

R. ASERO

In patients with LTP syndrome food-specific IgE show a predictable hierarchical order

Ambulatorio di Allergologia, Clinica San Carlo, Paderno Dugnano (MI), Italy

KEY WORDS

Food allergy; lipid transfer proteins; specific IgE

Corresponding author

Riccardo Asero
Ambulatorio di Allergologia
Clinica San Carlo
Via Ospedale 21
20037 Paderno Dugnano (MI), Italy
Phone: +39 02 990 38 470
Fax: +39 02 990 38 223
E-mail: r.asero@libero.it

Summary

Background. Lipid transfer protein (LTP) is a widely cross-reacting allergen in plant foods. **Objective.** To assess whether IgE to vegetable foods show predictable trends in LTP allergic patients. **Methods.** Clinical allergy to plant foods other than peach was sought in 15 consecutive peach-allergic adults monosensitized to LTP. IgE specific for peach, apple, hazelnut, walnut, peanut, lentil, maize, soybean, tomato, sesame, mustard melon, kiwi, and celery as well as to mugwort pollen was measured. **Results.** Peach-specific IgE levels exceeded IgE to all other study foods. The higher were peach-specific IgE levels, the higher was the probability that other plant-derived foods scored positive. Mean IgE levels specific for all study foods were strongly correlated to peach specific IgE. Food-specific IgE followed a rather precise hierarchy, both in terms of number of positive in-vitro tests and of IgE levels, with apple at the second place after peach, followed by walnut, hazelnut, peanut, lentil, maize, soybean, tomato, kiwi, sesame, mustard, melon, and celery. Such hierarchy was not necessarily paralleled by clinical allergy as lentil, maize, and soybean scored positive in the majority of patients, but induced allergy in 0, 1, and 0 patients, respectively. IgE levels were not necessarily correlated with the severity of clinical allergy. Little or no IgE reactivity to mugwort pollen was found. **Conclusion.** In LTP syndrome, IgE reactivity to foods other than peach is in most cases predictable and follows a regular sequence that probably depends on the degree of homology with Pru p 3. The reasons why some foods are tolerated by most patients despite elevated IgE reactivity remains to be elucidated.

Introduction

Lipid transfer protein (LTP), the major food allergen in the Mediterranean basin, is widely distributed throughout the plant kingdom. LTPs from distinct plant-derived foods show a moderate to high degree of cross-reactivity, due to a sequence homology ranging between 35% and 95% (1). Most allergists agree that the peach is the primary source of sensitization to this protein, and dominates the immune response to this allergen (2,3), although a possible influence by sensitization to LTP present in some pollens, particularly

those of plane tree and mugwort has been suggested (3-5). The clinical spectrum of LTP allergy ranges from patients allergic uniquely to the peach to patients allergic to a large array of fruits and vegetables. Previous studies found that the level of IgE specific for peach LTP, Pru p 3, is critical for the occurrence of cross-reactivity to botanically related (*Rosaceae*) and unrelated plant foods (6,7), although subsequent investigations were unable to detect sharp cutoff levels able to discriminate allergic patients from subjects with clinically irrelevant sensitization (8). However, it has to be considered that the reactivity of the patients varies with the amount of

allergen ingested, the affinity of IgE antibodies, the exposure to other allergens, the degree of processing and storage of food stuff. Whether the IgE levels to botanically distinct plant-derived foods show predictable trends in LTP-allergic patients has not been investigated so far. In the present study, IgE specific for several different vegetable foods were measured in parallel in patients sensitized to LTP.

Methods

The study was carried out on sera from 15 consecutive peach-allergic adults (M/F 5/10; mean age 34,2 years, range 19-78 years) seen at the allergy outpatient service of the Clinica San Carlo and diagnosed as being most probably monosensitized to lipid transfer protein. The diagnosis of peach allergy was based on a history of oral allergy syndrome (defined as the occurrence of oral itching with or without angioedema of the lips and/or tongue, some minutes after the ingestion of a food), urticaria with or without angioedema, anaphylaxis, digestive symptoms, and/or respiratory symptoms (rhino-conjunctivitis and/or asthma) following the ingestion of peach associated with an unequivocally positive SPT with fresh peach by the prick-prick technique. The diagnosis of probable monosensitization to LTP was based on positive SPT with a commercial peach extract (ALK-Abellò, Madrid, Spain) in the absence of skin reactivity to a commercial extract of birch pollen (Allergopharma, Rainbeck, Germany; 50 000 SBU/ml) and to a commercial date palm pollen extract enriched in profilin (ALK-Abellò; 50 µg protein/ml), thus ruling out sensitization to the other two main cross-reacting plant food pan-allergens, namely PR-10 and profilin. In previous studies, the commercial peach extract was found to react exclusively to LTP-hypersensitive patients and did not induce any skin reaction in patients sensitized either to Pru p 1, the peach allergen homologous to the major birch pollen allergen Bet v 1, or to Pru p 4, the peach profilin (9). This extract contains 30µg/ml of peach LTP, Pru p 3. The clinical allergy to other plant-derived foods was ascertained by interview using the same criteria defined above. It was also ascertained whether patients ate regularly the foods that they reported to tolerate.

IgE specific for peach, apple, hazelnut, walnut, peanut, lentil, maize, soybean, tomato, sesame, mustard melon, kiwi, and celery was measured in sera from all patients by ImmunoCAP FEIA (Thermo-Fisher Phadia, Uppsala, Sweden) following manufacturer's recommendations. Further, IgE specific for mugwort pollen, a pollen that has been regarded as a possible source of LTP sensitization (3,4,5), was measured. Levels were expressed as kUA/L; levels exceeding 0.35 kUA/L were regarded as positive. The correlation between peach-specific IgE levels and levels of IgE specific for all other study foods was assessed

using Pearson's coefficient method. A probability value < 5% was considered statistically significant.

Since the study was retrospective and based on routine investigations performed on patients spontaneously presenting at the clinic for suspect food allergy, no institutional ethical permission was needed.

Results

IgE measurement results are shown in **table 1**. As expected, all patients scored positive for the peach and peach-specific IgE levels were higher than IgE specific for all other study foods in all patients but two showing high specific IgE levels, in whom apple IgE were slightly higher than peach IgE. Further, the higher were peach-specific IgE levels, the higher was the probability that other plant-derived foods scored positive. In effect, the mean IgE levels specific for all study foods except kiwi were strongly correlated to peach specific IgE levels (correlation coefficient r ranging from 0.814 for peanut to 0.996 for apple; $p < 0.001$ for all foods). Kiwi IgE levels also were statistically correlated with peach IgE levels ($p < 0.01$) but the correlation coefficient was lower ($r = 0.701$). Food-specific IgE followed a rather precise hierarchy both in terms of number of positive in-vitro tests and of specific IgE levels, with apple at the second place after peach, followed by walnut, hazelnut, peanut, lentil, maize, soybean, tomato, kiwi, sesame, mustard, melon, and celery (**figure 1**). The offending foods in study patients are shown in **table 2**. Although clinical allergy was more frequently induced by Rosaceae and nuts, who also showed the highest levels of IgE in most patients, the IgE hierarchy was not necessarily associated with clinical allergy to the single foods; in fact, lentil, maize, and soybean scored positive in the majority of patients but only 1 patient had a history of maize allergy, whereas lentil and soybean were tolerated by all patients, just as was sesame that scored positive in 5 cases. Further, allergen specific IgE levels were not necessarily correlated with the severity of clinical allergy, with patients showing very high specific IgE levels and only local symptoms (e.g., patient # 2 in **tables 1** and **2**), and patients with low specific IgE levels and a history of systemic reactions to foods (e.g., patient # 14 in **table 1** and **2**). Finally, in patient # 7 who had a clinical history of allergy to hazelnut, peanut, tomato, and melon the ImmunoCAP failed, scoring negative for all these 4 foods, in the face of extremely strong reactions on SPT with commercial extracts (mean wheal diameter 8 mm, 11.5 mm, 12 mm, and 15.5 mm, respectively).

IgE reactivity to mugwort pollen was detected in sera from 8/15 patients. In 7/8 cases mugwort IgE levels were by far lower than peach specific IgE (**table 1**). Seven patients did not show any mugwort reactivity.

Table 1 - Level of IgE to the study foods measured in 15 patients monosensitized to LTP.

Patients	Peach	Apple	Walnut	Hazelnut	Peanut	Lentil	Maize	Soybean	Tomato	Kiwi	Sesame	Mustard	Melon	Celery	Mugwort pollen
1	58,1	59,2	43,3	7,66	20,9	23	21,9	12,9	7,37	1,92	7,21	2,54	1,07	2	0,61
2	16,6	12	11,1	1,17	0,65	0,82	0	0,39	0	1,18	0	0	0	0	1,03
3	12,1	9,1	4,93	1,12	1,9	0,83	0,79	0,58	0,47	0,68	0	0,49	0	0	1
4	11,6	9,25	4,52	3,05	3,75	2,94	2,23	1,37	1,1	1,1	0	0,42	0	0	1,77
5	11,4	11,9	6,61	6,29	2,05	1,77	3,85	0,75	0,67	2,01	0,71	0	0	0,36	1,81
6	7,04	5,34	4,39	1,86	2,14	0,61	3,92	1,14	2,8	0,76	0,67	0	0	1,08	0
7	4,58	2,57	2,05	0	0	0,37	1,53	0	0	0,41	0	0	0	0	1,05
8	3,81	2,33	1,32	0,49	1,86	1,18	1,73	0,79	1,02	0	0,55	0	0	0	0
9	3,12	1,44	0,38	0	0	0	0	0	0	0	0	0	0	0	0,74
10	2,75	1,92	1,59	1,18	0,86	0,75	0,43	0,47	0	0,74	0,56	0	0	0	0
11	1,75	0,74	0	0	0	0	0	0	0	0	0	0	0	0	0
12	1,44	0,77	0	0	0	0,42	0	0	0	0	0	0	0	0	0
13	1,11	0,84	0	0	0	0	0	0	0	0,83	0	0	0	0	0
14	1,08	0,39	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0,41	0	0	0	0	0	0	0	0	0	0	0	0	0	0,61

Specific IgE values are expressed as kU/L (positive if > 0.35 kU/l). Patients have been ordered on the basis of peach-specific IgE levels.

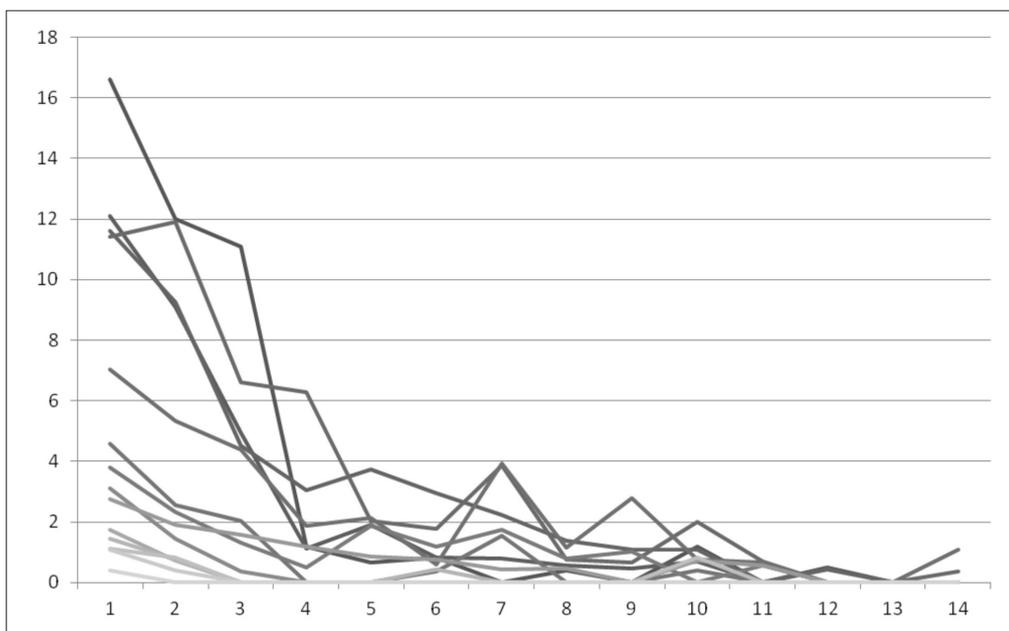


Figure 1 - Specific IgE levels in patients 2-15. Numbers under the horizontal line indicate the study foods (1: Peach; 2: Apple; 3: Walnut; 4: Hazelnut; 5: Peanut; 6: Lentil; 7: Maize; 8: Soybean; 9: Tomato; 10: Kiwi; 11: Sesame; 12: Mustard; 13: Melon; 14: Celery). Numbers along the vertical line are specific IgE levels in kU/L. Patient # 1 data were excluded due to the excess of specific IgE vs all other patients (see table 1).

Table 2 - Clinically offending foods in patients with LTP hypersensitivity.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	OF
1	X	X					X	X	X	X	X		X		X	X								X	X	X	
2	X				X				X	X	X												X				
3	X							X	X	X	X									X						X	X
4	X	X						X	X	X	X		X				X	X				X					X
5	X								X	X	X			X						X			X		X	X	
6	X	X							X	X			X	X		X				X	X				X		
7	X	X							X	X	X				X	X			X			X					
8	X																										
9	X		X	X					X																		
10	X	X	X						X			X															
11	X	X		X	X		X								X												
12	X		X	X																							
13	X	X																									
14	X		X		X																						
15	X																										

A: Peach; B: Apple; C: Cherry; D: Apricot; E: Plum; F: Pear; G: Strawberry; H: Almond; I: Walnut; J: Hazelnut; K: Peanut; L: Maize; M: Rice; N: Lettuce; O: Kiwi; P: Tomato; Q: Lentil; R: Saffron; S: Melon; T: Pistachio; U: Onion; V: Rucola; W: Cashew; X: Pineapple; Y: Fennel; Z: Other nuts (Brazil nut, coconut, macadamia nut); OF: Other Foods (Bell pepper, Pitaya).

A black box indicates systemic symptoms following the ingestion of a food. A grey box indicates local symptoms (Oral allergy syndrome) and a white box indicates a tolerated food.

Discussion

With the obvious limitations of being retrospective and based on a limited number of patients due to cost problems, this study shows that in patients with lipid transfer protein allergy syndrome, specific IgE levels follow a rather precise hierarchical order. Although this could theoretically be related to the amount of LTP present in the various extract for immunoassay, the possibility that it reflects the degree of homology between the proteins from different botanical sources and the LTP from the primary sensitizer, the peach, seems a more likely explanation. As observed before (3), the higher was the level of IgE to peach LTP, the higher was the likelihood of cross-sensitization to a large number of botanically unrelated plant-derived foods. However, hypersensitivity did not always reflect into clinical cross-reactivity; in fact, for several foods including lentil, soybean, maize and sesame the large majority of patients did not report any clinical reaction despite specific IgE levels that were frequently quite high. This might suggest either that in these foods the protein is less abundant than in the peach, or that it is absorbed more slowly in the gut, or that the thermal process-

ing reduces somehow the allergenicity of this protein in these foods. Both lentil and soybean belong to the *Leguminosae* family, and the good tolerance to legumes by LTP-allergic subjects was already observed several years ago (10). Further, some data suggest that LTP-containing foods may be more harmful if they are ingested alone (11). Finally, although LTP is known as an extremely heat-stable protein (12,13), some data seem to suggest that prolonged heat-treatments at high temperatures may significantly reduce its allergenicity (14). The case of kiwi is somehow special. Recent studies showed that kiwi-allergic patients living in southern Europe are mostly sensitized to profilin or to Act d 10, the kiwi lipid transfer proteins (15). In this study 9/15 patients showed IgE to kiwi, but only 3 patients reported allergic reaction to this fruit and, notably, one of them did not show any IgE reactivity to kiwi both on ImmunoCAP and on SPT, whereas in the remaining 2 specific IgE levels were low. Further, kiwi-specific IgE levels showed a significantly lower correlation with peach-specific IgE than all other study foods, suggesting that either patients were sensitized also to kiwi allergens other Act d 10, or that kiwi LTP shows a rather low homology to Pru p 3 causing a trend in specific IgE that differs from that of all

other plant-derived foods. Further studies are needed to elucidate this point.

Regarding the hypothesis that mugwort pollen may represent the primary allergen source for LTP sensitization, the results of the present study seem to rule out this possibility. In fact 7/15 patients did not show any IgE reactivity to this pollen, and in 7 out of the 8 remaining patients mugwort IgE were so much lower than peach IgE to make the hypothesis of a primary airborne, mugwort pollen-driven LTP sensitization unrealistic. Of course, it is possible that these findings are geographically specific for the population studied.

In conclusion, in patients with lipid transfer protein syndrome, IgE reactivity to foods other than peach is in most cases predictable and follows a regular sequence that depends on the degree of homology with Pru p 3. The reasons why some foods are tolerated by most patients despite elevated IgE reactivity remains to be elucidated.

References

1. 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;57:900-6.
2. Schulten V, Nagl B, Scala E, et al. Pru p 3, the nonspecific lipid transfer protein from peach, dominates the immune response to its homolog in hazelnut. *Allergy*. 2011;66:1005-13.
3. Palacín A, Gomez-Casado C, Rivas LA, Aguirre J, Tordesillas L, Bartra J, et al. Graph based study of allergen cross-reactivity of plant lipid transfer proteins (LTPs) using microarray in a multicenter study. *PLOS ONE*. 2012;7:e50799.
4. Asero R, Mistrello G, Roncarolo D, Amato S. Relationship between peach lipid transfer protein specific IgE levels and hypersensitivity to non-Rosaceae vegetable foods in patients allergic to lipid transfer protein. *Ann Allergy Asthma Immunol*. 2004;92:268-72.
5. Hartz C, Lauer I, Del Mar San Miguel Moncin M, et al. Comparison of IgE-binding capacity, cross-reactivity and biological potency of allergenic nonspecific lipid transfer proteins from peach, cherry and hazelnut. *Int Arch Allergy Immunol*. 2010;153:335-46.
6. Asero R, Arena A, Cecchi L, et al. Are IgE levels to foods other than Rosaceae predictive of allergy in lipid transfer protein-hypersensitive patients? *Int Arch Allergy Immunol*. 2011;155:149-54.
7. Garcia-Selles FJ, Díaz-Perales A, Sánchez-Monge R, Alcántara M, Lombardero M, Barber D, Salcedo G, Fernández-Rivas M. Pattern of reactivity to lipid transfer proteins of plant foods and Artemisia pollen: an in-vivo study. *Int Arch Allergy Immunol*. 2002Jun;128(2):115-22.
8. Lombardero M, García-Sellés FJ, Polo F, Jimeno L, Chamorro MJ, García-Casado G, Sánchez-Monge R, Díaz-Perales A, Salcedo G, Barber D. 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*. 2004Sep;34(9):1415-21.
9. Asero R. Plant food allergies: a suggested approach to allergen-resolved diagnosis in the clinical practice by identifying easily available sensitization markers. *Int Arch Allergy Immunol*. 2005;138:1-11.
10. Asero R, Mistrello G, Roncarolo D, Amato S, Falagiani P. Why do lipid transfer protein-hypersensitive patients tolerate bean (and other legumes)? *Int Arch Allergy Immunol*. 2005;137:236-40.
11. Arena A. Anaphylaxis to apple: is fasting a risk factor for LTP-allergic patients? *Eur Ann Allergy Clin Immunol*. 2010;42:155-8.
12. Pastorello EA, Pompei C, Pravettoni V, Farioli L, Calamari AM, Scibilia J, Robino AM, Conti A, Iametti S, Fortunato D, Bonomi S, Ortolani C. 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;112:775-83.
13. Asero R, Mistrello G, Roncarolo D, Amato S, Falagiani P. Analysis of the heat stability of lipid transfer protein from apple. *J Allergy Clin Immunol*. 2003;112:1009-11.
14. Sancho AI, Rigby NM, Zuidmeer L, Asero R, Mistrello G, Amato S, González-Mancebo E, Fernández-Rivas M, van Ree R, Mills EN. The effect of thermal processing on the IgE reactivity of the non-specific lipid transfer protein from apple, Mal d 3. *Allergy*. 2005;60:1262-8.
15. Le TM, Bublin M, Breiteneder H, Fernández-Rivas M, Asero R, Ballmer-Weber B, et al. Kiwifruit allergy across Europe: clinical manifestation and IgE recognition patterns to kiwifruit allergens. *J Allergy Clin Immunol*. 2013;131:164-71.