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Allergy to peanut lipid transfer protein (LTP): frequency and cross-reactivity between peanut and peach LTP

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KEY WORDS

Food allergy, lipid transfer protein, peanut

SUMMARY

Background: Lipid transfer protein (LTP) is a widely cross-reacting plant pan-allergen, and sensitized patients may react to many foods. Although peanut allergy is frequently reported by LTP-allergic patients, the evidence of the presence of an allergen homologous to LTP in peanuts is limited. **Objective:** To assess the prevalence of peanut allergy in patients sensitized to LTP, detect any allergen homologous to LTP in peanuts, and assess its cross-reactivity with peach LTP. **Methods:** Spanish and Italian adults monosensitized to LTP were interviewed for possible peanut allergy and underwent skin prick tests (SPTs) with peanut extract. Sera from 32 peanut-allergic patients were assayed for peanut-specific IgE by direct ELISA and the Real Test; the serum showing the strongest reactivity was used in immunoblot analysis. **Results:** 74/114 (65%) patients were sensitized to peanuts, and 37 (32% of the whole population; 50% of those sensitized) were clinically allergic. Positive histories were validated by open oral food challenges in 13/13 cases. No SPT-negative patients reported clinical allergy to peanuts. Thus, in this selected population, sensitivity and negative predictive value of peanut SPTs were 100%, whereas specificity and positive predictive value were poor (52% and 32%, respectively). Only 2/32 sera scored positive in both in vitro assays and 4 reacted in the Real Test alone. In immunoblot, the serum studied reacted at about 10 kDa against the peanut extract; pre-adsorption with purified peach LTP totally inhibited such reactivity. **Conclusions:** Peanut sensitization is frequent among LTP-allergic patients and is clinically significant in about 50% of cases. Peanut tolerance should be assessed in LTP-allergic patients positive on peanut SPTs. Peanut LTP seemingly shares all allergenic determinants with peach LTP.

Introduction

During the last few years, lipid transfer protein (LTP), the major allergen in the Rosaceae family for patients not sensitized to birch pollen (1-5), has acquired the status of a widely cross-reacting plant pan-allergen (6, 7). Proteins homologous to peach LTP, which is generally considered the most likely primary sensitizer to this allergen, have been detected and characterized in a number of plant-derived foods, including Rosaceae, maize, grape, tree nuts, asparagus, beer, spelt, wheat, orange, lettuce, and cabbage (4,5,8-17). It is now generally accepted that subjects sensitized to LTP may experience allergic reactions following the ingestion of a number of foods and that the likelihood of an allergic reaction to foods which are botanically distant from Rosaceae is directly related to the amount of circulating IgE specific for peach LTP (18). Surprisingly enough, peanuts, one of the foods frequently reported as offending by LTP-allergic patients (6, 7, 18), have not been extensively investigated so far. In a recent international allergy congress (19), hypersensitivity to peanut lipid transfer protein (Ara h 9) was reported, but only a single case report dealing with the clinical significance of peanut LTP, based on ELISA inhibition experiments, has appeared in medical literature (20). The present study aims to assess the prevalence of sensitization and clinical allergy to peanuts among patients sensitized to LTP and to assess the cross-reactivity between peanut and peach LTP.

Patients and methods

Patients

The clinical part of the study was carried out in 4 distinct clinical centers: 1 in Spain (Madrid), and 3 in Italy (Rome, Troina, and Paderno Dugnano). Adult patients monosensitized to LTP seen in the 4 participating centers were included in the study. Monosensitization to LTP was diagnosed in the presence of (a) an unequivocal clinical history of oral allergy syndrome and/or urticaria angioedema and/or anaphylaxis on more than one occasion following the ingestion of peaches, (b) negative skin prick tests (SPTs) with birch pollen extract, and (c) clear-cut positive SPT with a commercial peach extract containing 30 µg/ml of LTP (ALK-Abello, Madrid, Spain). Previous studies showed that this extract lacks both the Bet v 1-homologous allergen, Pru p 1, and profilin (6, 7). Further, although the presence of other unknown allergens cannot

totally be ruled out, all patients showing skin reactivity to this extract who were also investigated in vitro (by Uni-CAP with Pru p 3 or by immunoblot) in other studies reacted to Pru p 3 or to a 10 kDa protein band.

The reasons why peach was chosen as an index food are (a) that this is the fruit most frequently implicated in allergic reactions in patients sensitized to LTP and probably contains the highest amounts of this proteins (18), and (b) that, based on current knowledge, peach lacks other stable cross-reacting plant food allergens including those known to be involved in primary peanut allergy, such as seed storage proteins (legumins, vicilins, and 2S-albumins).

The prevalence of both sensitization and clinical allergy to peanuts was assessed in this population. Patients showing positive SPTs with commercial peanut extract (ALK-Abello 1:20 w/v) but tolerant to peanuts were considered as sensitized, but clinically tolerant. Those showing both positive SPTs and an unequivocal clinical history of peanut allergy were considered as clinically allergic. Italian patients from the latter group were asked to undergo an open oral food challenge (OFC) with peanuts, in order to validate the clinical history (see below). All those who accepted gave an informed written consent before the start of the procedure.

All the patients consented to participate in the study. Since examinations, SPTs, as well as OFCs were carried out as part of the routine diagnostic workup in the 4 participating centers, no Ethical Committee approval was required in Italy. Ethical Committee approval was obtained in Spain.

Twenty-three patients with other types of food allergy (8 shrimp, 5 kiwi, 4 latex-fruit allergy, 2 fish, 2 sunflower seed, 1 tomato, 1 buckwheat) underwent SPTs with the same peanut extract as controls.

Skin tests

Commercial extracts of peach and peanut (both by ALK-Abello) were used to carry out SPTs. SPT were performed on the volar side of the forearm with sterile, disposable 1-mm-tip lancets (ALK-Abello), pricking through a drop of the extract. SPTs with normal saline and histamine at 10 mg/ml were used as negative and positive controls, respectively. Readings were made 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 a positive result (21).

In vitro studies

Sera from 32 patients diagnosed as having clinical allergy to peanuts were used in the in vitro part of the study.

Peanut extract - Peanuts were ground in a mixer and then defatted by several passages in diethyl ether. The defatted powder was extracted as a 10 wt/vol suspension in 0.1M phosphate-buffered saline, pH 7.4.

Protein concentration of the extract, measured according to Bradford (22) (BioRad, Milan, Italy), was 10 mg/ml.

Detection of peanut-specific IgE and inhibition studies - IgE specific for peanuts were detected both by direct ELISA, as previously described (23), and by a reverse enzyme allergosorbent test which is not influenced by specific IgG (Real Test, Lofarma, Milan, Italy) (24) using the peanut extract prepared as described above. Both tests were performed at Lofarma Laboratories (Milan, Italy). ELISA and Real Test results were expressed as optical density (OD); based upon the mean value of 4 normal sera (< 400 OD), OD values > 800 were considered positive.

The serum showing the strongest IgE reactivity to peanuts was used in immunoblot analysis.

SDS-PAGE, immunoblot and immunoblot inhibition

Immunoblot analysis was carried out under reducing conditions. Peanut extract was mixed with LDS sample buffer (Nupage Bis-Tris, Novex, Prodotti Gianni, Milan) and 5% b-mercaptoethanol. The samples were then denatured by heating at 100°C for 5 min. Electrophoresis of extract (25 µg/lane) was carried out in a 10% polyacrilamide precast gel (Nupage Bis-Tris, Novex, Invitrogen, Milan) at 180 mA for 1 h. The resolved proteins were transferred for 1 h onto a nitrocellulose membrane according to Towbin et al. (25). 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 serum and 500 µl of saturation buffer). After 3 washings, bound specific IgE was de-

tected by peroxidase-conjugated anti-human IgE antibodies from goat (Biospecific, Emeryville, CA, USA; diluted 1:3500 in saturation buffer) and using an ECL western blotting kit (Amersham, Milan).

In inhibition studies, IgE reactivity was inhibited by pre-absorption of the serum with either 10 µg of recombinant peach LTP (26), 60 µg of the peanut extract, or 60 µg of house dust mite extract.

Statistical analysis

In order to assess the clinical usefulness of SPTs with commercial peanut extract in LTP-hypersensitive patients, sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) of SPTs were calculated by established methods (27).

Results*Frequency of peanut allergy among LTP-allergic patients and results of peanut SPTs*

The findings in each of the 4 participating centers are shown in Table 1. Out of a total of 114 adult patients monosensitized to LTP, 74 (65%) were positive in SPTs with the peanut extract, and 37 (32%) of the latter reported a convincing clinical history of peanut allergy. Thus, overall 50% of patients sensitized to peanuts (positive SPTs) were peanut allergic. No patient negative in SPTs with peanuts reported clinical allergy to them. These findings were very similar in all the participating centers with the prevalence of peanut sensitization ranging between 53% and 75%, and the prevalence of peanut allergy ranging from 27% to 39%.

Altogether, the SPTs with peanuts showed an excellent SE (100%) and NPV (100%), whereas SP and PPV were poor (52% and 32%, respectively).

No control subjects showed a positive SPT with the peanut extract.

Table 1 - Prevalence of sensitization and clinical allergy to peanuts among patients monosensitized to LTP in the 4 participating centers

| Center | No. of patients | No. positive in peanut SPTs (%) | No. with clinical allergy to peanuts (%) |
|-----------------|-----------------|---------------------------------|--|
| Paderno Dugnano | 55 | 41 (75%) | 15 (27%) |
| Madrid | 23 | 14 (61%) | 8 (35%) |
| Rome/Troina | 36 | 19 (53%) | 14 (39%) |

Validation of positive clinical histories by open oral food challenges (OFCs)

Of 29 patients with a clinical history of peanut allergy seen in Rome/Troina and Paderno Dugnano, 13 (8 from the Rome/Troina group, 5 from the Paderno Dugnano group) with a history of oral allergy syndrome accepted to undergo confirmation open OFCs with one peanut, and all (100%) experienced an oral allergy syndrome a few minutes after the ingestion. No patient experienced systemic reactions following OFCs.

In vitro studies

In vitro tests were carried out on sera from 32 out of 37 peanut reactors. Only 2/32 patients scored positive on both direct ELISA and the Real Test with the peanut extract, and 4 additional sera showed IgE reactivity to peanuts in the Real Test alone. The remaining 26 sera scored negative in both tests.

In immunoblot analysis (Fig. 1), the serum showing the strongest IgE reactivity to peanuts in ELISA reacted to a protein of about 10 kDa in peanut extract. Such reactivity was totally inhibited if the serum was pre-adsorbed with either purified peach LTP or peanut extract itself, but did not change following pre-adsorption with the house dust mite extract (Fig. 1). A normal control serum did not show any IgE reactivity to peanuts.

In view of the marked differences between the in vivo and in vitro tests, the SDS profiles of the peanut extracts used for the SPTs (ALK-Abello) and for ELISA (Lofarma) were compared. No difference was observed (Fig. 2).

Discussion

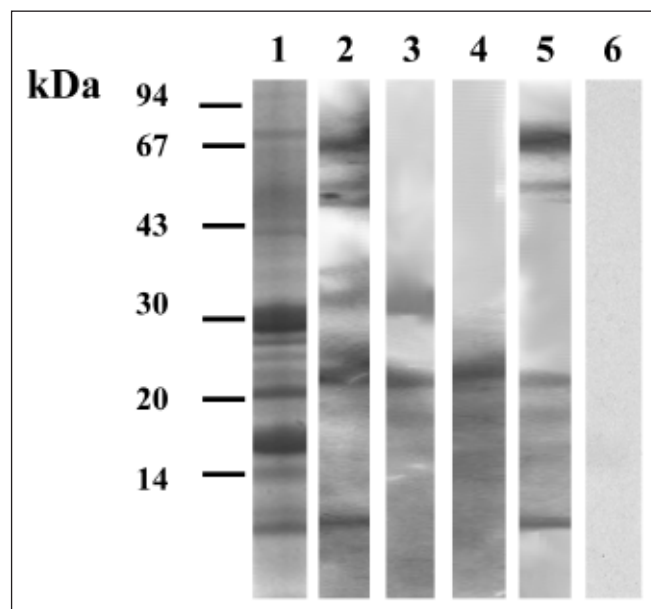
To our knowledge, this is the first study that specifically tries to establish the frequency of both sensitization and clinical allergy to peanuts in patients sensitized to LTP. With the selection criteria adopted, we are confident that patients with both peach and peanut allergy were not sensitized to allergens that have been frequently involved in peanut allergy, namely profilin and Ara h 8, the protein homologous to Bet v 1 (28, 29), but we cannot exclude co-sensitization to other peanut allergens, such as seed storage proteins, although this seems rather unlikely. More than half of LTP allergic patients from the 4 participating centers showed sensitization to peanuts and comparable percentages had clinical allergy to peanuts, suggesting that despite the geographical differences, the populations studied were homogeneous. These data, which

are in line with previous observations (6, 7, 18), suggest that clinical allergy to peanuts occurs in about one third of patients sensitized to LTP. It should be noted that, in this selected population, SPTs with commercial peanut extract showed an excellent NPV, which can be very useful in clinical practice; by contrast, the PPV of SPTs was rather poor, as frequently observed also with different food allergies.

Regarding cross-reactivity between peach and peanut LTP, one study has already provided some evidence using ELISA cross-inhibition experiments (7), while another one found that sera from LTP allergic patients may contain IgE that react to a 10 kDa protein in peanuts (20) and showed cross-reactivity among pomegranate, peanuts,

Figure 1 - SDS-PAGE and immunoblot analysis of peanut extract using the sera from one LTP-allergic patient and from a negative control. Lane 1: SDS-PAGE of peanut extract (25 µg/lane); lane 2: IgE reactivity of the LTP-allergic patient to peanut extract; lane 3: IgE reactivity of the LTP allergic patient to peanut extract after pre-incubation of serum with recombinant peach LTP (10 µg); lane 4: IgE reactivity of the LTP allergic patient to peanut extract after pre-incubation of serum with peanut extract (60 µg); lane 5: IgE reactivity of the LTP allergic patient to peanut extract after pre-incubation of serum with mite extract (60 µg); lane 6: IgE reactivity of normal serum on peanut extract.

The allergic patient's serum clearly shows IgE reactivity at about 10 kDa, which is totally inhibited after pre-adsorption with peach LTP or peanut extract, but persists following pre-adsorption with mite extract. The normal control serum does not show any IgE reactivity to peanut extract.



and hazelnuts. In the present study, we have used recombinant peach LTP as an inhibitor and have observed that, in our patient, peach LTP totally inhibited IgE reactivity to peanut LTP in vitro. This finding confirms recent observations showing that recombinant peanut LTP (Ara h 9) strongly cross-reacts with peach LTP (19).

Another aspect that deserves discussion is the much inferior sensitivity of both in vitro methods for detecting specific IgE to peanuts as compared to SPTs in patients sensitized to LTP. Although we did not carry out specific tests in this sense, the presence of low levels of serum specific IgE might be a good reason for this discrepancy, whereas qualitative difference between the extracts used for in vivo and in vitro tests seems rather unlikely, as the SDS-PAGE profiles demonstrate that the two extracts are very similar. The much higher SE of SPTs with respect to in vitro tests has been observed in other food allergies as well (30). It is tempting to speculate that the low sensitivity of in-vitro methods (caused either by the low amount of LTP in peanut, by intrinsic technical difficulties in extracting adequate quantities of this allergen, or by other causes) may be the reason why, despite a rather significant prevalence of clinical allergy to peanuts in LTP allergic patients, so few studies on peanut LTP have appeared in the medical literature, and the only immunological study carried out to date has been performed

using recombinant Ara h 9 rather than natural peanut extract (19).

In conclusion, peanut sensitization is frequent among LTP allergic patients, and such sensitization leads to clinical allergy in about half of the cases. In view of the extreme stability of this allergen, which can cause severe systemic allergic reactions, we suggest that clinicians carefully evaluate peanut tolerance in LTP allergic patients positive in SPTs with peanuts. Further, although this is based on the findings with the serum from a single patient, it seems that peanut LTP shares all allergenic determinants with peach LTP, as is the case with all other homologous proteins in fruits and vegetables that have been studied before.

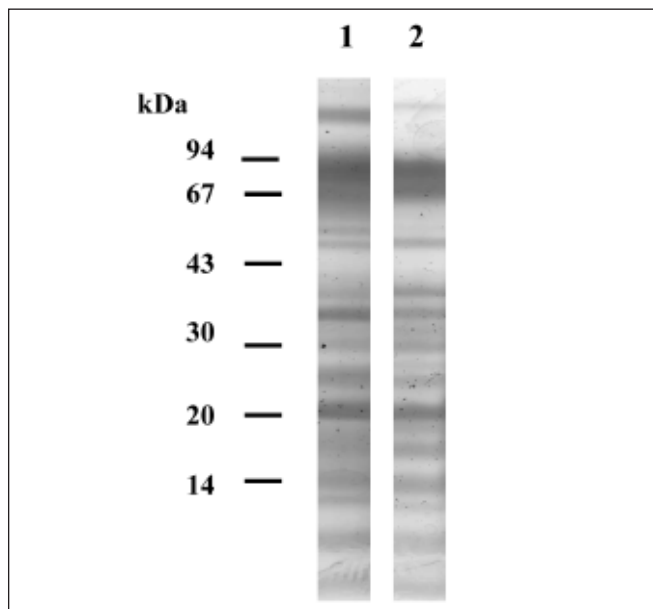
Acknowledgments

The authors wish to thank Eloina Gonzalez-Mancebo and M. Dolores Alonso Diaz de Durana, who collaborated to the clinical evaluation of some of the Spanish patients.

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Figure 2 - SDS page of the peanut extracts used for SPTs (left) and ELISA (right). The two extracts show identical profiles.



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