

Why Do Lipid Transfer Protein-Hypersensitive Patients Tolerate Bean (and Other Legumes)?

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Key Words

Lipid transfer protein · Food allergy · Cross-reactivity · Heat stability · Bean

Abstract

Background: Patients allergic to nonspecific lipid transfer protein (LTP) frequently score positive on SPT with legumes but virtually never report adverse reactions eating these foods. **Objective:** This study investigated the IgE reactivity to legumes of LTP-allergic patients and aimed to establish whether legumes can be considered safe in LTP-allergic patients. **Methods:** Skin reactivity as well as clinical allergy to bean and pea were evaluated in a large cohort of LTP-hypersensitive patients. Sera from 12 patients showing clinical allergy to a number of botanically unrelated plant-derived foods and high levels of IgE to peach LTP were employed in in vitro studies (ELISA, ELISA inhibition, SDS-PAGE/immunoblot) aiming to investigate IgE reactivity to bean. **Results:** Preabsorption of patients' sera with boiled bean extract did not cause any loss of IgE reactivity to peach LTP (whereas boiled apple totally abolished it). Immunoblot analysis did not show any IgE reactivity to bean proteins at about 10 kDa, and the SDS profile of bean showed little or no staining at 10 kDa. **Conclusion:** Sera from LTP-allergic patients do not seem to show any IgE reactivity to 10-kDa

proteins in bean. Whether this is caused by epitopic differences between Rosaceae and bean LTPs or by the fact that LTP is not expressed in bean remains to be established. This study explains why virtually all LTP-allergic patients tolerate legumes and suggests that these foods should be considered safe for patients sensitized to this protein.

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Introduction

Nonspecific lipid transfer proteins (LTPs), belonging to the PR 14 family of pathogenesis-related proteins, have been identified as the major allergen in Rosaceae in subjects not sensitized to birch pollen [1–5]. LTP is ubiquitous throughout the botanical kingdom [4–9], and recent studies demonstrated both in vitro [10] and in vivo [11] that this highly conserved protein is a widely cross-reacting pan-allergen. LTP is a potentially dangerous allergen as, due to its extreme pepsin stability, it probably reaches the intestinal tract in an almost unmodified form [10]. Further, recent studies found that LTP of peach [12], apple [13], hazelnut [14, 15], cereals [16, 17] and other plants [18] is heat stable, showing that also heat-processed foods may pose a risk to patients sensitized to this protein [13, 15, 19].

Legumes (beans, lentils, peas and chick peas) represent one of the most popular vegetable foods all over the world and are a relevant source of nutrients for many populations. Patients sensitized to LTP may score positive on SPT with commercial extracts of legumes, suggesting possible cross-reactivity between LTP in legumes and in Rosaceae. In a previous study, we found that 26 out of 37 (70%) LTP-hypersensitive patients showed positive SPT with bean, pea and/or soybean [10]. However, surprisingly enough, only a small minority of LTP-allergic patients showing skin reactivity to legumes report clinical symptoms after eating such vegetables [11]. In our series, the only LTP-allergic patient who experienced an anaphylactic episode following the ingestion of string beans turned out to be sensitized to a protein other than LTP [20]. The present study investigated the immunological reactivity to bean in LTP-allergic patients and tried to assess whether legumes can be considered safe food for these patients.

Patients and Methods

Patients

Starting from a population of 607 subjects clinically allergic to plant-derived foods seen at this allergy center during the last 9 years, 51 consecutive patients presumed to be monosensitized to LTP were selected. In these subjects, sensitization to LTP was diagnosed on the basis of a positive SPT with a commercial plum extract (1:20 w/v; Dome-Hollister/Stier, Spokane, Wash., USA) or peach extract (30 µg/ml of LTP as declared by the manufacturer; ALK/Abello, Lainate, Italy). We previously demonstrated that these extracts lack labile, pollen-related allergens and virtually contain only LTP [21]. No patient was sensitized to cross-reacting allergens other than LTP as shown by negative SPT with both birch pollen (5,000 SBU/ml; Allergopharma, Reinbeck, Germany) and natural rubber latex extracts (0.5 mg/ml; ALK-Abello). Patients underwent SPT with commercial extracts of pea and bean (1:20 w/v until 2000, Dome-Hollister/Stier; thereafter, 1:20 w/v, ALK/Abello); these were positive in 23 (45%) and 26 (51%) patients, respectively. Moreover, all patients were thoroughly interviewed to ascertain their tolerance to legumes (pea and bean), and all of them (either positive or negative SPT) reported regular intake of legumes (peas, beans, lentils, chick peas and/or string beans) without any consequence.

ELISA/ELISA Inhibition Studies

Sera from 10 patients showing positive SPT with both bean and pea were selected for ELISA/ELISA inhibition studies. As shown in table 1, these patients had a clinical history of allergic reactions (oral allergy syndrome and/or systemic urticaria/angioedema and anaphylaxis) following the ingestion of a large spectrum of botanically unrelated plant-derived foods.

LTP was purified from peach peel by gel chromatography as previously described [10]. IgE to peach LTP was measured by ELI-

Table 1. Offending foods in 10 patients strongly positive on SPT with commercial extracts of legumes

1	Rosaceae (peach, almond), walnut, hazelnut, fennel
2	Rosaceae (peach, apricot), walnut, hazelnut
3	Rosaceae (peach), rice, onion
4	Rosaceae (peach), walnut, rice, maize, salad
5	Rosaceae (peach, apple, plum, cherry), hazelnut, tomato, artichoke
6	Rosaceae (peach, strawberry), hazelnut, tomato, pineapple, kiwi
7	Rosaceae (peach, apple, cherry, strawberry, almond), walnut, hazelnut, pumpkin flower
8	Rosaceae (peach), maize
9	Rosaceae (peach, apricot), walnut, hazelnut, celery, fennel, tomato, maize, pea, bean
10	Rosaceae (peach), walnut, hazelnut, celery, mustard

SA using 0.1 µg/well of natural peach peel LTP as solid-phase antigen. Boiled (100°C × 60 min) beans and apple were homogenized and extracted as previously described by Bjorksten et al. [22]. In inhibition tests, ELISA was carried out after absorption of 65 µl of serum from all 10 patients with 55 µl of either boiled bean extract (630 µg protein/ml) or boiled apple extract (450 µg protein/ml) for 150 min at 25°C [10]. The wells were then filled with 100 µl of mixture (serum and bean extract) and incubated for 120 min at 25°C. Bound specific IgE were detected by peroxidase-conjugated antihuman IgE serum 1:1,500 in saturating solution, after 1-hour incubation at 25°C. Results were expressed as optical density (OD). Based upon the results obtained with a series of normal sera, values >100 OD were considered positive. In ELISA inhibition studies, based upon the results obtained in previous experiments [11], reductions >75%, 50–75% or <50% of reactivity to peach LTP were considered as total, partial or no inhibition, respectively. Inhibition experiments using a pool of sera from LTP-allergic patients and either ovalbumin (1 mg/ml) or whole peach (420 µg/ml protein) as inhibitors were carried out as well as controls.

SDS-Page/Immunoblot

Sera from 2 further patients (A and G) showing very high levels of IgE specific for peach LTP (OD 4,000 in both cases) were employed in immunoblot experiments. Serum from patient G was also tested against a whole peach peel extract as control. As a further control, the levels of IgE specific for raw bean pulp, raw bean peel and cooked bean peel and pulp in these 2 sera were measured by ELISA using 2 µg/well of extract as solid-phase antigen.

Electrophoresis of raw bean pulp, raw bean peel and cooked bean peel and pulp (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. [23]. 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 sera. After 3 washings, bound specific IgE were detected by peroxidase-conjugated antihuman IgE antibodies from goat (1:1,000 in saturation buffer), using an ECL Western blotting kit (Amersham, Milan, Italy) as substrate.

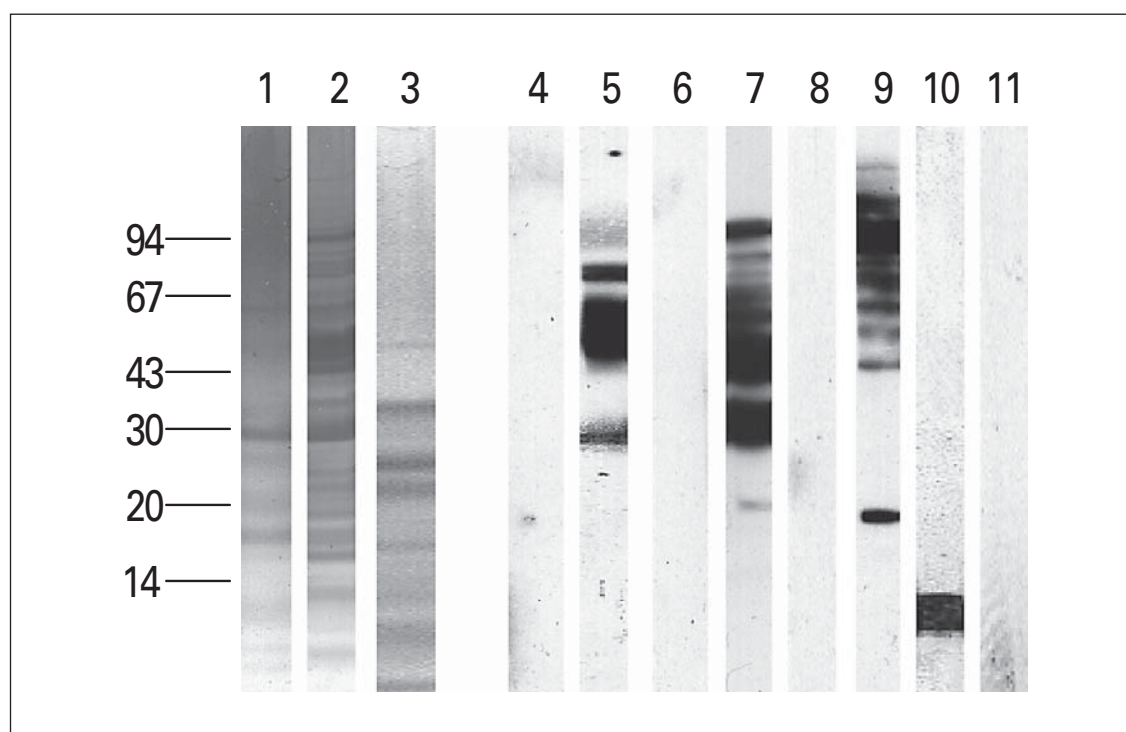


Fig. 1. SDS-PAGE results of bean extracts. Lanes 1–3: SDS-PAGE of raw bean peel, raw bean pulp and whole cooked bean. Immunoblots: lanes 4 and 5: IgE reactivity of patients A and G against raw bean peel; lanes 6 and 7: IgE reactivity of patients A and G against raw bean pulp; lanes 8 and 9: IgE reactivity of patients A and G against whole cooked bean; lane 10: IgE reactivity of patient G against whole peach extract; lane 11: IgE reactivity of a normal serum against whole cooked bean. Patients' sera do not show any reactivity against 10-kDa proteins in bean extracts. Patient G serum shows IgE reactivity against high-molecular-weight bean proteins and strong reactivity against a 10-kDa protein (LTP) in whole peach extract.

Table 2. ELISA/ELISA inhibition studies

No.	Uninhibited IgE to peach LTP	% inhibition by cooked apple	% inhibition by cooked bean
1	1,816	100	10
2	3,174	97	13
3	2,057	100	41
4	858	100	0
5	3,638	100	38
6	2,833	79	17
7	2,711	100	15
8	1,606	100	38
9	3,630	100	37
10	1,794	97	41
Pool	2,256	100 (peach)	24 (ovalbumin)

IgE levels are expressed as OD (positive if >100).

Results

Elisa Inhibition Studies

Results of ELISA inhibition experiments are shown in table 2. Sera from all 10 patients showed strong IgE reactivity to peach LTP. Preabsorption of sera with boiled apple caused a total or near-total loss of IgE reactivity to peach LTP in all cases, whereas preabsorption with boiled bean extract did not exert any inhibitory effect. Preabsorption of a pool of sera from LTP-allergic patients with whole peach extract totally abolished IgE reactivity to peach LTP, whereas no inhibition was observed following absorption with ovalbumin.

SDS-Page and Immunoblot

The SDS profile of cooked bean and immunoblot analysis are shown in figure 1. Patient A serum did not show any IgE reactivity to bean proteins. Immunoblot with se-

rum from patient G serum showed some reactivity to high molecular weight proteins, but no reactivity at about 10 kDa was observed. Patient G showed a strong IgE reactivity to peach LTP.

Bean-specific IgE levels measured by ELISA were in keeping with immunoblot results: patient A serum did not show any reactivity to raw or cooked bean (6, 96 and 30 OD units to raw pulp, raw peel and whole boiled bean, respectively), whereas the serum from patient G showed IgE reactivity against both raw and cooked bean pulp (239 and 112 OD units, respectively) but no reactivity to bean peel (58 OD units). Normal serum did not show any IgE reactivity to raw bean pulp, raw bean peel and whole cooked bean extracts (6, 6 and 35 OD units, respectively).

Discussion

Recent studies found that patients showing high levels of IgE to peach LTP show a marked propensity to cross-react to botanically unrelated plant-derived foods [24]. Moreover, as LTP is heat stable [18], hypersensitive patients may experience potentially severe allergic reactions upon ingestion of heat-processed foods, including commercial fruit juices, fresh cooked fruits, beer, peanuts and maize [10–13, 17, 19]. In view of the wide distribution of LTP throughout the botanical kingdom, it was considered possible that legumes could pose a risk for some LTP-al-

lergic patients. This was further prompted by the rather frequent skin reactivity to commercial bean and pea extracts among LTP-allergic patients. All these observations were in contrast with the very low prevalence of clinical allergy to legumes in LTP-hypersensitive subjects. The findings of this study seem to clarify this observation. While absorption of patients' sera with cooked apple extract totally inhibited their IgE reactivity to peach LTP (thus confirming cross-reactivity between peach and apple LTP), absorption with boiled bean extract did not cause any inhibition. Moreover, immunoblots did not show any reactivity against 10-kDa proteins in bean extracts. This finding is in keeping with those of a previous study of string bean-induced anaphylaxis in another LTP-allergic patient; also, in that case, no IgE reactivity at about 9–10 kDa was found on immunoblot and the patient's serum strongly reacted against proteins at higher molecular weights [20]. Altogether, this study suggests that sera from LTP-allergic patients showing positive SPT with legume extracts do not react to any homologue protein in bean. Our experiments with both raw and cooked bean as well as with boiling water seem to rule out a loss of the protein during the heat treatment. Whether the lack of reactivity is due to epitopic differences between peach and bean LTP or to the fact that LTP is not expressed in the legume remains to be established. This study suggests that bean (and possibly other legumes such as pea and lentil) should be regarded safe for LTP-allergic patients.

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