

Original article

Immunological cross-reactivity between lipid transfer proteins from botanically unrelated plant-derived foods: a clinical study

Background: Lipid transfer proteins (LTP) are highly conserved and widely distributed throughout the plant kingdom. Recent studies demonstrated immunological cross-reactivity between LTP from many botanically unrelated fruits and vegetables and concluded that LTP are pan-allergens. This study aimed to evaluate the clinical relevance of such cross-reactivity in a group of subjects monosensitized to LTP.

Methods: Twenty LTP-hypersensitive patients were selected from a population of about 600 subjects with history of *Rosaceae* allergy by means of: 1) negative skin prick test (SPT) with a commercial birch pollen extract; 2) positive SPT with a commercial plum extract, rich in LTP but virtually lacking both Bet v 1-like proteins and profilin; 3) *in-vitro* IgE reactivity to the 9–10 kDa fraction of peach peel or immunoblot with peach peel showing a single band at 10 kDa; and 4) total inhibition of reactivity to whole peach extract (containing Bet v 1-related allergen, profilin, and LTP) by purified peach LTP on enzyme-linked immunoassay (ELISA). Allergy to foods other than *Rosaceae* was ascertained by careful interview and analysis of medical recordings. SPT with a large series of plant-derived foods were carried out as well. The cross reactivity between LTPs from botanically unrelated plant-derived foods was assessed by ELISA inhibition tests using walnut and peanut extracts as substrate, and peach LTP as inhibitor.

Results: All patients reported allergic reactions after the ingestion of at least one from a large number of vegetable foods other than *Rosaceae*, and in several cases clinical reactions were very severe (anaphylaxis, asthma, urticaria/angioedema). Nuts and peanuts were the most frequently reported causes of allergic reactions (80% and 40% of patients, respectively). All patients showed positive SPT to several non-*Rosaceae* food extracts. SPT with nuts, peanut, legumes, celery, rice, and corn were positive in the majority of patients. In ELISA inhibition studies, absorption of sera with peach LTP caused complete inhibition of IgE reactivity to walnut and peanut in all cases.

Conclusion: LTP is a clinically relevant pan-allergen. Most *Rosaceae*-allergic, LTP-hypersensitive patients experience adverse reactions after ingestion of botanically unrelated plant-derived foods as well. In view of the high prevalence and severity of the allergic reactions induced, hazelnut, walnut, and peanut should be regarded as potentially hazardous for these patients.

R. Asero¹, G. Mistrello²,
D. Roncarolo², S. Amato²,
G. Caldironi³, F. Barocci³, R. van Ree⁴

¹Ambulatorio di Allergologia, Ospedale Caduti Bollatesi, Bollate (MI); ²Lofarma SpA, Milan;

³Centro Trasfusionale, Ospedale Rho, Rho (MI), Italy;

⁴CLB, Amsterdam, the Netherlands

Key words: food allergy; fruits; lipid transfer protein; nuts; peach; peanuts; *Rosaceae*; vegetables.

Dr Riccardo Asero
Ambulatorio di Allergologia
Ospedale Caduti Bollatesi
Via Piave 20
20021 Bollate (MI)
Italy

Accepted for publication 3 April 2002

Lipid transfer proteins (LTP), the major allergens in *Rosaceae* fruits (namely peach, apple, and apricot) in patients without birch pollinosis (1–5) are widely distributed throughout the plant kingdom (6–16). LTP from botanically unrelated vegetable foods show a moderate to high (35–95%) degree of sequence homology, and this fact suggests that LTP represent novel plant pan-allergens (3,17). In effect, cross-reactivity between LTP from distinct members of the *Prunoideae* subfamily has been demonstrated (18). Moreover, we recently found that *Rosaceae*-allergic patients who are hypersensitive to LTP frequently show both skin and serological reactivity to a number of other plant-derived foods as well; for the

first time we convincingly demonstrated immunological cross-reactivity between LTP from botanically unrelated fruits and vegetables, concluding that LTP are true pan-allergens (19). Subsequently, other workers have confirmed our findings, showing that LTP from *Rosaceae*, *Artemisia* pollen, and chestnut cross-react (20), and that LTP is the major allergen of maize that cross-reacts with both peach and rice LTP (21). LTP-sensitive patients with *Rosaceae* allergy frequently report adverse reactions after eating vegetable foods that do not belong to this botanical family (19,22); however, in our previous studies we could not clearly establish the clinical relevance of cross-reactivity between LTP because several patients

were also sensitized to other cross-reacting allergens such as Bet v 1 and/or Bet v 2 (profilin) (19). LTP is a potentially hazardous allergen: due to its extreme pepsin-stability (19); sensitive patients may experience severe allergic reactions upon the ingestion of offending foods (19, 21–24). On the other hand, patients sensitized to vegetable foods may result “false positive” in *in-vivo* and/or *in-vitro* tests (i.e. these subjects may eat fruits and vegetables that scored positive on *in-vivo/in-vitro* tests without any clinical consequence). Thus, identification of botanically unrelated plant-derived foods that are more likely to induce clinical symptoms in *Rosaceae*-allergic, LTP-sensitive patients may help to prevent the most severe reactions, avoiding the need to restrict patients’ diets unnecessarily. This study aimed to investigate the clinical effects of immunological cross-reactivity between LTP in a group of monosensitized patients. The study followed the revised nomenclature for allergic diseases recently proposed by the EAACI committee (25).

Material and methods

Selection of patients monosensitized to LTP

The study was carried out on LTP-monosensitive patients with a clinical history of allergic reactions following the ingestion of *Rosaceae* fruits (apple, pear, peach, cherry, apricot, plum, or almond). Symptoms of food allergy included oral allergy syndrome, urticaria/angioedema, gastrointestinal symptoms, asthma, rhinitis, or anaphylaxis. LTP-reactive patients were screened from a population of about 600 *Rosaceae*-allergic individuals on the

basis of a positive skin prick test (SPT) with commercial plum extract (Dome/Hollister-Stier, Spokane, WA; 1:20 w/v) (19). As previously shown, this extract contains LTP but virtually lacks both Bet v 1-like proteins and profilin (22). LTP reactivity was confirmed by ELISA using the 9–10 kDa fraction of peach peel separated by gel chromatography (19) as substrate, or by immunoblot analysis with peach extract showing IgE reactivity at 9–10 kDa.

In order to avoid interference from hypersensitivity to allergens other than LTP able to mediate cross-reactivity between birch pollen and foods (26–28), only patients monosensitized to LTP were studied. Selection of such patients was carried out on the basis of:

1. Negative SPT with a commercial birch pollen extract (Allergopharma; Reinbeck, Germany)
2. Lack of IgE reactivity to recombinant Bet v 1 and recombinant birch profilin, and Bet v 2 by RAST (CAP-system, Pharmacia, Uppsala, Sweden).
3. ELISA inhibition experiments showing complete loss of IgE reactivity to whole peach peel extract (prepared at Lofarma laboratories, Milan, Italy) containing Pru p 1, profilin and LTP, as shown by SPT on monosensitized patients, following absorption of serum with peach LTP (19). In these experiments the serum from a Pru p 1-positive/LTP-negative patient was used as control. Alternatively, IgE reactivity to a single band at 9–10 kDa had to be present on immunoblots with whole peach peel extract (19).

Twenty patients met these inclusion criteria and were therefore studied. Their sensitivity to seasonal and/or perennial airborne allergens as well as to *Rosaceae* is shown in Table 1. Patients’ monosensitivity to LTP was further confirmed by immunoblot analysis; pooled sera showed reactivity to a single band at about 10 kDa against an apple peel extract (Fig. 1).

Table 1. Skin prick test results for reported offending *Rosaceae*, and respiratory allergies for each patient

Patient	Peach (Lofarma)	Apple (PbP)	Pear (PbP)	Cherry (DHS)	Plum (DHS)	Apricot (DHS)	Almond (DHS)	Birch pollen (Allergopharma)	Respiratory allergy
1	+0	–			+	0	–	–	None
2	+0				+		+	–	Mite
3	+C/U	+G		+G	+	C	+	–	Pellitory
4	+U	+	+	+U	+U	+U	+	–	Pellitory
5	+C/U	–	+		+		+C	–	Grass
6	+U	+U		+U	+U	+U	+	–	None
7	+U	–			+		+	–	Grass; mite
8	+0	+0	0	+	+	0	–	–	None
9	+0			0	+	0	+	–	None
10	+0	+U	+	+	+		+	–	Grass
11	+0			+	+		+	–	Grass; mugwort
12	+U				+		+	–	Grass
13	+U	–	+	+	+		+U	–	None
14	+U	+U		+	+		+	–	None
15	+0	–		–	+0	0	–0	–	Mugwort
16	+G/S	+		+	+	+	+	–	Grass
17	+U				+		+	–	None
18	+U	+		+	+		+	–	None
19	+U	+U			+		–	–	None
20	+U	+0		+0	+U		+	–	Grass; pellitory

C = contact urticaria; G = gastroenteritis; 0 = oral allergy syndrome; S = asthma; U = urticaria.

– = negative SPT; + = positive SPT.

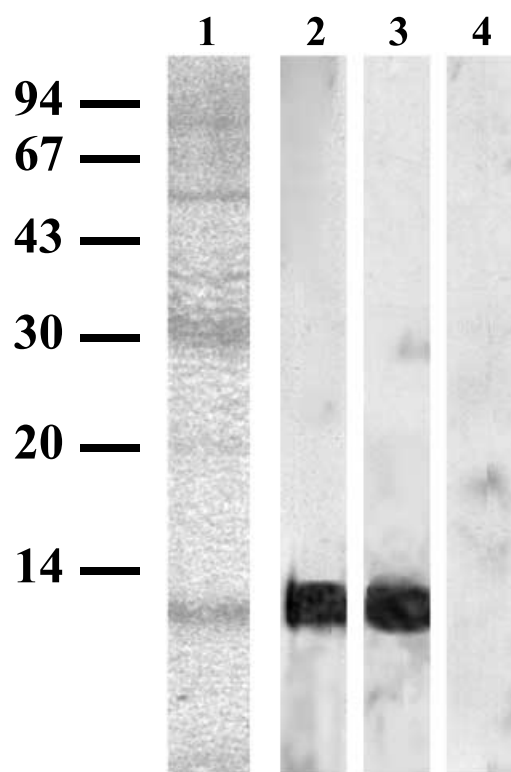


Figure 1. SDS-PAGE profile of apple peel extract and immunoblot analysis. Lane 1: SDS profile; Lane 2: IgE reactivity of pooled sera from patients 1–10; Lane 3: IgE reactivity of pooled sera from patients 11–20; Lane 4: IgE reactivity of pooled sera from normal controls. Patients' sera show marked IgE reactivity to a single protein band at about 10 kDA.

Clinical investigations

Patients underwent a thorough interview that aimed to ascertain all episodes of food allergy (oral allergy syndrome, urticaria/angioedema, gastrointestinal symptoms, asthma, rhinitis, or anaphylaxis) following the ingestion of non-*Rosaceae* plant-derived foods. Twelve patients needed emergency-room care due to the severity of food-induced reactions. Offending foods were clearly identified by all patients: in several cases the culprit food was the only food ingested, and in the other cases the onset of systemic symptoms was preceded by oral allergy syndrome caused by the offending food. Eight patients had a history of oral allergy syndrome caused by the same food that induced a systemic reaction. As in other recent studies of LTP (21), in view of frequently reported severe reactions (anaphylaxis, urticaria/angioedema, asthma; Table 2) and because of ethical reasons, patients were not submitted to confirmatory double-blind, placebo-controlled food challenge (DBPCFC) with the offending foods.

Skin tests

All selected patients underwent SPT with the following commercial extracts of non-*Rosaceae* vegetable foods (Dome/Hollister-Stier): cereals (wheat, rye, barley, corn, oat and rice), legumes (peanut, green pea, soybean, and white bean), *Apiaceae* (celery, carrot), nuts (walnut, hazelnut), potato, tomato, banana, garlic, onion, and orange.

SPT were carried out on the volar side of the forearm with a sterile, 1 mm-tip lancet (Dome/Hollister-Stier), pricking through the drop of the extract. Readings were taken after 15 min and results were assessed by comparison with the wheal area induced by histamine 10 mg/ml (29).

ELISA/ELISA inhibition

ELISA/ELISA inhibition studies were carried out in order to detect whether the patients' clinical hypersensitivity to foods other than *Rosaceae* resulted from cross-reactivity between the LTP. ELISA was used to measure IgE reactivity to extracts of two of the most commonly offending foods, namely walnut ($n = 17$) and peanut ($n = 7$) (Table 1). Extracts were prepared as previously described (19) and were used at a protein concentration of 0.6 mg/ml. IgE reactivity was expressed as absorbance (optical density, OD); values < 200 were considered negative. In inhibition studies, 50 μ l of serum were preincubated for 1 h with 40 μ g of peach LTP, prior to addition to the coated plate. A fall of $> 80\%$ in IgE reactivity, or a reduction to < 200 OD, was considered as complete inhibition.

Statistical analysis

In order to assess clinical usefulness of SPT with commercial food extracts in LTP-hypersensitive patients, sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) of SPT were calculated by established methods (30):

$$SE = TP / (TP + FN)$$

$$SP = TN / (TN + FP)$$

$$PPV = TP \times P / [TP \times P + FP (1 - P)]$$

$$NPV = TN (1 - P) / [TN (1 - P) + FN \times P]$$

where P (prevalence) = patients with positive history/total patients; TP (true positive) = patients with positive history and positive SPT; FP (false positive) = patients with negative history and positive SPT; TN (true negative) = patients with negative history and negative SPT; FN (false negative) = patients with positive history and negative SPT.

Results

Clinical findings

Offending foods other than *Rosaceae* as well as SPT results are shown in Table 2. All but one patient reported allergic reactions after ingesting at least one non-*Rosaceae* plant-derived food; most patients reported reactivity to several foods, and in many cases clinical reactions were very severe (anaphylaxis, asthma, urticaria/angioedema). Two patients (numbers 1 and 7) experienced four episodes of anaphylaxis after eating four different non-*Rosaceae* foods. Most systemic reactions were directly observed, recorded, and treated by physicians in the emergency rooms. Nuts, peanuts, and *Apiaceae* were the most frequently reported culprits of allergic reactions. The prevalence of food allergy to nuts (including hazelnut, walnut and pistachio), peanut, and *Apiaceae* (including,

Table 2. Reported offending foods other than Rosaceae, and skin prick test results

	Patient																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Walnut	-O	+U	+	+U	+U	-	+	-	+	-U	+U	-	+O	-U	+O	+US	+	+	+	+O
Hazelnut	+X	+	+G	+U	+U	+U	+	+U	+U	+O	+U	+	+O	+	+O	+US	+	+	+	+O
Pistachios	/ N	/ N	/ N	/ N	/ N	/ N	/ X	/	/ N	/ N	/ N	/ N	/ N	/ N	/ O	/ N	/ N	/ N	/ N	/ N
Bean/pea	+	+	+	-	+	-	+	-	+	+	+	-	+	-	+ O/U	+	+	+	-	+
Lentils	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/ O/U	/	/	/	/	/
Soybean	+	+	+	-	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+
Lupine	/	/	/	/	/	/ U	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Peanut	+	+	+	-	+	-	+ X	-	+ O	+ O	+ U	+	+ O	+ U	+ O	+	+	+	+	+ O
Cashew	/ N	/ N	/ N	/ N	/ N	/ N	/ X	/ N	/ N	/ N	/ N	/ N	/ N	/ N	/ N	/ N	/ N	/ N	/ N	/ N
Celery	-	+	+	-	+	-	+	-	-	-	+	-	-	-	+ O	+	+	+	+	+ O
Carrot	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- C	-
Fennel	/	/	/	/	/	/	/ O	/	/	/	/	/	/	/	/ O	/	/	/	/	/ O
Parsley	/	/	/	/	/	/	/	/	/	/	/ U	/	/	/	/	/	/	/	/	/
Beer	/	/	/	/	/	/	/	/	/	/	/ U	/	/	/	/	/	/	/ U	/	/
Wheat/barley	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
Corn	-	+ U	+	-	+	-	+	-	-	-	-	+ U	-	-	+ U	+	+	+	+	+ U
Rice	-	+ C	+	-	+	-	+	-	-	-	+	-	-	-	+	+	+	+	+	+
Lemon	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/ U	/	/	/	/	/
Orange	-	+	+	-	+	- U	-	-	-	-	-	-	-	+	-	+	+	+	-	-
Watermelon	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/ S	/	/	/	/
Banana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fig	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/ O	/	/	/	/	/ O
Grapes	/	/	/	/	/	/	/	/	/	/	/	/	/	/ O	/	/ GR	/	/	/	/
Pineapple	/	/	/	/	/	/	/	/	/	/	/	/	/ C	/	/	/	/	/	/	/
Tomato	-	+	+	+	+	-	-	-	-	-	-	-	-	-	+ O	+	+	+	-	-
Mustard	/	/	/	/	/	/	/	/	/	/	/	/	/ O	/	/	/	/	/	/	/
Potato	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Onion	-	+	+	-	+	-	-	-	-	-	-	-	+	-	-	+	+ U	+	-	+ U
Eggplant	/	/	/	/	/	/	/	/	/	/	/	/	/	/ O	/	/	/	/	/	/ O
Bell pepper	/	/	/	/	/	/	O	/	/	/	/	/	/	/	/	/	/	/	/	/

C = contact urticaria; G = gastroenteritis; O = oral allergy syndrome; R = rhinitis; S = asthma; U = urticaria; X = anaphylaxis.

N = tolerance unknown.

(/) SPT not done; (-) negative SPT; (+) positive SPT.

celery, fennel, carrot, and parsley) in the study population was 80% (16/20), 40% (8/20), and 25% (5/20), respectively. One further patient (number 19), who reported contact urticaria on her hands from preparing (scratching) carrots, tolerated the ingestion of raw scratched carrots. Of the 20 subjects, four (20%) experienced maize allergy, and two (10%) had a history of allergic reactions to legumes other than peanuts. A large spectrum of other plant-derived foods induced allergic reactions in individual cases (Table 2); these included fig, beer, onion, grapes, eggplant (two cases each), mustard, watermelon, rice, lemon, orange, pineapple, tomato, and bell-pepper (one case each).

All patients showed positive skin reactions to one ($n = 1$) or more than one ($n = 19$) extracts of non-*Rosaceae* foods. Notably, SPT with hazelnut extract was positive in all 20 patients. SPT with walnut, peanut, legumes, celery, rice, and corn were positive in the majority of patients. A minority reacted against onion, tomato, orange, and other cereals, and SPT with carrot, potato, and banana extracts scored negative for all

patients. SE, SP, PPV, and NPV of SPT with extracts of the main offending foods are shown in Table 3. SE of SPT was excellent in most cases, whereas SP was often poor due to the high prevalence of "false positive" results.

Inhibition studies

Results of the ELISA inhibition studies are shown in Table 4. In all cases showing significant IgE reactivity to

Table 3. Analysis of the clinical value of skin prick tests with commercial extracts of the most common offending foods

	Positive on SPT (%)	Prevalence of clinical allergy	Sensitivity	Specificity	Predictive value	
					Positive	Negative
Hazelnut	20 (100%)	65%	100%	0%	78%	0%
Walnut	14 (70%)	55%	73%	33%	62%	45%
Peanut	17 (85%)	40%	100%	25%	37%	100%
Maize	11 (55%)	20%	100%	56%	13%	100%

Table 4. Inhibition studies: IgE reactivity to whole fruit extracts before and after inhibition with peach lipid transfer proteins (LTP)

	ELISA LTP (OD)	Peach reactivity (OD)			Walnut reactivity (OD)			Peanut reactivity (OD)		
		Before	After	%	Before	After	%	Before	After	%
Patient										
1	2441	1652	278	83	1602	0	100			
2	2046	381	96	75	neg					
3	3346	2331	230	90	1416	0	100			
4	615	480	65	86	neg					
5	1688	794	140	82	229	0	100			
6	980	697	165	77	neg					
7	2940	1196	111	91	530	0	100	600	0	100
8	2070		nd			nd				
9	2152	933	48	95	neg			806	0	100
10	962	427	59	86	neg			neg		
11	3069	3247	37	99	1800	0	100	2238	44	98
12	1249	448	27	94	272	53	81			
13	1097	587	91	84	1218	53	96	326	0	100
14	2341	775	33	96	711	60	92			
15	1888	2131	326	85	1729	51	97	2578	54	98
16	WB*		nd							
17	1064	763	139	82	904	0	100			
18	3576		nd			nd				
19	1720	1040	56	95	264	0	100			
20	3405	2419	118	95	555	145	74	1560	0	100
Controls										
Negative	Neg	148			14			0		
Positive†	Neg	1038	865	17	nd	nd	nd			

OD = optical density; ELISA negative if OD < 200; nd = not done; % = percentage inhibition induced by LTP.

* Monosensitivity to peach LTP shown by immunoblot analysis.

† Bet v 1+/LTP- patient.

walnut and/or peanut, preabsorption of sera with peach LTP resulted in complete inhibition of IgE reactivity to both these plant-derived foods. Inhibition tests were not performed on sera from three subjects; in one case (number 8) this was because of lack of serum, and in two cases (numbers 16 and 18) because complete inhibition of walnut, hazelnut and peach IgE reactivity by carrot recombinant LTP had already been demonstrated in our previous paper (19).

Discussion

LTP occur widely throughout the plant kingdom and there is cross-reactivity between LTP from botanically unrelated sources, and this study showed that most LTP-hypersensitive patients with *Rosaceae* allergy may experience adverse reactions after the ingestion of a large spectrum of non-*Rosaceae* plant-derived foods. Allergic reactions induced by LTP may be very severe; thus, for ethical reasons, DBPCFC were not carried out in this study. The ethical policy of avoiding confirmatory DBPCFC in order to spare patients possibly severe adverse reactions was recently adopted in another study of LTP (21). Moreover, most of our patients had spontaneously excluded the offending foods from their

diets after experiencing adverse reactions and would not have accepted oral challenge.

Nuts and peanuts were the most common non-*Rosaceae* offending foods. Indeed 80% of our patients reported clinical reactivity to nuts (hazelnut, walnut, pistachio); in 11 cases (55%) nuts caused systemic symptoms, with urticaria/angioedema in nine cases and anaphylaxis in two. Such a high prevalence of clinical allergy suggests great cross-reactivity between LTP contained in *Rosaceae* and nuts. The finding that all our patients scored positive on SPT with hazelnut extract supports this conclusion.

The association between allergy to *Rosaceae* and nuts in patients sensitive to LTP confirms our previous preliminary observations (22). Clinical allergy to other plant-derived foods such as legumes (peanut, soybean, beans, pea, chick pea, and lupine), *Apiaceae* (celery, carrot, fennel), maize, and others, was less frequent and less predictable on the basis of skin-test results. Peanuts and *Apiaceae* induced adverse reactions in 40% and 25% of patients, respectively. Maize and beer were not tolerated by four (20%) and two (10%) patients respectively, which confirms both the reported cross-reactivity between maize and peach LTP (21) and our recent finding that the 10 kDa allergen found in beer (31) is LTP (32). Two patients experienced immediate gastroenteritis/rhi-

nititis and oral allergy syndrome, respectively, after ingesting grapes. Nine cases of grape allergy, all reacting against LTP and showing complete cross-reactivity with peach LTP, have been described very recently (33).

In general, the sensitivity of SPT with many food extracts was excellent, whereas both specificity and the positive predictive value were frequently low due to the high prevalence of "false positive" results. Falsely positive SPT are frequently found in subjects hypersensitive to cross-reacting vegetable food allergens. In these individuals the onset of clinical symptoms upon ingestion of a potentially offending food may depend on several factors:

- 1) It has been shown that the ability to synthesize some cross-reacting allergens (e.g. Mal d 1) may vary greatly from one cultivar to another (34).
- 2) Extracts for SPT are usually prepared using raw material; if the relevant allergen is totally or partially heat-labile, it is not surprising that patients showing positive SPT may tolerate potentially offending foods after cooking—legumes are a typical example. Most patients in this study showed positive SPT with pea, bean, and/or soybean extracts, however only two patients experienced symptoms after eating legumes. Similarly, maize was frequently positive on SPT, but only four patients reported maize allergy, and all of them experienced their reactions after ingesting canned corn or salted maize snacks, whereas all tolerated "polenta" (a long-boiled cornbread dish) without any problem. These observations suggest that LTP is certainly pepsin-resistant and only partially heat-stable, but are not in keeping with previous findings (21,35). The heat-stability of LTP is presently under investigation in a specifically designed study; results will be presented elsewhere.
- 3) The distribution of the allergen within the offending food may also play a role. LTP is more highly represented in the superficial layer (peel) than in the pulp of vegetables (36), and we recently observed a marked difference in reactivity to pulp and peel extract of *Rosaceae* in LTP-allergic subjects (37). Therefore, certain food-preparation habits such as peeling (e.g. apple, pear, peach) and hygienic procedures, such as scratching of carrots, may cause the loss of most of

the allergenic activity of a potentially hazardous food. For instance, one patient in this study (number 19) experienced contact urticaria while preparing (scratching) raw carrots, but tolerated their ingestion. Additionally, the distribution of LTP may influence SPT results; carrot, potato and banana extracts for SPT employed in this study scored negative in all patients. However, Sterk et al. (38) demonstrated the presence of LTP in carrots, and we recently showed that a recombinant carrot LTP was able to inhibit patients' seroreactivity to LTP from a number of plant-derived foods (19).

In inhibition studies, sera from some patients positive on SPT with walnut ($n = 5$) and peanut ($n = 1$) extracts were negative on ELISA with the same foods. In general, this occurred for sera showing low IgE reactivity to whole peach extract (Table 4); the presence of lower concentrations of LTP in walnut and peanut extracts than in peach extract might be the basis of this phenomenon.

In clinical practice, most LTP-reactors are quite easily detected by an absence of birch-pollen sensitivity, and by skin reactivity to commercial food extracts (e.g. the commercial plum extract used in this study) that are generally known to produce negative SPT in allergic patients with birch pollinosis (22). In view of the high prevalence and severity of the allergic reactions induced, hazelnut, walnut, and peanut should be considered potentially hazardous for LTP sensitive patients with a history of *Rosaceae*-allergy, particularly in those showing positive SPT.

Cashew and pistachio induced an anaphylactic reaction in a single patient. Cashew nuts are rarely eaten in Italy. In our study group this patient was the only one to have eaten cashew nuts; most of the other patients had not even heard of it. Moreover, apart from the patient who experienced anaphylaxis, only two of 19 patients used to eat pistachios, and one of these experienced oral allergy syndrome. Although we did not assess exactly the prevalence of pistachio and cashew-nut allergy by DBPCFC, and therefore cannot draw definitive conclusions, our observations do suggest that cashew and pistachio nuts might also pose a potential hazard to some LTP-allergic patients.

References

1. LLEONART R, CISTERÒ A, CARREIRA J, BATISTA A, MOSCOSO DEL PRADO J. Food allergy: identification of the major IgE-binding component of peach (*Prunus persica*). *Ann Allergy* 1992;**69**:128–130.
2. PASTORELLO EA, FARIOLI L, PRAVETTONI V, et al. The major allergen of peach (*Prunus persica*) is a lipid transfer protein. *J Allergy Clin Immunol* 1999;**103**:520–526.
3. SANCHEZ-MONGE R, LOMBARDEO M, GARCIA-SELLES FJ, BARBER D, SALCEDO G. Lipid transfer proteins are relevant allergens in fruit allergy. *J Allergy Clin Immunol* 1999;**103**:514–519.
4. PASTORELLO EA, PRAVETTONI V, FARIOLI L, et al. Clinical role of a lipid transfer protein that acts as a new apple-specific allergen. *J Allergy Clin Immunol* 1999;**104**:1099–1106.
5. PASTORELLO EA, PIRELLO D'AMBROSIO F, PRAVETTONI V, FARIOLI L, GIUFFRIDA G, et al. Evidence for a lipid transfer protein as the major allergen of apricot. *J Allergy Clin Immunol* 2000;**105**:371–377.
6. PYEE J, YU H, KOLATTUKUDY PE. Identification of a lipid transfer protein as the major protein in the surface wax of broccoli (*Brassica oleracea*) leaves.

- Arch Biochem Biophys 1994;**311**:460–468.
7. TASSIN S, BROEKAERT WF, MARION D, AELAND DP, PTAK M, et al. Solution structure of Ace-AMP1, a potent antimicrobial protein extracted from onion seeds. Structural analogies with plant non-specific lipid transfer proteins. Biochemistry 1998;**37**:3623–3637.
8. SHIN DH, LEE JL, HWANG KY, KIM KK, SUH SW. High resolution crystal structure of the non-specific lipid transfer protein from maize seedlings. Structure 1995;**3**:189–199.
9. TORRES-SCHUMANN S, GODOY JA, PINTOR-TORO JA. A probable lipid transfer protein gene is induced by NaCl in stems of tomato plants. Plant Mol Biol 1992;**18**:749–757.
10. OSTERGAARD J, HOJRUP P, KNUDSEN J. Amino acid sequences of three acyl-binding/lipid transfer proteins from grape seedlings. Biochim Biophys Acta 1995;**1254**:169–179.
11. MEIJER EA, DE VRIES SC, STERK P, GADELLA DWJ, WIRTZ KW, HENDRICKS T. Characterization of the non-specific lipid transfer protein EP2 from carrot (*Daucus carota*). Mol Cell Biochem 1993;**123**:159–166.
12. STRIVER K, LEAH R, MULLER-URI F, OLSEN FL, MUNDY J. Structure and expression of the barley lipid transfer protein gene. Plant Mol Biol 1992;**18**:585–589.
13. DIERYCK W, GAUTIER MF, LULLIEN V, JOUDRIER P. Nucleotide sequence of a cDNA encoding a lipid transfer protein from wheat (*Triticum durum* Desf.). Plant Mol Biol 1992;**19**:707–709.
14. VIGNOLS F, LUND G, PAMMI S, et al. Characterization of a rice gene coding for a lipid transfer protein. Gene 1994;**142**:265–270.
15. PELESE-SIEBENBOURG F, CAELLES C, KADER JC, DELSENY M, PUIGDOMENECH P. A pair of genes coding for lipid transfer proteins in *Sorghum vulgare*. Gene 1994;**148**:305–308.
16. COUTOS-THEVENOT P, JOUENNE T, MAES O, GUERBETTE F, GROSBOIS M, et al. Four 9-kDa proteins excreted by somatic embryos of grapevine are isoforms of lipid transfer proteins. Eur J Biochem 1993;**217**:885–889.
17. SALCEDO G, DIAZ-PERALES A, SANCHEZ-MONGE R. Fruit allergy: plant defence proteins as novel potential panallergens. Clin Exp Allergy 1999;**29**:1158–1160.
18. PASTORELLO EA, ORTOLANI C, FARIOLI L, PRAVETTONI V, ISPANO M, et al. Allergenic cross-reactivity among peach, apricot, plum, and cherry in patients with oral allergy syndrome: an *in vivo* and *in vitro* study. J Allergy Clin Immunol 1994;**94**:699–707.
19. ASERO R, MISTRELLO G, RONCAROLO D, DE VRIES SC, GAUTIER MF, et al. Lipid transfer protein: a pan-allergen in plant-derived foods that is highly resistant to pepsin digestion. Int Arch Allergy Immunol 2000;**122**:20–32.
20. DIAZ-PERALES A, LOMBARDO 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.
21. PASTORELLO E, FARIOLI L, PRAVETTONI V, ISPANO M, 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.
22. ASERO R. Detection and clinical characterization of patients with oral allergy syndrome caused by stable allergens in Rosaceae and nuts. Ann Allergy Asthma Immunol 1999;**83**:377–383.
23. FERNANDEZ-RIVAS M, VAN REE R, CUEVAS M. Allergy to Rosaceae fruits without related pollinosis. J Allergy Clin Immunol 1997;**100**:728–733.
24. NAVARRO AM, ORTA JC, SANCHEZ MC, DELGADO J, BARBER D, LOMBARDO M. Primary sensitization to *Morus alba*. Allergy 1997;**52**:1144–1145.
25. JOHANSSON SGO, et al. Position paper. A revised nomenclature for allergy. Allergy 2001;**56**:813–824.
26. KARAMLOO F, SCHMITZ N, SCHEURER S, FOETISCH K, et al. Molecular cloning and characterization of a birch pollen minor allergen, Bet v 5, belonging to a family of isoflavone reductase-related proteins. J Allergy Clin Immunol 1999;**104**:991–999.
27. KARAMLOO F, WANGORSCH A, KASAHARA H, DAVIN LB, et al. Phenylcoumaran benzylic ether and isoflavonoid reductases are a new class of cross-reactive allergens in birch pollen, fruits and vegetables. Eur J Biochem 2001;**268**:5310–5320.
28. MAHLER V, FISCHER S, HEISS S, DUCHENE M, KRAFT D, VALENTA R. cDNA cloning and characterization of a cross-reactive birch pollen allergen: identification as a pectine esterase. Int Arch Allergy Immunol 2001;**124**:63–66.
29. AAS K. Some variables in skin prick testing. Allergy 1980;**35**:250–254.
30. GOLDMAN L. Quantitative aspects of clinical reasoning. In: Principles and Practice of Internal Medicine. New York: McGraw-Hill 1987, 5–11.
31. FERNANDEZ-ANAYA S, CRESPO JF, RODRIGUEZ JR, et al. Beer anaphylaxis. J Allergy Clin Immunol 1999;**103**:959–960.
32. ASERO R, MISTRELLO G, RONCAROLO D, AMATO S, VAN REE R. A case of allergy to beer showing cross-reactivity between lipid transfer proteins. Ann Allergy Asthma Immunol 2001;**87**:63–67.
33. PRAVETTONI V, CONTI A, FARIOLI L, et al. Identification of the major allergens of grape. Presented at 8th International Symposium on Immunological, Chemical, and Clinical Problems of Food Allergy, Venezia, Italy, March 11–13, 2001, 66 (Abstr).
34. VIETHS S, JANKIEWICZ A, SCHONING B, AULEPP H. Apple allergy. The IgE-binding potency of apple strains is related to the occurrence of the 18 kDa allergen. Allergy 1994;**49**:262–271.
35. BRENNAN O, POMPEI C, ORTOLANI C, PRAVETTONI V, FARIOLI L, PASTORELLO EA. Technological processes to decrease the allergenicity of peach juice and nectar. J Agric Food Chem 2000;**48**:493–497.
36. FERNANDEZ-RIVAS M, CUEVAS M. Peels of Rosaceae fruits have a higher allergenicity than pulps. Clin Exp Allergy 1999;**29**:1239–1247.
37. ASERO R, MISTRELLO G, RONCAROLO D, CASARINI M, FAGAGNIANI P. Allergy to non-specific lipid transfer proteins in Rosaceae. A comparative study of different *in-vivo* diagnostic methods. Ann Allergy Asthma Immunol 2001;**87**: 68–71.
38. STERK P, BOUJ H, SCHELLEKENS GA, VAN KAMMEN A, DE VRIES SC. Cell-specific expression of the carrot EP2 lipid transfer protein gene. Plant Cell 1991;**3**:907–921.