

Detection of Some Safe Plant-Derived Foods for LTP-Allergic Patients

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Key Words

Banana • Carrot • Cross-reaction • Food allergy • Lipid transfer protein • Melon • Potato

Abstract

Background: Lipid transfer protein (LTP) is a widely cross-reacting plant pan-allergen. Adverse reactions to Rosaceae, tree nuts, peanut, beer, maize, mustard, asparagus, grapes, mulberry, cabbage, dates, orange, fig, kiwi, lupine, fennel, celery, tomato, eggplant, lettuce, chestnut and pineapple have been recorded. **Objective:** To detect vegetable foods to be regarded as safe for LTP-allergic patients. **Methods:** Tolerance/intolerance to a large spectrum of vegetable foods other than Rosaceae, tree nuts and peanut was assessed by interview in 49 subjects monosensitized to LTP and in three distinct groups of controls monosensitized to Bet v 1 (n = 24) or Bet v 2 (n = 18), or sensitized to both LTP and birch pollen (n = 16), all with a history of vegetable food allergy. Patients and controls underwent skin prick test (SPT) with a large spectrum of vegetable foods. The absence of IgE reactivity to foods that were negative in both clinical history and SPT was confirmed by immunoblot analysis and their clinical tolerance was finally assessed by open oral challenge (50 g per food). **Results:** All patients reported tolerance and showed negative SPT to carrot, potato, banana and melon; these foods scored positive in SPT and elicited clinical symptoms in a significant proportion of patients from all three control groups. All patients tolerated these four foods on

oral challenge. Immunoblot analysis confirmed the lack of IgE reactivity to these foods by LTP-allergic patients. **Conclusion:** Carrot, potato, banana and melon seem safe for LTP-allergic patients. This finding may be helpful for a better management of allergy to LTP.

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Introduction

Lipid transfer protein (LTP), the major allergen in Rosaceae in geographic areas where the prevalence of birch pollen allergy is low [1–5], is a widely cross-reacting plant pan-allergen.

This protein has been detected in an array of vegetable sources [6–11], and adverse reactions to a variety of non-Rosaceae plant-derived foods including tree nuts, peanut, beer, maize, mustard, asparagus, grapes, mulberry, cabbage, dates, orange, fig, kiwi, lupine, fennel, celery, tomato, eggplant, lettuce, chestnut and pineapple [12–24] have been recorded in patients sensitized to LTP. In most LTP-allergic subjects, skin reactivity and clinical allergy are limited to Rosaceae, tree nuts and peanut [12, 13]. However, a subset of patients, particularly those showing the highest levels of IgE to peach LTP [25], show skin reactivity to a much larger spectrum of plant-derived foods in skin prick test (SPT) and carry therefore a potential risk for (possibly severe) adverse reactions upon ingestion of foods that are well tolerated by the majority of LTP-al-

lergic subjects. In the management of these subjects, it would be extremely useful to know whether some plant-derived foods are safe for all LTP-allergic patients. The present study aimed to detect some vegetable foods to be regarded as safe for all LTP-allergic patients.

Patients and Methods

Patients

The study includes 49 patients (21 males, 28 females; mean age 29 years) diagnosed as being probably monosensitized to LTP at this allergy unit. Patients were considered as presumably monosensitized if they fulfilled the following criteria: (a) a positive SPT response to a commercial peach extract (ALK-Abello, Madrid, Spain); this extract reportedly contains 30 µg/ml of LTP, and previous studies showed that it lacks both Bet v 1-like protein and profilin [26]; (b) a negative SPT response to a commercial birch pollen extract (50,000 SBU/ml; Allergopharma, Reinbeck, Germany) containing both Bet v 1 and birch profilin, Bet v 2 [27], and (c) a negative SPT with natural rubber latex extract (0.5 mg/ml; ALK-Abello).

All 49 patients had a clinical history of peach allergy (oral allergy syndrome, OAS, urticaria, angioedema, asthma and/or anaphylaxis). A large proportion of patients reported allergic reactions to Rosaceae other than peach (apple, pear, cherry, plum, apricot and/or almond), tree nuts and/or peanut, too.

Controls

Three distinct groups of control subjects were enrolled in this study.

Group A. Group A included 24 subjects monosensitized to birch pollen with a history of OAS following the ingestion of fruits and/or vegetables. These subjects were negative to grass pollen, mugwort pollen, natural rubber latex and commercial peach extract in SPT. Sera from these subjects were found to contain IgE to rBet v 1 but did not show any reactivity to rBet v 2 [27].

Group B. Group B consisted of 18 subjects monosensitized to profilin with a history of OAS following the ingestion of plant-derived foods. These subjects scored positive to most seasonal airborne allergens in SPT and were negative to commercial peach extract in SPT. Their sera were found to contain IgE specific for rBet v 2 but not for rBet v 1 [27].

Group C. Group C comprised 16 subjects sensitized to both LTP (by positive SPT with commercial peach extract) and birch pollen with or without hypersensitivity to other seasonal airborne allergens.

Clinical History and Skin Tests

A history of intolerance to vegetable foods other than Rosaceae, tree nuts and/or peanut was ascertained both in patients and controls by a thorough interview. Legumes (pea, soybean, bean, lupine, chick pea and lentil), Apiaceae (carrot, fennel, celery and parsley), potato, tomato, garlic, onion, rice, maize, kiwi, banana, gourds (melon, watermelon, cucumber and zucchini), fig, orange, grapes, eggplant, artichoke, pineapple, beer, lettuce cabbage, dates, mustard, poppy seed and sesame seed were considered. A history of typical OAS (immediate itching of the lips, oral and/or

pharyngeal mucosa), urticaria, angioedema, asthma and/or anaphylaxis following the ingestion of a certain food was considered as a probable allergic reaction.

Patients and controls underwent SPT with a large panel of commercial vegetable food extracts (other than those frequently causing adverse reactions in LTP-allergic subjects), including rice, maize, pea, bean, soybean, carrot, celery, plum, melon, banana, tomato and orange (1:20 wt/vol; Dome-Hollister/Stier, Spokane, Wash., USA, or ALK/Abello); SPTs with fresh material were carried out in those reporting allergic reactions to foods other than those tested by commercial SPT. All skin tests were carried out on the volar side of the forearm with sterile, disposable 1-mm-tip lancets (Stallergenes). Readings were taken after 15 min. Reactions were expressed as the mean wheal diameter (adding the longest diameter to the orthogonal diameter and dividing by 2). A mean wheal diameter of 3 mm or more was considered positive [28]. Histamine (10 mg/ml) and saline were used as positive and negative controls, respectively.

SDS-Page and Immunoblot Analysis

In all LTP-allergic patients, the absence of IgE reactivity to foods that did not induce any adverse reaction according to clinical history nor any wheal-and-flare reaction in SPT was confirmed by immunoblot analysis. These foods were homogenized and extracted as previously described [29]. Protein concentration of extracts was measured according to Bradford [30]. Electrophoresis of all extracts (25 µg/lane) was carried out in a 10% polyacrylamide precast gel (Nupage Bis-Tris, Novex, Prodotti Gianni, Milan, Italy) at 180 mA for 1 h. The resolved proteins were transferred for 1 h onto a nitrocellulose membrane according to Towbin et al. [31]. The membrane was saturated with 0.1 M Tris-buffered saline containing 5% fat-free milk powder and incubated for 16 h at 4°C with sera (800 µl with 200 µl of saturation buffer). After three washings, bound specific IgE was detected by peroxidase-conjugated anti-human IgE antibodies from goat (1:1,000 in saturation buffer; Biospecific, Emeryville, Calif., USA) and using an ECL Western blotting kit (Amersham, Milan, Italy) as substrate. IgE reactivity against the foods challenged was assessed by immunoblot analysis. Due to serum shortage, a pool of sera from 11 patients and individual sera from 3 further patients were tested. All these patients showed a broad cross-reactivity to botanically unrelated plant-derived foods including Rosaceae, maize, rice, tree nuts, peanut and legumes. A pool of sera from non-atopic individuals was used as a negative control.

Oral Challenges

Oral challenges with foods that were both reportedly tolerated by all patients and always negative in SPT were also performed in patients monosensitized to LTP. Challenges were carried out in an open fashion; patients were asked to ingest about 50 g of each food at the allergy clinic on separate days, and to remain under control at the clinic for 2 h afterwards. Patients were carefully instructed to report any adverse reaction immediately. OAS, urticaria, angioedema, asthma and/or anaphylaxis were considered as a positive response. All patients gave a written informed consent before the start of the procedure.

Statistics

Proportions were compared by χ^2 test with Yates' correction. Probability values <5% were considered significant.

Results

Offending foods other than Rosaceae, nuts and peanuts that were reported by LTP-allergic patients as well as SPT results are shown in table 1. Altogether, the prevalence of reported allergy to individual foods was comparatively low, ranging from 0% for several foods to 16% (fennel). SPT (with commercial extracts or with fresh foods) scored positive in 32/34 (94%) patients with a history of adverse reactions, thus showing excellent sensitivity; only 2 patients reporting tomato and kiwi allergy, respectively, had negative SPTs to the offending foods. In contrast, specificity of SPT was poor, since many patients showed skin reactivity to foods that they reportedly ate without any problem, including legumes, onion, garlic, celery, orange and cereals.

Since all LTP-allergic patients reported tolerance to carrot, potato, banana and melon, and these foods also scored negative in SPT in all cases, these four foods were chosen for both open oral challenges and immunoblot analysis. On open oral challenge to 50 g of fresh raw carrot, banana pulp and melon pulp and of peeled boiled potato was negative in all patients.

For immunoblot analysis, extracts of fresh peach peel, melon pulp, banana pulp, raw whole carrot and boiled, peeled potato were used at a starting concentration of 735, 745, 440 and 616 µg/ml and 1.8 mg/ml, respectively. Both the patients' serum pool and sera from the 3 individual patients did not show any reactivity at 10 kDa against carrot, potato, banana and melon; in contrast, the serum pool showed an evident IgE reactivity to a 10-kDa allergen in peach extract (fig. 1) and also some IgE reactivity at molecular weights >10 kDa against peach, banana and potato. The normal serum pool did not react against any of the food extracts (fig. 1). The SDS-PAGE profile of peach, banana, melon, carrot and potato extracts is shown in figure 2. Peach extract showed a clear-cut band at 10 kDa that could not be observed in the other four foods.

A comparative analysis of the reported reactivity to these four foods in patients and controls is shown in table 2. Carrot allergy was reported by 17% of the controls sensitized to Bet v 1 ($p < 0.05$ vs. LTP-monosensitized patients) and by 17% of controls sensitized to Bet v 2 ($p < 0.05$ vs. LTP-monosensitized patients); moreover, profilin reactors showed a high prevalence of allergy to both melon ($p < 0.001$ vs. LTP-monosensitized patients) and banana ($p < 0.001$ vs. LTP-monosensitized patients). Interestingly, melon allergy was reported by 25% of group C controls (subjects sensitized to both LTP and either Bet

Table 1. Reported offending foods (other than Rosaceae, tree nuts and peanut) and SPT results in 49 patients monosensitized to LTP

	History+	History +		History -	
		SPT+	SPT-	SPT+	SPT-
Pea	1 (2%)	1	0	22	23
Soybean	0	–	–	30	19
Bean	1 (2%)	1	0	28	17
Lupine	3 (6%)				
Lentil	3 (6%)				
Chick pea	1 (2%)				
Celery	4 (8%)	4	0	20	25
Carrot	0	–	–	0	49
Fennel	8 (16%)	5	0	2	7
Parsley	2 (4%)				
Potato	0	–	–	0	49
Garlic	0	–	–	8	28
Onion	2 (4%)	2	0	14	25
Tomato	5 (10%)	4	1	11	32
Lettuce	2 (4%)				
Mustard	2 (4%)				
Kiwi	3 (6%)	2	1	3	9
Banana	0	–	–	0	49
Melon	0	–	–	0	48
Watermelon	0				
Cucumber	0				
Zucchini	0				
Fig	5 (10%)				
Orange	2 (4%)	1	0	8	3
Tangerine	0				
Lemon	1 (2%)				
Grapes	3 (6%)				
Pistachio	1 (2%)				
Dates	1 (2%)				
Eggplant	2 (4%)				
Artichoke	1 (2%)	1			
Pineapple	2 (4%)	1			1
Persimmon	0				
Poppy seed	0	–	–	2	2
Sesame seed	0	–	–	1	3
Rice	2 (4%)	2	0	19	23
Maize	6 (12%)	6	0	18	18
Beer	2 (4%)	2	0	0	0

v 1 and/or profilin) versus 0% of patients monosensitized to LTP ($p < 0.01$).

SPT results in patients monosensitized to LTP and group C controls are compared in table 3. No statistical difference was observed between the two groups except for carrot, potato, banana and melon that scored negative in patients and induced a wheal-and-flare reaction in 60, 33, 20 and 31% of controls, respectively. This difference was statistically significant in all cases.

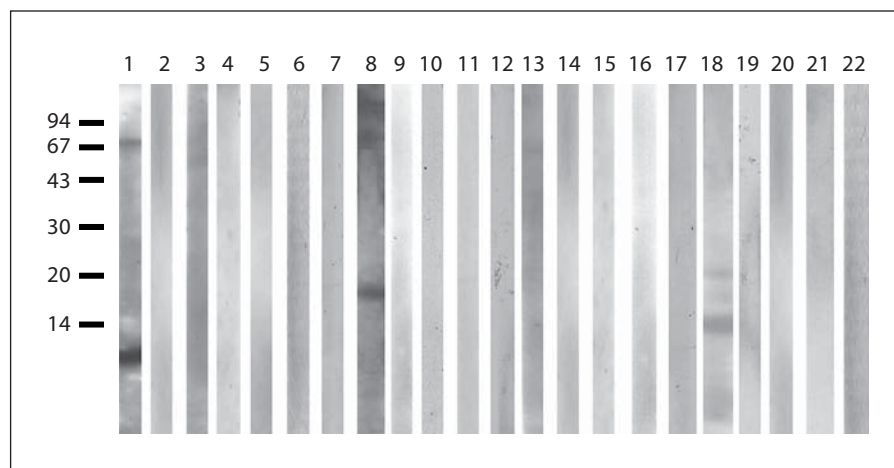


Fig. 1. Immunoblot analysis: IgE reactivity of a pool of sera from 11 patients considered as monosensitized to LTP against peach, melon, banana, carrot and potato (lanes 1, 3, 8, 13 and 18, respectively); IgE reactivity to the same plant-derived foods of a pool of sera from non-atopic individuals is shown in lanes 2, 7, 12, 17 and 22, respectively. IgE reactivity of 3 individual sera (B, C and R) from LTP-monosensitized patients against the same foods is shown in lanes 4–6 (melon), 9–11 (banana), 14–16 (carrot) and

19–21 (potato), respectively. The reactivity of the patients' serum pool to a peach allergen at 10 kDa is clearly visible whereas no IgE reactivity at the same molecular weight is observed against the other foods. The patients' serum pool shows also some IgE reactivity at molecular weights >10 kDa against peach (lane 1), banana (lane 8) and potato (lane 18). Sera from individual patients as well as the negative serum pool do not show any IgE reactivity against the four study foods.

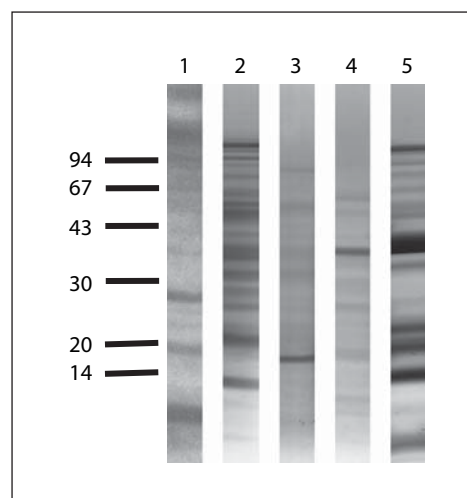


Fig. 2. SDS-PAGE of peach (lane 1), banana (lane 2), carrot (lane 3), melon (lane 4) and potato (lane 5) extracts. The gel is stained with Coomassie colloidal blue (Invitrogen, Milan, Italy). A protein band at 10 kDa is clearly visible in peach extract; no similar bands can be observed at the same molecular weight in the extract from the other foods.

Discussion

LTP is a widely cross-reacting allergen, and a proportion of patients sensitized to this protein experience allergic reactions upon the ingestion of an array of botanically unrelated vegetable foods [12–24]. Due to the extreme heat and pepsin stability of this protein, such reactions are frequently severe. This study aimed to de-

tect some plant-derived foods to be regarded as possibly safe for all patients allergic to LTP. To this end, the tolerance to a number of popular foods other than Rosaceae, tree nuts and peanut (which are the main culprits for adverse reactions in patients sensitized to LTP) [13, 25] was assessed by interview in a large group of patients probably monosensitized to LTP. Clearly, the admission criteria adopted to select patients did not rule out sensitization to

Table 2. History of allergy to some vegetable foods in patients and controls

Food	Patients (n = 49)	Control groups		
		A (n = 24)	B (n = 18)	C (n = 16)
Carrot	0	4 (17%)*	3 (17%)*	1 (6%)
Potato	0	0	0	1 (6%)
Banana	0	2 (8%)	12 (67%)*	1 (6%)
Melon	0	0	10 (56%)*	4 (25%)*

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Group A = Controls monosensitized to Bet v 1; group B = controls monosensitized to profilin; group C = controls sensitized to both LTP and birch pollen.

Table 3. Positive SPT results in LTP-allergic patients and LTP-allergic controls

	Patients	Controls (group C)	p
Pea	23/46 (50%)	11/16 (69%)	NS
Soybean	30/49 (61%)	13/15 (87%)	NS
Bean	29/46 (63%)	13/15 (87%)	NS
Celery	24/49 (49%)	10/16 (63%)	NS
Carrot	0/49 (0%)	9/15 (60%)	<0.001
Potato	0/49 (0%)	4/12 (33%)	<0.001
Garlic	8/36 (22%)	4/13 (31%)	NS
Onion	16/41 (39%)	6/14 (43%)	NS
Tomato	15/48 (32%)	8/16 (50%)	NS
Banana	0/49 (0%)	3/15 (20%)	<0.025
Melon	0/48 (0%)	5/16 (31%)	<0.001
Rice	21/44 (48%)	12/15 (80%)	NS
Maize	24/42 (57%)	12/13 (92%)	NS

allergens other than Bet v 1-like protein, profilin or latex-homologous proteins, e.g. cross-reactive carbohydrate determinants (CCD) or thaumatin-like proteins (TLP). However, it is unlikely that this fact may have biased the results of this study in some way. It is generally accepted that CCD have little or no clinical relevance, and the clinical relevance of TLP, which have been detected as the offending allergen in some cases of apple or cherry allergy, is still debated and difficult to evaluate; in the recent EU-funded study SAFE carried out on about 400 apple-allergic patients, TLP hypersensitivity was virtually always associated with sensitization to other allergens (Mal d 1, profilin or LTP). Not surprisingly, the en-

rolled patients reported adverse reactions to a rather large spectrum of vegetable foods although individual foods induced symptoms in a small proportion of cases, thus confirming that foods other than Rosaceae, nuts and peanuts are harmful only in a subset of LTP-allergic patients [25]. On the whole, in previous studies, SPT with commercial food extracts or with fresh foods proved very sensitive in LTP-allergic patients [26]. The frequent finding of a positive SPT in reportedly tolerant patients should be regarded as the probable consequence of immunologic cross-reactivity with peach LTP and, hence, as a marker of potential clinical risk. Therefore, in view of the aims of the present study, only four foods that were both reportedly tolerated and negative on SPT, namely carrot, potato, banana and melon, were finally considered for oral challenges. These foods were tolerated by all the patients. In view of the study design, we cannot exclude that some potentially safe foods other than banana, carrot, melon and potato have been overlooked. Further studies will better clarify this point. Interestingly, carrot, potato, banana and melon frequently caused OAS and scored positive in SPTs in all control groups, including group C (i.e. subjects sensitized to both LTP and Bet v 1 and/or Bet v 2) thus showing the relevance of allergens other than LTP in clinical allergy to these foods. In effect, previous studies showed that both a Bet v 1-homologous protein (namely, Dau c 1) and a profilin are able to induce clinical symptoms in carrot-allergic subjects [32, 33], that profilin is a major melon allergen [34] and that profilin-sensitized patients frequently experience OAS following the ingestion of banana [27]. The possibility that commercial extracts of carrot, potato, banana and melon used to carry out SPT did not contain LTP due to a loss of this protein during extraction procedures seems rather unlikely. LTP is extremely resistant to both heat and pepsin digestion [12, 35, 36] and has been detected in a number of commercial vegetable food extracts [13, 26]. The association of clinical tolerance and negative SPT rather suggests the absence of LTP in the edible part of carrot, potato, banana and melon. Although LTP has been isolated from carrot plant [37] and in a previous study a recombinant carrot LTP was able to inhibit patients' seroreactivity to LTP from a number of plant-derived foods [12], the findings in our patients monosensitized to LTP are in keeping with the results of a recent study that did not detect LTP in carrot root extract [38]. Based on our immunoblot findings it is possible to hypothesize that the same also holds true for the other three vegetable foods studied here. In effect, in previous studies, Grob et al. [39] did not detect any LTP-allergic subject among patients with clin-

ical allergy to banana not sensitized to natural rubber latex, no patient allergic to melon, watermelon and other members of the gourd family without clinical pollinosis have been observed so far [40–43], and no LTP hypersensitivity has been observed in potato-allergic children [44]. Since LTPs are generally located in the epidermal layers of plant-derived foods we might not ingest LTP in banana, melon and potato as these foods are usually con-

sumed in peeled form. Further studies will establish whether this hypothesis is correct.

Recent studies from this allergy clinic demonstrated that legumes are safe for LTP-allergic patients [45]. This study suggests that carrot, banana, potato and melon should also be regarded as safe in patients allergic to LTP who are not sensitized to birch pollen or to natural rubber latex allergens.

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