

PEACH FUZZ CONTAINS LARGE AMOUNTS OF LIPID TRANSFER PROTEIN: IS THIS THE CAUSE OF THE HIGH PREVALENCE OF SENSITIZATION TO LTP IN MEDITERRANEAN COUNTRIES?

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Background: Allergy to lipid transfer protein (LTP) is quite common in the Mediterranean countries but virtually absent in Northern Europe. The reasons for this latitude-dependent distribution are unclear. One hypothesis is that peach, the primary sensitizer to LTP, may lose in part its allergenicity as a consequence of treatments (handling, brushing, washing, and packaging) preceding marketing in Northern European. Peach surface fuzz might represent a potential vehicle of LTP.

Objective: To detect LTP in peach fuzz, and compare IgE reactivity to peach fuzz and peel of sera from LTP-allergic patients.

Methods: IgE reactivity to peach peel and peach fuzz extract was measured by ELISA using sera from 2 LTP-allergic patients. Purified peach LTP was used in inhibition studies.

Results: Both sera strongly reacted both to peach peel and fuzz but reactivity to fuzz was stronger than to peel. Pre-absorption of one serum with peach LTP caused an 87% reduction of IgE reactivity to peach fuzz extract.

Conclusion: Peach fuzz contains large amounts of LTP and might be a potential vehicle of this allergen causing sensitization in genetically predisposed subjects. Fuzz loss during pre-marketing handling of peaches might be at the basis of the geographic differences that characterize allergy to LTP.

Key-words: Food allergy - Lipid transfer protein - Peach.

INTRODUCTION

Non-specific lipid transfer proteins (LTP) are ubiquitous allergens in plant-derived foods belonging to pathogenesis related proteins group 14 (1). Due to their extreme stability to heat and to pepsin digestion (2-5) these proteins behave as fully developed food allergens. Although recent studies have demonstrated that LTP-allergic patients may be sensitized to a large number of plant-derived foods due to cross-reactivity between LTP in botanically unrelated sources (2, 6), it is generally believed that the primary sensitizer to LTP is the peach. Such concept is based on the following observations:

➤ Virtually no LTP-allergic patient not sensitized to peach has been reported so far; the few that were negative on SPT with peach (7) had been tested with fresh fruit only, a method that in this particular type of food allergy is less sensitive than SPT with commercial food extract (8);

➤ Peach-allergic, LTP-hypersensitive patients frequently tolerate other Rosaceae as well as non-Rosaceae vegetable foods and are negative on SPT and RAST with these foods;

➤ Cross-reactivity to non-Rosaceae vegetable foods is strongly dependent on the level of IgE to peach LTP (9). One of the most surprising aspects of this kind of food allergy is its geographical distribution. Virtually all reports of LTP allergy come from Southern Europe, a trend that has been confirmed by the EU-funded project SAFE that was recently carried out in 4 European countries, namely Austria, the Netherlands, Spain, and Italy (10). The first case report of allergy to LTP in Northern Europe has appeared only recently in the literature (11). The reasons for this latitude-dependent distribution are unclear. A preliminary study of baby and infant food consumption habits in different European Countries did not show any clear cut-difference in the presence of commercial products containing peach (12). Some Spanish allergologists suggest that the reason for this phenomenon might be that peach is introduced very early in Spanish children diet (Dr Montserrat Fernandez-Rivas, personal communication). However, most LTP-allergic patients seen at this allergy center so far were adults who developed their hypersensitivity in adult age. An explanation based uniquely on genetic factors seems unlikely. Although their marketing season might be slightly shorter than in Mediterranean countries peaches are largely popular in Northern Europe; as a consequence one can assume that people living in northern

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Europe should be exposed to fresh peaches to a similar extent as people living in Mediterranean countries. Thus, we considered the possibility that pre-marketing treatment, including handling, packaging, and shipping might provoke some changes in the freshly picked fruits resulting in a marked reduction of allergenicity before marketing in Northern Europe.

LTP allergic patients frequently report local urticaria or pruritus upon contact with fresh peaches; in a Spanish study (notably a geographic area where birch trees are virtually absent and the prevalence of allergy to LTP is very high) 61% of 70 peach-allergic subjects had contact urticaria from peaches, and this was the most frequent symptom of peach allergy after oral allergy syndrome (13). Interestingly, in our experience (2,6) patients with peach-induced contact urticaria do not experience similar symptoms after handling botanically related fruit such as nectarines or other *Rosaceae* such as apple, pear, cherry or plum suggesting possible differences in the very surface layers of these fruits. Freshly picked peaches are characterized by abundant surface fuzz. Fuzz adheres to the fingers if the fruits are slightly rubbed, and can be nearly totally removed if they are washed and dried with a soft cloth. Italy is one of the main peach suppliers to northern Europe. We performed a field investigation in the surroundings of Ferrara, a relevant growing area for peaches in Italy. This revealed that peaches grown by large producers are refrigerated soon after picking and subsequently brushed, washed and dried in order to reduce surface fuzz before being packaged and exported or sold in the supermarkets. At the end of this process the fruits have lost most fuzz. In contrast, peaches produced by small producers are picked, (inconstantly) refrigerated and frequently sold in local markets without undergoing brushing, washing and drying. Moreover, interestingly, in the surroundings of Ferrara many peach pickers report contact urticaria and have to wear gloves during their working hours. In contrast no peach allergies are reported among personnel working into plants where peaches are automatically washed, weighed, sized up, and packaged. Thus, peach fuzz might theoretically play a role as a carrier able to propagate the allergen that totally lacks in peaches sent abroad as well as in other *Rosaceae*. We assessed whether peach fuzz contains LTP and compared IgE reactivity of sera from LTP-allergic patients to peach fuzz and peach peel.

PATIENTS AND METHODS

Preparation of allergenic material

Freshly picked ripe peaches showing abundant surface fuzz were provided by CIV (Consorzio Italiano

Vivaisti) for the study. Fruits' fuzz was carefully removed from 15 peaches (about 200 g weight each) using a razor blade paying much attention to avoid any damage to the peel and to squeeze the fruit. Afterwards peach peel was carefully removed. Collected materials (fuzz and peel) were homogenized separately and used for subsequent analysis.

Peach fuzz was extracted (5%,w/v) in 0.1M phosphate-buffered saline, pH 7.4 (PBS) shaking over-night at 4°C. Peach peel was extracted as previously described by Bjorksten (14). Protein content of all extracts was determined according to Bradford (15) using commercial BioRad Protein Assay Dye Reagent (Bio Rad, Milan, Italy) and BSA as reference standard. The protein concentrations of peach peel and fuzz extracts were 481 mg/ml and 145 mg/ml, respectively.

SDS-PAGE and Immunoblot analysis

Peach peel and fuzz extracts underwent SDS-PAGE analysis (25 µg/lane), as previously described (2). IgE reactivity against both peach peel and fuzz extracts of a pool of sera from patients monosensitized to LTP was assessed by immunoblot analysis. Electrophoresis of both extracts (25 µg/lane) was carried out in a 10% polyacrilamide 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.* (16). The membrane was saturated with 0.1 mol/L tris-buffered saline containing 5% fat-free milk powder and incubated for 16h at 4°C with the serum pool diluted 1:2. After 3 washings, bound specific IgE were detected by peroxidase-conjugated anti-human IgE antibodies from goat (1:1000 in saturation buffer) and using an ECL western blotting kit (Amersham, Milan, Italy) as substrate.

Elisa

The presence of LTP in collected material was assessed by ELISA. Sera from 2 patients previously shown (6) as being monosensitized to LTP by immunoblot analysis and with a clinical history of peach-induced urticaria/angioedema were used. The serum from 1 normal subject served as control. In direct ELISA assays 3 µg of protein of either peach peel extract or peach fuzz extract per microtiter plate well were used. 100 µl of undiluted serum were added per well. In inhibition studies serum from patient 1 (diluted 1:3) was absorbed with 5 µg of purified peach LTP (2) or 10 µg of house dust mite extract before the analysis (0.1 µg peach hair protein/well). Dried *D. pteronyssinus* mites were obtained from CSL (Melbourne,

Australia). Mites were extracted for 12 hours in PBS at 4°C (5% w/v extract). The extract was filtered through a 0.22-µm membrane. Results were expressed as optical density units (OD). Values less than 500 OD were considered negative.

RESULTS

SDS-PAGE and immunoblot analysis are shown in figure 1. Both peach peel and fuzz showed a large amount of protein at about 10 kDa (lanes 1 and 2, respectively). The immunoblot analysis showed a strong IgE reactivity against skin and fuzz LTP (lanes 3 and 4, respectively). ELISA results are shown in table 1. Sera from both LTP-allergic patients showed a strong IgE reactivity against both peach peel and peach fuzz. Interestingly, in both cases fuzz reactivity was significantly stronger than peel reactivity. The normal serum did not show any IgE reactivity to peach fuzz or peel.

In inhibition studies (table 2) pre-absorption of patient 1 serum with peach LTP caused an 87% reduction of IgE reactivity to peach fuzz extract; in contrast, pre-absorption with an uncorrelated extract (house dust mite extract) did not induce any reduction in peach reactivity.

DISCUSSION

The explanations provided so far for the geographic distribution of allergy to LTP, including earlier exposure of babies to foods containing peach or larger consumption of peaches in countries where this aller-

gy is frequent, are all but convincing. Clearly this allergy is prevalent in areas where peaches are grown, and exposure is always a major risk for sensitization. Nonetheless there are no data suggesting that within Mediterranean countries this allergy is exclusively present or more frequent in the surroundings of peach orchards. Moreover, LTP is still present in peel after pre-marketing treatment as shown by a number of previous studies. Our field investigations revealed that peaches produced by large producers to be sold in supermarkets or exported in northern Europe undergo treatments aiming to eliminate surface fuzz, whereas peaches produced by small producers that are sold directly in itinerant markets do not undergo washing and brushing. Fuzz can be quite easily detached from peach peel, and in the present study we demonstrated that peach fuzz contains large amounts of LTP. Therefore our findings suggest that peach fuzz might be a potential vehicle able to sensitize genetically predisposed subjects to LTP. Whether this occurs via the inhalant, transdermal, or gastrointestinal route has still to be established. The fact that no LTP allergic patient seen at this allergy center during the last 10 years reported rhinoconjunctivitis and/or bronchial asthma in the presence of fresh peaches suggests that the former possibility is unlikely. Many patient report contact urticaria as the first symptom of peach allergy (13) which suggests that sensitization might occur via the transdermal route.

In conclusion our results might offer a possible explanation for the geographic differences that characterize allergy to lipid transfer protein. If our hypothesis is

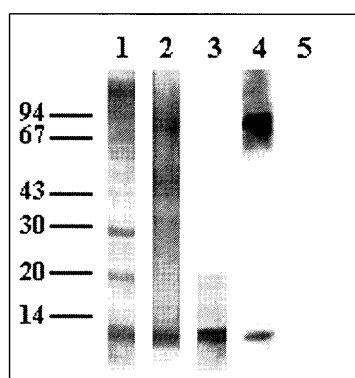


Figure 1: SDS-PAGE of peach peel (lane 1) and fuzz (lane 2) extracts, and IgE reactivity of a pool of sera from LTP allergic patients to peach peel (lane 3) and fuzz (lane 4) extracts. Lane 5: IgE reactivity to peach fuzz of a normal serum. Patients' serum pool shows marked IgE reactivity at 10 Kda, the molecular weight of LTP.

	Peach peel	Peach fuzz
Patient 1	2796	>4000
Patient 2	1807	3326
Control serum	326	448

Table 1: IgE reactivity to peach peel and fuzz by ELISA.

Values are expressed as OD (negative if < 500).

	Uninhibited	Pre-absorbed with Peach LTP	Pre-absorbed with House dust mite
Patient 1 serum 1:3	1396	180 (87%)	1429 (0%)
Normal serum	320		

Table 2: ELISA inhibition studies. IgE reactivity to peach fuzz.

Values are expressed as OD (negative if < 500). Percentages in parentheses represent the degree of inhibition.

correct de-novo sensitization rate to this allergen in Mediterranean countries might be strongly reduced if surface fuzz was systematically removed before peaches are put on the market.

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