

Anaphylaxis to Wheat Flour-Derived Foodstuffs and the Lipid Transfer Protein Syndrome: A Potential Role of Wheat Lipid Transfer Protein Tri a 14

Arantxa Palacin^a Joan Bartra^b Rosa Muñoz^b Araceli Diaz-Perales^a
Antonio Valero^b Gabriel Salcedo^a

^aDepartamento de Biotecnología, E.T.S. Ingenieros Agrónomos, Universidad Politécnica, Madrid,

^bUnitat d'Al·lèrgia, Servei de Pneumologia i Al·lèrgia Respiratòria, Hospital Clínic, Universitat de Barcelona, Barcelona, España

Key Words

Wheat food allergy • Lipid transfer protein • Tri a 14 •
Anaphylaxis • Pollen food cross-reactions • Peach Pru p 3

Abstract

Background: Food allergy to wheat-derived foodstuffs is on the rise. Tri a 14, a wheat flour lipid transfer protein (LTP) allergen, has been described as a major allergen associated with baker's asthma and wheat food allergy. Cross-reactivity among LTP allergens leads to the so-called 'LTP syndrome'.

Methods: Eight adult patients showing anaphylaxis after ingestion of wheat-derived foodstuffs were selected. A homemade wheat extract, purified natural (n) and recombinant (r) Tri a 14, and peach fruit and *Artemisia* pollen LTP allergens Pru p 3 and Art v 3 were subjected to skin prick test, specific IgE determination (ELISA) and IgE immunodetection assays.

Results: All tests were positive in the 8 selected patients with the homemade extract. Positive skin prick test responses to nTri a 14, Pru p 3 and Art v 3 were found in 5/8, 6/8 and 4/4 patients, respectively. Specific IgE determined by ELISA assays was detected in 6 to nTri a 14 and rTri a 14, in 4 to Pru p 3 and in 3 to Art v 3 out of 8 individual sera tested, whereas all these sera showed IgE binding to nTri a 14 and Pru p 3 in immunodetection after SDS-PAGE separation. **Conclusions:** Tri a 14 seems to be a relevant allergen in patients with ana-

phylaxis after ingestion of wheat flour foodstuffs, according to in vitro and in vivo results. Clinical history of the analyzed patients, together with sensitization to peach Pru p 3 and *Artemisia* pollen Art v 3, suggests that 6 of them suffer from LTP syndrome.

Copyright © 2009 S. Karger AG, Basel

Introduction

Wheat is among the 3 cereal crops that dominate world agriculture, providing a high fraction of the dietary supply of protein in western countries [1]. Consequently, the high consumption of wheat-derived foodstuffs has led to its association with food allergies in children [2], and recently, also in adult patients [2–6]. A wide array of wheat flour proteins, both salt-soluble proteins and prolamins, and particularly members of the α -amylase/trypsin inhibitor family and ω 5-gliadin (in wheat-dependent exercise-induced anaphylaxis), have been linked to food wheat allergy [3, 5, 7–9]. Several of these allergen groups have also been involved in baker's asthma, an important occupational disease caused by inhalation of cereal, especially wheat, flours [2, 7]. Lipid transfer proteins (LTPs) are among these allergen families (wheat flour Tri a 14) recently identified as a relevant allergen associated with

Table 1. Clinical symptoms to wheat-derived foodstuffs and sensitization to other plant foods and pollens of the selected patients

Patient No.	Age years/sex	Symptoms to wheat ¹	Other sensitizations ²		Anaphylaxis after ingestion of other plant foods
			pollens	plant foods	
1	44/F	An (1)	pl, ar, ch	ma, pe, ha, mu, le, kw	ha
2	53/F	An (2)	pl, ar, ch, pa	ma, mu, le	le
3	21/F	An (3)	pl, ar, ch, pa	ma, pe, ha, mu, le, kw	pe, ha, le
4	20/M	An (3)	pl, ar, pa	ma, pe, ha, mu, le, kw	pe, ha, le
5	22/M	An (1, 3)	pl, ar, ch	ma, pe, ha, mu, le, kw	pe, le
6	36/F	An (4)	pl, ar, ch	ma, pe, ha, le, kw	ha
7	34/M	An (3, 4)	ol	no	no
8	52/M	An (5)	pl, ar, ch	no	no

¹ Anaphylaxis (An) induced by ingestion of whole bread (1), whole biscuit (2), pizza (3), bread (4), bread, pizza or biscuit associated with exercise (5).

² Positive SPT responses to commercial extracts of pollens, including plane tree (pl), *Artemisia* (ar), *Chenopodium* (ch), *Parietaria* (pa) and olive (ol), or of plant foods, including maize (ma), peach peel (pe), hazelnut (ha), mustard (mu), lettuce (le), kiwi (kw), or not detected (no).

both baker's asthma [10, 11] and wheat IgE-mediated food allergy [3, 5].

LTPs are small (around 9 kDa) and basic polypeptides, which form a panallergen family identified in several plant foods (Rosaceae fruits, nuts, vegetables and cereal seeds) and pollens [12]. Allergenic LTPs, mainly peach fruit Pru p 3, are particularly important in the Mediterranean adult population [13, 14] and have been proposed as model of true food allergens, based on their resistance to digestive proteases and induction of severe and systemic symptoms in many patients [12, 15]. Besides being sensitizers by the oral route, an increasing body of experimental data indicates that some LTPs can also act as inhalant allergens from cereal flours (Tri a 14) or pollens [12, 16].

A wide cross-reactivity among food LTP allergens has been found [17, 18], and therefore, sensitization to multiple foods is common in LTP-allergic patients, leading to the so-called 'LTP syndrome' [19]. Furthermore, an immunological relationship between Tri a 14 (inhalant –) and Pru p 3 (food –) allergens has been described in baker's asthma patients, as well as between Pru p 3 and cabbage Bra o 3 and pollen LTPs, particularly Art v 3 from *Artemisia* [17, 20, 21] and Pla a 3 from plane tree [22].

We report sensitization to Tri a 14, determined by in vivo and in vitro methods, in most of the 8 patients with anaphylaxis to wheat flour-derived foodstuffs, who showed a clinical history and food and pollen sensitization profiles suggestive of LTP syndrome.

Materials and Methods

Patients and Sera

Eight patients with anaphylaxis after ingestion of wheat flour-derived products (bread, pizza and/or biscuit) were selected from a bulk of 180 subjects diagnosed of plant food allergy during the period 2007–2009 at the Unitat d'Allèrgia, Hospital Clínic, Barcelona, Spain. All selected patients showed positive skin prick test (SPT) response and specific IgE to a homemade wheat extract (see below; table 1). Additionally, total IgE determined by the Pharmacia CAP System FEIA (Phadia, Uppsala, Sweden) and SPT responses to commercial extracts (ALK-Abello, Madrid, Spain) from pollens (grass, *Artemisia*, *Chenopodium*, *Parietaria*, olive, *Cupressus* and plane tree) and plant foods (maize, peach peel, hazelnut, mustard, lettuce, kiwi and lentil) were also measured in the 8 selected subjects. Written informed consent was obtained from all patients, and the study was approved by the Committee on Ethics of the Hospital Clínic, Barcelona, Spain.

A pool of sera from the 8 selected patients was used in IgE immunodetection assays and each individual serum in direct ELISA and immunodetection test.

Wheat Flour Extract and Purified Allergens

A homemade wheat extract (0.1 M Tris-HCl, pH 7.5, 10 mM EDTA) of ground and defatted whole seeds of *Triticum aestivum* (cultivar Astral) was obtained as previously described [10].

Natural (n) and recombinant (r) Tri a 14 were produced and isolated as in Palacin et al. [10, 11], and nPru p 3 and rPru p 3, as well as nArt v 3, following Diaz-Perales et al. [17, 23]. Isolated proteins were quantified by the commercial bicinchoninic acid test (Pierce, Cheshire, UK). Purity of the isolated allergens was checked by protein staining after SDS-PAGE and N-terminal amino acid sequencing.

Table 2. SPT responses and specific IgE to wheat extract, crude gliadin and purified natural and recombinant wheat Tri a 14, natural peach fruit Pru p 3 and natural *Artemisia* pollen Art v 3

Patient No.	SPT response, mm					Specific IgE							
	WE	gliadin	nTri	nPru	nArt	ELISA ¹ , OD units					IgE immunodetection		
						WE	nTri	rTri	nPru	nArt	nTri	rTri	rPru
1	3	–	6	10	7	0.48	0.23	0.27	–	0.55	+	+	+
2	5	–	6	3	n.d.	0.24	0.26	0.28	–	–	+	+	+
3	5	–	–	7	n.d.	0.33	–	–	1.23	0.62	+	+	+
4	6	–	3	10	6	0.38	0.32	0.30	0.35	0.25	+	±	+
5	4	–	3	7	6	0.34	–	–	0.58	–	+	+	+
6	6	–	3	13	6	0.35	0.30	0.28	0.23	–	+	±	+
7	9	–	–	–	n.d.	0.46	0.46	0.36	–	–	+	+	+
8	9	7	–	–	n.d.	0.92	0.44	0.50	–	–	+	+	+
Positive n	8	1	5	6	4	8	6	6	4	3	8	6	8
%	100	12	62	75	100	100	75	75	50	37	100	75	100

WE = Wheat extract; – = negative; n.d. = not done; ± = weak band (fig. 1).

¹ Values are means (n = 3); the cutoff value = 0.21 OD units (see Materials and Methods).

Skin Prick Test

SPTs with the homemade wheat extract [2 mg/ml of protein in a 1:1 (v/v) PBS buffer/glycerol solution], a commercial crude gliadin preparation (1 mg/ml; Leti, Madrid, Spain) and purified natural Tri a 14, Pru p 3 and Art v 3 (20 µg/ml) were performed by standard procedures. Histamine dihydrochloride (10 mg/ml) and PBS buffer/glycerol solution served as positive and negative controls, respectively. A mean wheal diameter of ≥3 mm (15 min after puncture) compared with that of the negative control was considered a positive response.

Specific IgE Determination

The 8 individual sera (1:2 dilution) from wheat-allergic patients were tested by a direct ELISA assay previously reported [11, 23] to determine specific IgE to wheat extract (15 µg/ml in solid phase) and purified nTri a 14, rTri a 14, nPru p 3 and nArt v 3 (5 µg/ml each in solid phase). Blocking solution (Sigma, St. Louis, Mo., USA) without solid phase [n = 6; mean ± SD = 0.15 ± 0.02 optical density (OD) units] was used as negative control, and OD values >0.21 OD units (mean + 3 × SD of negative control) were considered positive (table 2).

IgE Immunodetection Assays

The wheat extract (15 µg) and purified nTri a 14, rTri a 14 and nPru p 3 (3 µg each) were separated by SDS-PAGE on Bio-Rad Miniprotein III System gels (15% polyacrylamide), according to the method of Laemmli [24]. Separated samples were then electrotransferred to polyvinylidene difluoride membranes. After blocking, membranes were incubated overnight with a serum pool or each individual serum (1:3 dilution) from wheat-allergic patients, washed and then treated with goat anti-human IgE-peroxidase conjugate (1:3,000 dilution; Sigma). Detection of IgE-binding components was achieved by chemiluminescence, ac-

cording to the manufacturer's instruction (Amersham Biosciences, Little Chalfont, UK).

Statistical Analysis

Correlation between specific IgE levels to the different allergens tested was analyzed by one-way intraclass correlation coefficient (ICC). The comparison and the correlation were performed at a significant level of p < 0.05. All tests were performed using SPSS statistical software version 12 for Windows (SPSS Inc., Chicago, Ill., USA).

Results

Clinical Characteristics of Selected Patients

Eight selected patients were adults (mean age 35.2 years, range 20–53; 4 females/4 males), all of them with anaphylaxis after ingestion of wheat flour-derived foodstuffs, such as whole or flour bread, pizza or biscuit (table 1). Interestingly, 6 patients (Nos. 1–6 in table 1) had also suffered anaphylactic episodes after consumption of plant foods, such as hazelnut (Nos. 1, 3, 4 and 6), lettuce (Nos. 2–5) and peach (Nos. 3–5). In contrast, patients No. 7 and 8 (the only case of wheat-dependent exercise-induced anaphylaxis in the selected group) were not allergic to other plant-derived foods. Accordingly, with the clinical symptoms, all patients were sensitized to wheat, and most of them to maize (n = 6), lettuce (n = 6), peach (n = 5) and hazelnut (n = 5; table 1). Mustard (n = 5) and kiwi

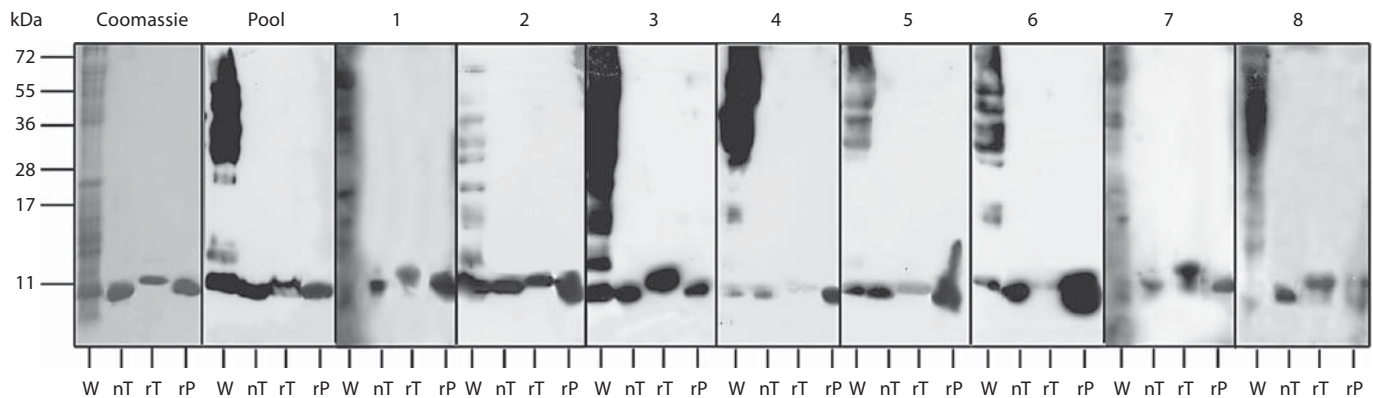


Fig. 1. Protein staining (Coomassie) and IgE immunodetection with a serum pool or individual sera (Nos. 1–8, as in tables 1 and 2) from wheat-food-allergic patients of the following samples separated by SDS-PAGE: homemade wheat extract (W), purified natural (nT) and recombinant (rT) Tri a 14, and recombinant Pru p 3 (rP).

($n = 5$) were other prevalent food sensitizations in the group of selected patients.

Regarding pollen sensitizations, almost all subjects ($n = 7$) showed positive SPT responses to plane tree and *Artemisia* (table 1).

Specific IgE and in vivo Response to LTP Allergens

ELISA assays allowed to detect specific IgE to the wheat extract in all patients, and in 6 of them (75%), to natural and recombinant Tri a 14 (table 2). The immunological equivalence of both wheat allergen forms was supported by their similar IgE-binding capacity ($ICC = 0.932$; $p < 0.001$). Additionally, significant specific IgE levels to natural peach Pru p 3 and *Artemisia* pollen Art v 3 were found in 4/8 and 3/8 patients, respectively. However, no correlation was found between the IgE levels to nPru p 3 and nTri a 14 ($ICC = -0.544$; $p = 0.894$). These results were further confirmed and extended by IgE immunodetection experiments (fig. 1). The 8 individual sera detected a low-molecular-size band in the wheat extract, which most probably corresponded to Tri a 14. Besides, other IgE-binding components with an apparent molecular mass between 12 and 90 kDa were also recognized, indicating sensitization to other allergens included in the extract. In fact, the relative intensity of the putative Tri a 14 band was weaker than that corresponding to some of these additional IgE-binding components in some individual sera. However, the strong recognition of purified nTri a 14 in all sera (except in patient No. 4), and of nPru p 3 in all cases, pointed to the high IgE reactivity of LTP allergens.

In vivo responses (SPTs; table 2) supported sensitization to wheat flour of the selected patients (100% positive with the homemade wheat extract) and the reactivity to LTP allergens in most of them: 5/8 to nTri a 14, 6/8 to nPru p 3 and 4/4 to Art v 3. In contrast, only patient No. 8 showed a positive response to the gliadin preparation assayed.

Discussion

Wheat food allergy in adult patients has been considered a minor and rare IgE-mediated hypersensitivity, in contrast with baker's asthma, noticed as an important occupational disease among bakery workers [2, 7, 25]. However, different European clinical groups have reported a substantial number of adult subjects with allergy to wheat flour-derived products, including those showing wheat-dependent exercise-induced anaphylaxis, in the last years [3–6, 26].

On the other hand, extensive clinical, immunochemical and biochemical evidence produced during the last decade has highlighted the pivotal role of LTP allergens in plant food allergy, particularly in the adult population of Southern European countries [5, 12–14, 19]. The presence of cross-reactive allergenic members of this protein family in commonly consumed plant foods, and even in some pollens, seems to be the molecular basis of a complex pattern of multiple sensitizations to foods in some patients, known as 'LTP syndrome' [12, 18, 19]. Wheat is not among the main foods considered in this syndrome, but recent data point to its involvement in LTP sensitiza-

tion profiles. Thus, Tri a 14 has been identified as a major allergen associated with baker's asthma in Spanish patients, and its partial cross-reactivity with peach Pru p 3 was uncovered [10, 11]. More important, sensitization to wheat LTP has been detected in more than 50% of Italian subjects with reactions to wheat-containing products [5].

Six of the 8 selected patients recruited in the current study display a paradigmatic sensitization profile related to the LTP syndrome. Besides a reaction to wheat flour-derived foodstuffs, 6 of them showed anaphylaxis after ingestion of hazelnut, lettuce and/or peach. Accordingly, positive SPT responses to hazelnut, lettuce and peach, together with maize, mustard and kiwi, have been obtained in most patients. Relevant LTP allergens have been defined in almost all these plant foods, namely hazelnut Cor a 8 [27], walnut Jug r 3 [28], lettuce Lac s 1 [29], peach Pru p 3 [30] and maize Zea m 14 [31]. Mustard cross-reactions with cabbage, peach and *Artemisia* pollen probably also implicated allergenic LTPs [21], whereas no member of the family has been located in kiwifruit at present [32]. Pollen sensitizations of the selected patients complete this scenario: 7 out of 8 reacted in SPTs with plane tree and *Artemisia*, the only 2 pollens clearly linked with Pru p 3 cross-reactivity and the LTP syndrome [12, 20–22].

Interestingly, only 1 positive SPT response (patient No. 8) to a commercial crude gliadin preparation was found in the selected subjects. This patient was the only one showing an anaphylaxis clearly associated with exercise. These results suggest that most patients were not sensitized to gliadins, which have been identified as relevant allergens in other groups of patients with wheat food allergy [5, 7], and are in line with the well-known role of ω 5-gliadin in wheat-induced exercise-induced anaphylaxis [7, 26].

Joint consideration of the in vitro and in vivo results (table 2; fig. 1) clearly demonstrated sensitization to wheat Tri a 14 in all selected patients. Furthermore, these results, together with the well-established association between LTP allergens and severe and systemic symptoms [12, 13, 15, 19], strongly suggested the implication of Tri a 14 in their clinical reactions. Specific IgE to the natural allergen was detected in 100 and 75% of patients by IgE immunodetection and ELISA assays, respectively. Positive SPT responses to nTri a 14 were obtained in 62% of patients, in line with the known differential sensitivity of different diagnostic assays. Sensitization was confirmed by testing rTri a 14, which further supports the immunological equivalence of both natural and recombinant forms of Tri a 14, previously reported in adults with bak-

er's asthma [11]. The high correlation of IgE levels to both allergen forms (ICC = 0.932) reported here points to the potential use of rTri a 14 as a molecular tool for diagnosis of wheat food allergy as well. However, it should be mentioned that IgE immunodetection assays indicate a lower reactivity to the recombinant form comparing with that to nTri a 14 in the same sera, in agreement with the slight decrease in IgE-binding potency previously found for rTri a 14 [11].

Besides the potential role of Tri a 14 in patients with anaphylaxis after ingestion of wheat-containing foodstuffs, the data presented allow at least 2 additional conclusions. Firstly, IgE immunoblots of the wheat extract reveal a band corresponding to wheat LTP in all individual sera, but also several IgE-binding components of higher molecular sizes, indicating that other wheat allergens are involved in the allergic response. Some of these allergens could be concerned in non-LTP-mediated cross-reactions between wheat and other plant foods and pollens included in the sensitization profiles of the studied patients, such as proteases recently found in kiwi/wheat cross-reactivity [33]. However, preliminary data obtained from the ISAC microarray assay (Phadia; J. Bartra et al., unpubl.) on 3 sera (Nos. 3, 4 and 5) used in the current study show a positive reaction with only the true LTP allergens (Pru p 3, Art v 3 and Cor a 8) and none with the 5 wheat allergens (or any other from plant sources) included in the array. Secondly, specific IgE determination and SPT results indicate that most patients were also sensitized to peach fruit Pru p 3 and *Artemisia* pollen Art v 3, 2 of the prototypic allergens inducing 'LTP syndrome' [12, 20, 21], and commonly involved in plant foods or pollen, foods cross-reactions in LTP-sensitized subjects. These data fit the clinical characteristic shown in table 1, as well as the 'LTP syndrome' proposed for the patients included in this report. Taking into account all these data, the possible sensitization to Tri a 14 in some patients by cross-reactivity with food (i.e., Pru p 3, Zea m 14, and others) or pollen (Pla a 3 or Art v 3) LTP homologous allergens, which could act as primary sensitizers, has to be considered.

Acknowledgements

We thank Dr. P. Gamboa (Hospital de Basurto, Bilbao, Spain) for assistance with the statistical analysis. Financial support was provided by the Instituto de Salud Carlos III, Redes de Investigación Cooperativa (RIRAFF; ref RD07/0064/03) and Ministerio de Ciencia e Innovación (Project BIO 2009-07050), Spain.

References

- Shewry PR: Wheat. *J Exp Bot* 2009;60:1537–1553.
- Palouso K: Update on wheat hypersensitivity. *Curr Opin Allergy Clin Immunol* 2003;3: 205–209.
- Battais F, Courcoux P, Popineau Y, Kanny G, Moneret-Vautrin DA, Denery-Papini S: Food allergy to wheat: differences in immunoglobulin E-binding proteins as a function of age or symptoms. *J Cereal Sci* 2005;42:109–117.
- Scibilia J, Pastorello EA, Zisa G, et al: Wheat allergy: a double-blind, placebo-controlled study in adults. *J Allergy Clin Immunol* 2006;117:433–439.
- Pastorello EA, Farioli L, Conti A, et al: Wheat IgE-mediated food allergy in European patients: α -amylase inhibitors, lipid transfer proteins and low-molecular-weight glutenins. *Int Arch Allergy Immunol* 2007;144:10–22.
- Salvatori N, Reccardini F, Convento M, et al: Asthma induced by inhalation of flour in adults with food allergy to wheat. *Clin Exp Allergy* 2008;38:1349–1356.
- Tatham AS, Shewry PR: Allergens in wheat and related cereals. *Clin Exp Allergy* 2008; 38:1712–1726.
- Sanchez-Monge R, Gomez L, Barber D, et al: Wheat and barley allergens associated with baker's asthma. Glycosylated subunits of the α -amylase-inhibitor family have enhanced IgE-binding capacity. *Biochem J* 1992;28: 401–405.
- James JM, Sixbey JP, Helm RM, et al: Wheat alpha-amylase inhibitor: a second route of allergic sensitization. *J Allergy Clin Immunol* 1997;99:239–244.
- Palacin A, Quirce S, Armentia A, et al: Wheat lipid transfer protein is a major allergen associated with baker's asthma. *J Allergy Clin Immunol* 2007;120:1132–1138.
- Palacin A, Varela J, Quirce S, et al: Recombinant lipid transfer protein Tri a 14: a novel heat and proteolytic resistant tool for the diagnosis of baker's asthma. *Clin Exp Allergy* 2009;39:1267–1276.
- Salcedo G, Sanchez-Monge R, Barber D, Diaz-Perales A: Plant non-specific lipid transfer proteins: an interface between plant defence and human allergy. *Biochim Biophys Acta* 2007;1771:781–791.
- Fernández-Rivas M, González-Mancebo E, Rodríguez-Pérez R, et al: Clinically relevant peach allergy is related to peach lipid transfer protein, Pru p 3, in the Spanish population. *J Allergy Clin Immunol* 2003;112:789–795.
- Asero R, Antonicelli L, Arena A, et al: Epide-mAAITO: features of food allergy in Italian adults attending allergy clinics: a multi-centre study. *Clin Exp Allergy* 2009;39:547–555.
- van Ree R: Clinical importance of non-specific lipid transfer proteins as food allergens. *Biochem Soc Trans* 2002;30:910–913.
- Zuidmeer L, van Ree R: Lipid transfer protein allergy: primary food allergy or pollen/food syndrome in some cases. *Curr Opin Allergy Clin Immunol* 2007;7:269–273.
- Diaz-Perales A, Lombardero M, Sanchez-Monge R, et al: Lipid-transfer proteins as potential plant panallergens: cross-reactivity among proteins of *Artemisia* pollen, *Castanea* nut and Rosaceae fruits, with different IgE-binding capacities. *Clin Exp Allergy* 2000;30:1403–1410.
- Asero R, Mistrello G, Roncarolo D, et al: Immunological cross-reactivity between lipid transfer proteins from botanically unrelated plant-derived foods: a clinical study. *Allergy* 2002;57:900–906.
- Pastorello EA, Robino AM: Clinical role of lipid transfer proteins in food allergy. *Mol Nutr Food Res* 2004;48:356–362.
- 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.
- 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.
- Lauer I, Miguel-Moncin MS, Abel T, et al: Identification of a plane pollen lipid transfer protein (Pla a 3) and its immunological relation to the peach lipid-transfer protein, Pru p 3. *Clin Exp Allergy* 2007;37:261–269.
- Diaz-Perales A, Sanz ML, Garcia-Casado G, et al: Recombinant Pru p 3 and natural Pru p 3, a major peach allergen, show equivalent immunologic reactivity: a new tool for the diagnosis of fruit allergy. *J Allergy Clin Immunol* 2003;111:628–633.
- Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680–685.
- Baur X, Degens PO, Sander I: Baker's asthma: still among the most frequent occupational respiratory disorders. *J Allergy Clin Immunol* 1998;102:984–997.
- Lehto M, Palosuo K, Varjonen E, et al: Humoral and cellular responses to gliadin in wheat-dependent, exercise-induced anaphylaxis. *Clin Exp Allergy* 2003;33:90–95.
- 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.
- Pastorello EA, Farioli L, Pravettoni V, et al: Lipid transfer protein and vicilin are important walnut allergens in patients not allergic to pollen. *J Allergy Clin Immunol* 2004;114: 908–914.
- San Miguel-Moncin M, Krail M, Scheurer S, et al: Lettuce anaphylaxis: identification of a lipid transfer protein as the major allergen. *Allergy* 2003;58:511–517.
- Sánchez-Monge R, Lombardero M, García-Sellés FJ, Barber D, Salcedo G: Lipid-transfer proteins are relevant allergens in fruit allergy. *J Allergy Clin Immunol* 1999;103:514–519.
- Pastorello EA, Farioli L, Pravettoni V, et al: The maize major allergen, which is responsible for food-induced allergic reactions, is a lipid transfer protein. *J Allergy Clin Immunol* 2000;106:744–751.
- Palacin A, Rodriguez J, Blanco C, et al: Immunoglobulin E recognition patterns to purified kiwifruit (*Actinidia deliciosa*) allergens in patients sensitized to kiwi with different clinical symptoms. *Clin Exp Allergy* 2008;38:1220–1228.
- Palacin A, Quirce S, Sánchez-Monge R, et al: Allergy to kiwi in patients with baker's asthma: identification of potential cross-reactive allergens. *Ann Allergy Asthma Immunol* 2008;101:200–205.