

Respiratory Allergy to Lipid Transfer Protein

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Established Facts

- Allergy to LTP is the most frequent primary food allergy in the Mediterranean countries, but is rather rare in Northern Europe.
- The reasons for such geographical distribution are still unclear.
- Recent studies show that peach fuzz contains large amounts of lipid transfer protein.

Novel Insights

- The results of the present study prompt that sensitization to LTP might occur via the respiratory tract, particularly in areas where peaches are grown and fresh, unprocessed peaches with abundant fuzz are available on the market.

Key Words

Food allergy · Lipid transfer protein · Peach · Respiratory allergy

Abstract

Background: Due to unclear reasons, allergy to lipid transfer protein (LTP) is frequent in Mediterranean countries but rare in Northern Europe. **Objective:** We report a paradigmatic case of primarily airborne sensitization to LTP that might explain the geographical distribution of this type of food allergy. **Methods:** A 21-year-old woman began having severe

perennial rhinitis 6 months after she started working in a wholesale fruit storehouse in Southern Italy where large amounts of fruits, including peaches, were handled; symptoms subsided when she left the workplace for >5 days and relapsed as soon as she was back at work. Later on, she developed severe food allergies to peach, hazelnut, peanut, apricot, plum and tomato. The patient underwent a nasal challenge with peach peel extract, and IgE reactivity was assessed by immunoblot analysis. **Results:** In vivo and in vitro analyses showed sensitivity to LTP. The nasal challenge with peach peel extract (6 µg protein) induced acute, severe respiratory symptoms. On immunoblot with peach peel ex-

tract patient's serum reacted uniquely against LTP, as demonstrated by inhibition assays with the recombinant peach protein. **Conclusion:** LTP may induce sensitization via the respiratory tract due to inhalation of air-dispersed food particles, and this may precede the onset of food allergy. If this way of sensitization were effective in the majority of LTP allergic patients (e.g. by exposure to peaches showing intact fuzz in areas where peaches are grown and directly sold on the market) our findings could explain the strange geographical distribution of this type of food allergy.

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Introduction

Lipid transfer protein (LTP), the major allergen in *Rosaceae* in areas where birch pollen is not present [1–5], has been detected in a large number of vegetable sources and is presently recognized as a widely cross-reacting plant pan-allergen. LTP is characterized by an extreme stability upon both heat processing [6–9] and enzyme digestion [10, 11], which makes it a potentially hazardous allergen. In effect, as a difference from patients with pollen-food allergy syndrome, LTP-sensitized patients frequently report severe clinical symptoms after eating processed foods, including commercial juices [9], jams, and fruit-containing cookies, sweets, wine and beer [12, 13]. Allergy to LTP is quite common in Mediterranean areas but rare in Northern Europe [12, 14]. The reasons for such geographic distribution are still debated. The peach represents the most likely primary sensitizer to LTP in most instances [15]. Recently, based on the high prevalence of contact urticaria among fruit pickers and workers in the food industry in areas where peaches are grown, one study suggested that repeated skin contact with peaches with intact fuzz (notably, peach fuzz is almost totally removed from the fruits by industrial brushing, drying, and packaging procedures that precede export of the fresh fruits from growing areas to supermarkets and foreign countries) might be the possible cause of sensitization to LTP via the dermal route [16]. On the other hand, other authors hypothesized that LTP allergy might start as respiratory sensitization to pollens containing LTP [17, and revised in 18]. In the present study, we report a case suggesting that inhalation of fruit-derived LTP might represent a possible route of sensitization.

Patient and Methods

Case Report

A 21-year-old woman who had been working as an administrative employee during the last 3 years into a wholesale fruit storehouse in Southern Italy presented with a 7-year history of mild seasonal rhinitis that had become severe and perennial during the last 2.5 years; further, she had experienced several episodes of generalized urticaria/angioedema following the ingestion of peach, hazelnut, peanut, apricot, plum and tomato, and of contact urticaria triggered by peach during the last 2 years. Respiratory symptoms showed a poor response to local steroids and systemic anti-histamines. Large amounts of fruits, including peaches, were handled in the storehouse. The woman did not have direct skin contact with the fruits; nonetheless, she was largely exposed to the environment's dusts because her workplace was an open-plan one.

Skin prick tests (SPT) with commercial extracts (Abello, Lainate, Italy) of both seasonal (grass, mugwort, ragweed, pellitory, birch, olive, and cypress) and perennial airborne allergens (house dust mite, cat and dog dander, and *Alternaria tenuis*) showed hypersensitivity to grass and mugwort pollen. SPT with commercial extracts of peach, walnut, peanut, tomato, orange, banana, and natural rubber latex scored strongly positive for peach, and weakly positive for walnut and peanut.

Specific IgE measurements (Phadia CAP system) showed high levels of IgE specific for peach (8.52 kUa/l) and surprisingly low levels of IgE to grass pollen (0.6 kUa/l) and olive tree (0.68 kUa/l); IgE levels to rPru p 3 (peach LTP, courtesy of Phadia) were 10.05 kUa/l. No specific IgE to walnut, natural rubber latex, and weeds was found.

The patient was asked to leave the workplace for at least 10 days. On day 3 she already felt much better and was able to stop the use of antihistamines. She totally recovered on day 5. Rhinitis recurred as soon as she went back at work. The patient is presently working in the same place very sporadically and controls symptoms by taking triamcinolone 2 mg and chlorfeniramine 4 mg orally at least 1 h before exposure.

Peach Peel Extract

Peach peel (including peel plus fuzz) was extracted as previously described by Bjorksten et al. [19]. Protein content was determined according to Bradford [20] using a commercial BioRad Protein Assay Dye Reagent (Bio Rad, Milan, Italy) and BSA as reference standard. The protein concentration of peach peel extract was 168 µg/ml.

Nasal Provocation with Peach Peel Extract

A nasal provocation test was carried out using a peach peel extract solution (60 µg protein/ml) prepared by Lofarma, Milan, Italy. After an informed written consent was obtained, a single puff (100 µl, corresponding to 6 µg peach peel protein) was administered in the right nostril. The same solution was administered in one nostril of a healthy volunteer (F.B.).

In vitro Studies

Patient's serum IgE reactivity to peach peel extract was assessed by immunoblot analysis under reducing conditions. Extract was mixed with LDS sample buffer (Nupage Bis-Tris, Novex, Prodotti Gianni, Milan, Italy) and 5% b-mercaptoetha-

nol. The sample was then denaturated by heating at 100°C for 5 min.

Electrophoresis of extract (25 mg/lane) was carried out in a 10% polyacrylamide precast gel (Nupage Bis-Tris) at 180 mA for 1 h. The resolved proteins were transferred for 1 h onto a nitrocellulose membrane according to Towbin et al. [21]. The membrane was saturated with 0.1 mol/l tris-buffered saline containing 5% fat-free milk powder and incubated for 16 h at 4°C with serum (700 µl of the serum pool and 500 µl of saturation buffer). After 3 washings, bound specific IgE were detected by peroxidase-conjugated anti-human IgE antibodies from goat (Biospecific, Emeryville, Calif., USA diluted 1:3500 in saturation buffer) and using an ECL western blotting kit (Amersham, Milan, Italy). In inhibition studies, patient's serum was pre-absorbed with 5 µg of recombinant peach LTP of peach obtained as previously described [22] or with 5 µg of bovine serum albumin (BSA).

Results

Nasal Provocation Test

About 10 s after the administration, the patient began sneezing repeatedly. About 1 minute later the right conjunctiva became intensely red and tearing, and after 5 min dysphagia along with total nasal block occurred. At this point epinephrine 0.3 mg was given intramuscularly along with intravenous corticosteroids (betamethasone 6 mg), oral antihistamine (cetirizine 10 mg) and olopatadine eye drops. Symptoms ameliorated in about 30 min. The nasal administration of the same peach peel extract solution to the non allergic control did not elicit any adverse reaction.

Immunoblot Analysis

Patient's IgE reactivity to peach peel is shown in figure 1. The patient reacted uniquely against a protein at about 10 kDa. Inhibition studies showed that this was the peach lipid transfer protein.

Discussion

Airborne allergy as well as sensitization to foods is not uncommon. Lipid transfer protein has been detected as the allergen responsible for severe respiratory symptoms caused by rice inhalation [23], and cases of anaphylaxis upon the first ingestion of millet or sunflower seed have been recorded among bird fanciers who most probably had become sensitized via the respiratory route [24, 25]. Further, anaphylaxis on the first ingestion of rambutan has been reported [26], and cereal-induced anaphylaxis in subjects occupationally exposed to psyllium has been

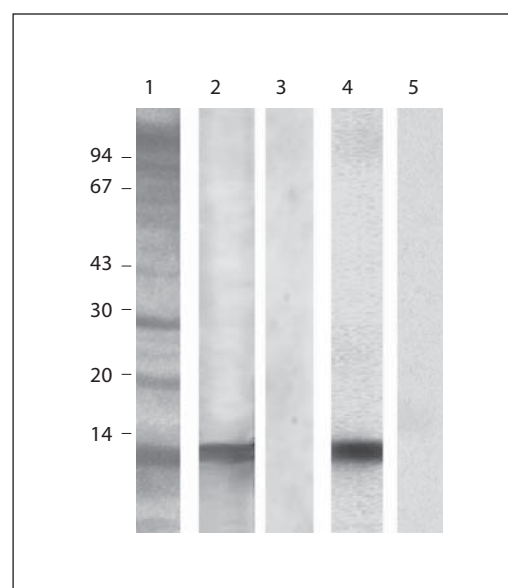


Fig. 1. SDS-PAGE and immunoblot inhibition experiments with peach peel extract. Lane 1 = SDS-PAGE of peach peel extract; lane 2 = uninhibited patient's serum; lanes 3, 4 = patient's serum pre-absorbed with 5 µg of recombinant peach LTP and 5 µg of BSA, respectively; lane 5 = serum pool from nonatopic subjects.

described [27]. Finally, very recently, lipid transfer protein has been recognized as a relevant wheat allergen in patients with baker's asthma [28]. Our patient had had grass pollen allergy for many years but she did not suffer from perennial rhinitis or from food allergy before she started working in the wholesale fruit storehouse. She developed a severe, perennial rhinitis after 6 months in an environment that was probably saturated with LTP of fruit-origin; and, most importantly, respiratory symptoms clearly preceded the onset of her food allergy. It seems therefore reasonable to suggest that workplace exposures were the primary trigger for LTP sensitization that occurred via the respiratory tract. The fact that respiratory symptoms were not associated with a particular season suggests an ongoing exposure to the allergen in an inhalable form. Whether peach fuzz dust is involved in the widespread diffusion of LTP as previous studies suggested [16] remains to be established; nonetheless, the physical/chemical features of this protein could maintain its allergenic potential over long periods of time, particularly indoors. On nasal challenge with peach peel extract, a surprisingly severe reaction occurred following exposure to an apparently low dose of allergen (of 6 µg of total peach peel protein administered LTP arguably represents

only a small fraction) suggesting a strong respiratory sensitization to LTP. This is the first report of respiratory sensitization to LTP induced by exposure to fruits that precedes the development of food allergy; the main reason why such phenomena have not been observed before is probably that, as a difference from our patient, the general population is exposed to much lower amounts of LTP. However, even minimal quantities of airborne allergens may induce sensitization though they are not able to elicit clinically appreciable respiratory symptoms; this has been previously observed with other food allergens [25, 29]. The present study suggests that, at least in some cases, primary sensitization to LTP occurs via the respiratory tract due to inhalation of air-dispersed food particles. It is tempting to speculate that if this were a major way of sensitization for LTP allergic patients, exposure to peaches showing intact fuzz (as is the case in areas where

peaches are grown and directly sold on the market without undergoing industrial processing) would at least partly explain the strange geographical distribution of this type of food allergy. Further, in view of this study and of other recent works highlighting the potential relevance of respiratory sensitization to LTP, in the future it might be helpful to measure the concentration of LTP particles in the air in special highly exposed workplaces by the use of filters (similarly to those used for pollen counts) and specifically designed in vitro tests.

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