	100	786	13	4,297	12.3	16,408	9.8
Sin a 3	NA		15	60	NA		NA		NA	
Tri a 14	34	11.8	31	100	NA		NA		NA	
Vit v 1	14	71.4	13	100	NA		NA		NA	
Zea m 14	NA		22	86.4	NA		NA		NA	
Inhalants										
Amb a 6	9	11	101	41.6	NA		NA		NA	
Art v 3	22	0	9	88.9	NA		4,297	7.7	NA	
Hev b 12	31	0	37	24.3	NA		NA		NA	
Ole e 7	786	14.9	134	55.2	786	14.9	NA		NA	
Par j 1	24	29.2	22	95	NA		NA		16,408	25.7
Par j 2	37	0	50	85.7	786	8.3	4,297	15.7	16,408	22.2
Pla a 3	NA		58	51.7	NA		NA		NA	

[a] The range of LTP sensitization prevalence is given according to data obtained from http://www.allergome.org

[b] Three epidemiologic studies enrolling >500 patients are reported

LTP lipid transfer protein; *NA* not available

depending on maturity, storage conditions, and cultivar of the fruit [14]. However, studies that would determine a threshold level for LTP sensitization for triggering allergic reactions are still lacking.

Plant food LTPs are also present in cutin-free plant tissues (eg, roots), which primarily express members of the LTP2 subfamily. Although both LTP1 and LTP2 proteins can be found in plant seeds, thus far, only LTP1 proteins have been characterized as allergens. In fact, the official allergen list of the International Union of Immunological Societies currently contains no entries for allergens belonging to the LTP2 subfamily. Recently, Ara h 9 has been described as major allergen in peanut-allergic patients from the Mediterranean area [15].

Studies on hazelnut allergy scored Cor a 8 between 0% and 71%, and among 4,297 Italian study participants, 5.7% tested positive for Cor a 8 in a microarray system (Table 2). Although most data on LTPs were gathered from the Mediterranean area, scattered reports show LTP reactivity worldwide, although at a very low prevalence [16]. For

example, a study evaluating sensitization to LTPs in Dutch children concluded that sensitization to Cor a 8 is a risk factor for severe symptoms in response to ingestion of hazelnut not only in the Mediterranean but also in birch-endemic areas [17].

Pollen Lipid Transfer Proteins

In addition to plant-derived foods, LTPs also constitute important pollen allergens of plant species native to the Mediterranean basin (eg, pellitory [18], olive [10], plane [19], and mugwort [20]). Among inhaled LTPs causing respiratory symptoms, the highest prevalence has been reported for Par j 1 and Par j 2 from *Parietaria* spp, ranging from 0% to 85%, depending on patient selection criteria and countries (Table 2). A large study using simplex IgE testing in a Spanish population (n=786) reported a prevalence rate of 8.3% for Par j 2 [10]. According to Scala et al. [11], the frequency of IgE reactivity to Par j 2 and Par j 1 measured by the Immuno Solid-phase Allergen Chip in a very large Italian cohort of unselected allergic participants (n=16,408) is 25.7% and 22.2%, respectively, ranking these molecules sixth and ninth among 75 tested allergens. Prevalence of Art v 3 sensitization was reported to range from 0% to 88.9%. A recent study reported a sensitization rate of 7.7% to Art v 3 in an unselected cohort of 4,297 allergic patients [21]. Concerning Ole e 7, Barber et al. [10] reported a prevalence rate of almost 15% among 786 participants. Pollen LTPs from ragweed and plane tree have been reported positive from 11% to 41% and 51.7%, respectively (Table 2). However, as only a few studies on a limited number of selected patients are available for these pollen LTPs, the prevalence is thought to be lower. Interestingly, LTPs also have been detected in the pollen of *Rosaceae* trees, thus constituting an additional source of exposure [22••].

The Sensitizer Question

Ever since LTPs were identified as important allergens, a longstanding debate about the sensitizing molecule began, emphasizing a predominant role for peach LTP. In fact, IgE reactivity to any LTP under investigation is largely accompanied by Pru p 3 sensitization. Although some cases of IgE reactivity to pollen without concomitant Pru p 3 recognition have been described [19], the high frequency of sensitization and allergenicity renders Pru p 3 a marker molecule for LTP allergy. LTPs are generally considered true or class 1 food allergens that are able to sensitize via the gastrointestinal tract but also have been discussed in the context of class 2 food allergies due to IgE cross-reactivity between homologous pollen and food allergens. Typically, pollen allergens act as primary sensitizers in class 2 food allergies. However, in the case of LTPs, this picture is rather ambiguous. For example, in mugwort peach allergy, both Pru p 3 and Art v 3 have been suggested as the primary sensitizer, depending on selection of the study population [20, 23]. According to current knowledge, no evidence suggests a clinical association between sensitization to LTP from food and pollen. Still, it has been suggested that exposure to olive, pellitory, plane tree, and mugwort in the absence of birch pollen, as well as fruit consumption at a young age might facilitate the development of and in parallel explain the geographic restriction of LTP allergy [12, 24]. This suggestion is supported by the observation that the risk of sensitization to apple LTP seems to be decreased in patients suffering from birch pollinosis, whereas it is increased in patients with mugwort or plane pollen allergy [12]. In addition to ingestion and inhalation, skin contact has been proposed as a third route of LTP exposure. For example, the high allergen content in peels and cutin layers of fresh fruits has been proposed to be responsible for transdermal sensitization and peach-induced urticaria [25]. However, sensitization to Hev b 12 was shown to have no clinical relevance in contact allergy to natural rubber latex [26].

In summary, LTPs can be considered as widely distributed and cross-reactive “eurallergens” capable of sensitizing via the gastrointestinal and respiratory tracts as well as potentially across the skin. The clinical significance of LTPs is mainly confined to the Mediterranean area, where they represent major allergens and are particularly responsible for allergies to plant-derived foods.

Antigenic and Allergenic Determinants

T-cell Epitopes

Recently, two independent studies identified T-cell epitopes of Pru p 3 using T-cell lines from Spanish and Italian patients [27•, 28]. Despite some slightly controversial results, residues 61 to 80 were identified in both studies. In contrast to other plant food and pollen allergens, Pru p 3-specific T-cell clones demonstrated low production of interleukin-10 and a high expression of integrin β_7, indicating that those cells were presumably primed in the gut [27•]. Although data on T-cell cross-reactivity are still missing, one could speculate that homologous LTPs present in various foodstuffs might be able to stimulate preexisting T cells, as was shown for Bet v 1-related food allergens [29].

B-cell Epitopes

Determination of linear IgE-binding epitopes using overlapping synthetic peptides yielded valuable information

primarily for Pru p 3. As depicted in Fig. 1, three major epitopes were localized at residues 11 to 25, 31 to 45, and 71 to 80, which mainly corresponded to positively charged regions on the molecule's surface [30]. In addition, residues R39, T40, and R44 were found to be critical for IgE recognition, as a Pru p 3 triple mutant displayed decreased antibody reactivity. A later study showed that LTPs of apple, apricot, and plum share consensus epitopes, which presumably accounts for pronounced IgE cross-reactivity between these molecules [31]. Interestingly, the sequence within residues 16 to 24 was found to be highly conserved among certain LTPs and seems to represent a *Rosaceae*-specific epitope. A consensual IgE-binding epitope (residues 39–44) in LTPs from various sources was predicted using a computational algorithm [32]. To determine conformational epitopes, mimotope mapping using a phage display library was applied for Pru p 3. The resulting mimotope recognized by patients with oral allergy syndrome was composed of $L_{37}R_{39}T_{40}P_{42}D_{43}R_{44}A_{46}P_{70}S_{76}Y_{79}$, whereas the mimotope for systemic patients consisted of $N_{35}N_{36}L_{37}R_{39}T_{40}D_{43}A_{46}S_{76}P_{78}$ [33]. Apart from the fact that LTP symptom severity obviously cannot be fully explained by recognition of different epitopes, identified residues are also involved in the formation of linear allergenic determinants. As another example, allergenic epitopes of the wheat flour LTP were mapped presenting similar epitopes as described for Pru p 3 mimotopes [34]. However, two different sequential epitopes were identified pointing at a specific sensitization toward the allergen source. In addition, the major *Parietaria* allergens Par j 1 and Par j 2 were shown to contain five and eight IgE epitopes, respectively, three of which were similar on both molecules [18]. Taken together, clear evidence indicates that LTPs contain both linear and conformational IgE epitopes. Although LTPs are extremely heat stable, the allergenicity of those molecules can be reduced or abolished upon excessive heat treatment [35]. Particularly at neutral buffer conditions, the fold of Pru p 3 was shown to be irreversibly denatured [36]. In addition, the allergological relevance of the disulfide bond-stabilized structure was demonstrated with cysteine mutants of Par j 1 [37].

IgE Cross-reactivity

Prediction of antibody cross-reactivity relies on simple comparison of linear sequence stretches or on the three-dimensional structure. Allergenic members of the LTP family usually demonstrate moderate primary sequence identities ranging from 45% to 65% but are well above the currently defined cross-reactivity threshold of 35%. However, the highly conserved three-dimensional structure gives rise to additional epitopes involved in IgE cross-reactivity. Because sensitization and thus cross-reactivity show an extremely complex pattern, some examples of IgE cross-reactivity demonstrated for LTPs from plant foods and pollen are presented in the following sections.

Cross-reactivity Between Foods

A high degree of IgE cross-reactivity has been observed for allergenic LTPs within the *Rosaceae* family [38], including the potent sensitizer molecule Pru p 3. In fact, most of the known LTPs to date are members of this botanical family showing high sequence identity. Recently identified allergenic LTPs from various sources (eg, mulberry [21], peanut [15], mustard [39], lettuce [40], green bean [41], or cabbage [42]) all demonstrated in vitro cross-reactivity with Pru p 3. Although some variability exists among patients and in the degree of cross-reactivity, most previous studies showed that Pru p 3 is the most potent inhibitory molecule, while inhibition to Pru p 3 by other LTPs can only be partially achieved. In general, it seems that this molecule possesses more epitopes and/or epitopes with higher IgE-binding affinity compared with other LTPs [43]. Moreover, Pru p 3 also demonstrates a higher mediator release capacity in biological assays for most investigated patients' sera. However, a weaker potency was found in a subgroup of lettuce allergic patients, suggesting a different source of sensitization [40].

Cross-reactivity Between Pollen and Food

The first evidence that LTPs from pollen and food sources demonstrate in vitro cross-reactivity came from studies that enrolled peach and mugwort LTP sensitized individuals. However, depending on different study populations, Pru p 3 [23] or Art v 3 [20] was considered the primary sensitizing molecule. A correlation between Art v 3 and Cas s 8 was found, as a positive skin prick test to chestnut LTP was only observed in Art v 3 sensitized patients [38]. The cabbage allergen, Bra o 3, was also found to cross-react with Art v 3 in inhibition experiments [42]. A study by Lauer et al. [19] showed that plane pollen LTP may also elicit allergic symptoms independent of preexisting Pru p 3 sensitization and may represent the sensitizing molecule in at least a subgroup of plane pollen allergic individuals [19]. Although Par j 1 and Par j 2 are considered members of the LTP family, they seem to play a unique role within this allergen group, with an evident restriction to *Parietaria* pollen allergy. In inhibition studies, Par j 2 demonstrates rather low or absent cross-reactivity with other allergenic LTPs [21, 44]. This may result from their low sequence identity (<35%) as well as structural differences due to longer protein sequences.

Clinical Picture of Lipid Transfer Protein Allergy

A broad spectrum of symptoms can be caused by ingestion or inhalation of LTPs, with severity ranging from rather mild to anaphylactic reactions. However, LTP sensitization without clinical translation also has been observed.

Symptoms Upon Ingestion

In most cases, the ingestion of plant food LTPs causes symptoms in the oral cavity. However, it became evident from very early reports on these allergens that they are also capable of eliciting severe generalized reactions. Unlike other allergen groups (eg. Bet v 1-related food allergens or profilins), LTPs can be responsible for severe reactions upon the ingestion of fresh as well as processed foods. In fact, it was shown that LTPs retain their allergenic activity during processing; thus, even cooked food, fruit juices, jams, and fermented drinks still display IgE-binding activity [22••]. It is noteworthy that LTPs were the only detectable allergens in different commercial tomato products [45]. In addition, several studies have demonstrated the resistance of LTPs to proteolysis by pepsin [22••], while gastric and duodenal digestion was assessed in detail for Vit v 1 [46]. Stability under such conditions is an important feature for class 1 food allergens, which can elicit systemic reactions and sensitize via the gastrointestinal tract. The remarkable physicochemical properties of LTPs render them potent elicitors of allergic reactions, including anaphylaxis. However, individuals do not necessarily react to all LTPs to which they are exposed. In the Italian population, most patients sensitized to Pru p 3 show clinical reactivity solely upon peach ingestion, whereas other fruits do not cause symptoms. In contrast, some Pru p 3-positive patients do react to a broad number of LTP-containing food sources. In between these two conditions is a wide range of clinical manifestations rendering the prediction of symptoms and diet recommendation difficult.

Symptoms Upon Inhalation

Individuals monosensitized to *Parietaria* pollen extract are now recognized to have IgE toward the two major allergens, Par j 1 and Par j 2. Due to the long-lasting pollen season causing the highest level of allergen exposure among allergenic pollen, up to 60% of the sensitized patients are affected by asthma symptoms associated with rhinoconjunctivitis [47]. Little is known about the relevance of other pollen LTPs (eg, Art v 3 or Pla a 3) in causing respiratory symptoms because patients often also display IgE antibodies against other allergens (eg, Art v 1, Pla a 1, and Pla a 2) from the same plants. To define the clinical profile of inhalant LTPs, the strategy could be to select sensitized patients who do not react to different and concurrent allergens.

Diagnostic Options and Treatment of Lipid Transfer Protein Allergic Patients

Diagnosis

Routine clinical allergy diagnosis using commercially available extracts is hampered by the fact that products are not routinely standardized on LTPs. Thus, those molecules may be present at different amounts or totally absent as a consequence of extraction procedures, leading to false-negative results. The currently most reliable way to diagnose sensitization to LTP is based on purified molecules using, for example, microarray systems. At present, several LTPs have been successfully produced in *Escherichia coli* and *Pichia pastoris*. Recombinant allergens resembling the natural molecule have the advantage of being free of contaminating allergens from the same source. In general, molecule-based approaches result in the best patient profiles and help to decide whether to exclude/include certain plant-derived foods to prevent unnecessary diet restrictions.

Therapeutic Approach

Allergen avoidance and pharmacologic therapy remain the pillars to prevent allergic reactions to food LTPs. However, two studies using extracts with quantified LTPs have been published dealing with immunotherapy of hazelnut and peach LTP allergic patients [48, 49••]. Such preliminary studies pave the way to food allergy treatment. In this context, a European Union-funded project assessing the safety and efficacy of Pru p 3-based immunotherapy is currently in its second year. Three centers in Greece, Italy, and Spain are enrolling patients under very stringent selection criteria to determine the feasibility of a treatment for these highly affected patients. In general, consumption of carrots, potatoes, bananas, and melons was found to be safe for LTP allergic individuals [50]. Regarding the treatment of patients allergic to pollen LTPs, specific immunotherapy is performed with extracts and was shown to be clinically efficient.

Conclusions

The prevalence of allergic disease has been drastically increasing during recent decades in industrialized countries. This has been paralleled by fundamental environmental changes, such as increased pollution, reduced microbial burden, or altered socioeconomics. Many of those environ-

mental changes may constitute abiotic stressors for plants and therefore influence expression and allergenicity of PR proteins. As a consequence, augmented exposure to LTPs could directly influence the frequency of sensitizations. However, this cannot explain the restriction of clinically relevant LTP allergy to the Mediterranean basin. Evidently, pollen of the predominant allergenic plants native to this area contains LTPs, whereas such pollen is rarely found in other geographic regions. In addition to Pru p 3, pollen LTPs should be considered more seriously with regard to the question of the primary sensitizer. Due to complex and individual sensitization patterns of LTP allergic patients, studies clearly addressing this topic are still missing. Although sensitization to LTPs displays a broad range of clinical manifestations, it is linked to severe anaphylactic symptoms in the Mediterranean population. In contrast to conventional allergy tests using extracts, molecule-based diagnosis allows the simultaneous testing of many allergenic LTPs, revealing patient-specific sensitization profiles. Such profiles would be useful for the clinician in providing adequate dietary advice and provide the basis for future therapeutic approaches.

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- • Of importance
- •• Of major importance

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