

Molecular characterization of contact urticaria in patients with melon allergy

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Summary

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Conflicts of interest

None declared.

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Background The relevance of contact allergy to plant-related food has recently emerged. Oral allergy syndrome is one of the most characteristic symptoms of fruit allergy, although it also causes systemic reactions. Plant-food allergy is increasing at the same time as pollen allergy, and fruit-induced allergic contact urticaria could be rising as well.

Objectives The present study was carried out in order to investigate whether one particular primary melon-peel allergen is responsible for contact urticaria.

Methods Fourteen patients presenting with contact urticaria after touching melon peel were evaluated. A melon-peel extract was prepared and analysed by immunoblotting using the patients' sera. Molecular characterization of IgE-binding bands was performed using mass spectrometry. Melon-peel lipid transfer protein (LTP) was purified. Inhibition studies and contact challenge with the protein were performed to confirm IgE reactivity to the purified allergen.

Results An IgE-binding band of ~8–9 kDa was observed in an immunoblotting assay with all the patients' sera and was identified as an LTP. The melon-peel LTP was purified in two chromatography steps. Inhibition studies confirmed LTP as a major allergen in patients with melon-peel contact urticaria. Contact challenge with melon-peel LTP was performed in five patients, all of whom had positive results, exhibiting itchy erythema and hives in the area of contact.

Conclusions This study confirmed our previous findings that melon-peel LTP is a major allergen and is responsible for contact allergy. This knowledge may be used to improve both diagnosis and treatment of patients allergic to melon.

What's already known about this topic?

- The relevance of contact allergy to plant-related food has recently emerged, and the number of patients reporting contact allergy to melon peel has increased strikingly; however, the allergens involved have not been studied.
- Allergic reactions attributed to melon-pulp ingestion can be caused by contact with the peel of this fruit while eating it.

What does this study add?

- This study demonstrates that melon-peel lipid transfer protein is responsible for contact urticaria in patients sensitized to melon.

The relevance of contact allergy to plant-related food has recently emerged. The incidence of sensitization to panallergens present in pollens and vegetables (profilins and lipid transfer proteins, or LTPs) is greatest in areas with high levels of pollen exposure. Plant-food allergy is increasing at the same time as pollen allergy, and fruit-induced allergic contact urticaria could be rising as well.¹

Fruits are the most common cause of food allergy in children aged > 5 years and in adults.² Oral allergy syndrome is one of the most characteristic symptoms of fruit allergy, but urticaria, angio-oedema, respiratory and gastrointestinal symptoms and even anaphylaxis are also frequent. In most cases, these symptoms occur after eating the fruit, but occasionally can occur simply by contact with or handling of the fruit. Indeed, not only the pulp but also the peel of certain fruits may cause symptoms.

Allergic reactions that develop after eating melon (*Cucumis melo*) are frequent. Oral allergy syndrome due to ingestion of melon pulp is the main manifestation of melon allergy, and systemic reactions have rarely been reported.^{3–5} Contact urticaria has been described as a typical clinical manifestation of peach-peel allergy, and it has been reported that contact urticaria caused by the fruit might be associated with sensitization to LTP (Pru p 3).⁶ To date, several allergens have been identified in melon pulp: cucumisin (Cuc m 1),⁷ profilin (Cuc m 2),⁸ pathogenesis-related protein (PR) of the PR-1 family (Cuc m 3),⁹ malate dehydrogenase¹⁰ and phloem lectin (Lec 17-1).¹⁰ Recently, our group was the first to describe the involvement of two new melon-peel proteins (one LTP and one thaumatin-like protein) in a case of contact urticaria and angio-oedema to this fruit.¹¹

The family of LTPs is widely distributed in plant-related food. The first LTP to be described was found in peach.¹² It is a low-molecular-weight protein (~8–9 kDa) that caused severe symptoms not associated with hay fever. This protein was designated as Pru p 3 and has a high cross-reactivity to LTPs from other fruits that belong to the Rosaceae family. However, LTPs have been described in botanically unrelated vegetable foods, and there is little homology between them.¹³

The present study was carried out in order to investigate whether one particular major melon-peel allergen is responsible for these symptoms.

Materials and methods

Patients

The selection criteria for the study included the following: clinical history of contact urticaria during or after touching melon peel, and a positive skin prick–prick test (SPPT) to melon peel¹⁴ according to European Academy of Allergy and Clinical Immunology guidelines.¹⁵ Fourteen patients (mean age 33 years, range 11–55) were recruited from the Hospital Universitario Fuenlabrada, the Hospital Clinic Barcelona and the Hospital General Universitario Ciudad Real (all located in Spain). A panel of aeroallergens, including profilin from ALK

Abello (Madrid, Spain) and plant food, was tested in all patients by performing a skin prick test (SPT). Specific IgE antibodies were determined using the CAP-System FEIA™ (Pharmacia Diagnostics AB, Uppsala, Sweden).

Five healthy and nonatopic volunteers with no history of type I allergy were included as controls. All subjects provided informed consent, and all protocols received local institutional review board approval.

Preparation of the melon-peel extract

Melon peel was cut into small pieces and homogenized in phosphate-buffered saline (PBS, 100 mmol L⁻¹ phosphate buffer, 100 mmol L⁻¹ NaCl; pH 7.5) at 30% (w/v); the proteins were extracted by agitating with magnetic stirring for 3 h at room temperature. The sample was centrifuged at 10 000 g for 30 min and then dialysed against distilled water at 8 °C using dialysis tubing. Protein extracts were lyophilized and stored at 4 °C. The total protein content was determined using the Coomassie Plus (Bradford) Assay (Pierce, Rockford, IL, U.S.A.).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the Hoefer SE 600 electrophoresis system (GE Healthcare, Amersham, U.K.). Polyacrylamide concentrations of 14% (w/v) and 5% (w/v) were used for separating and stacking gels, respectively. Twenty micrograms of protein extract was applied per lane. To ensure proper protein separation and visualization, the gels were stained with PageBlue Protein Staining Solution (Fermentas International, Inc., Burlington, ON, Canada) or used for immunoblotting as described below.

Immunoblotting analysis

Protein bands separated by SDS-PAGE were transferred by semidry blotting onto nitrocellulose sheets. Immunoblots were carried out as previously described.¹⁶ Immunoblotting assays were performed with the 14 individual sera from melon-allergic patients. In addition, a pool of sera from five melon-allergic patients was used as a positive control and a pool of sera from five nonatopic subjects was used as a negative control. For immunoblotting inhibition assays, sera from allergic patients were preincubated for 4 h at room temperature with 100 µg inhibitor per mL serum.

Protein identification and characterization by mass spectrometry

A protein band was extracted from the gel as previously described.⁷ The protein was identified by mass spectrometry (MS) using liquid chromatography–MS in tandem (MS/MS), as previously described,¹⁷ in the Proteomic Service of the

Complutense University of Madrid, a member of the ProteoRed Network.

Purification of the native lipid transfer protein

Melon-peel LTP was purified by following two chromatography steps using an AKTA Purifier System (GE Healthcare). Firstly, melon-peel extract was loaded onto a Superdex 75 10/300 GL column (GE Healthcare) equilibrated in PBS. A fraction containing LTP was dialysed against 20 mmol L⁻¹ Tris-HCl, pH 7.4, and loaded onto a Mono Q column (GE Healthcare). The allergen was eluted with a 0–1 mol L⁻¹ NaCl linear gradient.

Contact challenge test

A contact challenge test (rubbing test) was performed randomly on five patients. The test was performed on each patient's forearm by rubbing the skin with a purified melon-peel LTP (50 µg) for 30 s. At 15 and 60 min after the challenge, the patient was examined for local skin reactions, such as itching, redness, weals or systemic reactions. Symptoms were scored as 1 for local itching without any other manifestations, 2 for redness, 3 when weals became apparent, and 4 if a systemic reaction appeared.¹⁸ Five subjects who developed

no symptoms after touching melon peel were used as negative controls.

Results

Clinical features of melon peel-allergic patients

All patients reported itching and hives in areas that came into contact with melon peel, especially on the hands or lips (14/14, 100%). Some also developed palpebral angio-oedema and conjunctivitis if they touched their eyes after coming into contact with melon peel (two of 14, 14%). Two of them developed lip angio-oedema when they ate melon without separating the peel from the pulp (two of 14, 14%). The majority of the patients presented oral allergy syndrome while eating melon.

The results from the SPPT with melon peel were clearly positive [mean diameter (MD) 20 mm] and slightly positive with melon pulp (MD 5 mm) in all the patients. The SPT or serum-specific IgE levels to peach LTP (Pru p 3) were positive in 13 of 14 (93%) subjects. Profilin SPT was positive in only two of 14 (14%). SPT with pollen extracts was positive in 12 of 14 (86%) patients, mainly with Gramineae, *Olea europaea*, *Cupressus arizonica*, *Platanus acerifolia*, *Artemisia vulgaris* and *Parietaria judaica* pollens (Table 1).

Table 1 Clinical features of melon-peel allergic patients

Patient no.	Age (years)	Sex	Melon allergy symptoms	SPPT melon peel (mm)	Sensitization (SPT or specific IgE)		Sensitization	
					Melon pulp	LTPs (Pru p 3)	Other foods	Pollens
1	11	F	CU, PCA, C, LCA, OAS	25 × 9	Positive	Positive	Peach	G, O, Cu, Ptg
2	55	M	CU, PCA, C	10 × 9	ND	Positive	Pepper, strawberry, plum	—
3	15	F	CU, LCA, OAS	30 × 15	Positive	Positive	Courgette	G, O
4	39	M	CU	17 × 6	ND	Positive	Hazelnut	G, O, A
5	27	F	CU	7 × 5	Positive	Positive	Almond, chestnut, hazelnut, corn, nut, peanut, rice	A
6	39	F	CU, OAS	5 × 4	ND	Positive	Peach, hazelnut, walnut, corn, wheat, mustard, lettuce	Ptg, Pl, A
7	35	F	CU	4 × 4	ND	ND	—	—
8	37	F	CU, OAS	5 × 5	ND	Positive	Peach, hazelnut, walnut, corn, lettuce	P, Pl, A
9		F	CU, OAS	7 × 7	ND	Positive	Peach, hazelnut, walnut, corn, mustard, lettuce, green bean	P, Pl, A, G, Cu
10	47	F	CU, OAS	4 × 3	ND	Positive	Peach, hazelnut, corn	G, O, Pl, A
11	26	F	CU	5 × 5	ND	Positive	Peach	Cu
12	31	F	CU, OAS	4 × 4	ND	Positive	Peach, hazelnut, walnut, peanut, corn, wheat, mustard, apple, lettuce, green bean	Pl, A, O, Sa, Ptg, Ch
13	40	F	CU, OAS	5 × 5	ND	Positive	Peach, hazelnut, walnut, peanut, corn, wheat, lettuce, green bean	Pl, A, Ptg
14	24	M	CU, OAS	5 × 5	ND	Positive	Peach, hazelnut, walnut, corn, wheat, lettuce, green bean	P, Pl, A

LTP, lipid transfer protein; ND, not done; SPT, skin prick test; SPPT, skin prick–prick test; CU, contact urticaria; OAS, oral allergy syndrome; PCA, palpebral contact angio-oedema; C, conjunctivitis; LCA, lips contact angio-oedema; A, *A. vulgaris*; Ch, *Chenopodium album*; Cu, *C. arizonica*; G, grass mix; O, *O. europaea*; P, *P. judaica*; Pl, plane tree; Ptg, *Pl. lanceolata*; Sa, *Salsola kali*.

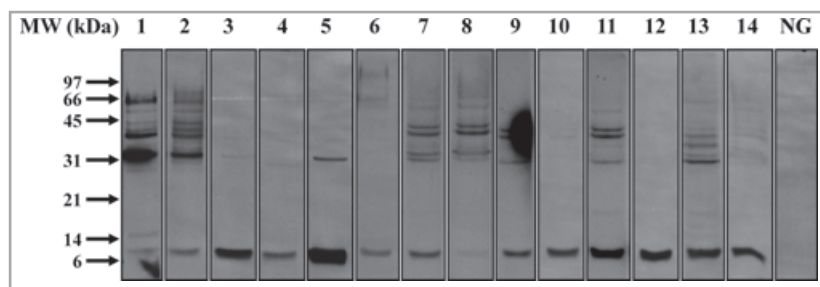


Fig 1. IgE-binding bands in melon-peel extract obtained by immunoblotting. Lanes 1–14, serum from 14 patients; lane NG, negative control (pool of sera from nonatopic subjects); MW, molecular weight markers (kDa).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotting

The potential allergenic components of melon-peel extract were detected by IgE immunoblotting after SDS-PAGE separation. An IgE-binding band with a molecular mass of ~7–8 kDa was detected with all the patients' sera (Fig. 1). This band from the melon-peel extract was manually excised from the gel, digested with trypsin, and analysed using MS/MS. MS/MS was performed and a sequence of internal peptides was obtained, SLNQAATTADRR for an 8-kDa protein, identified as the same melon-peel LTP we described previously.¹¹

IgE-binding activity of purified melon-peel lipid transfer protein

The purified melon LTP (Fig. 2a) was analysed by means of MS/MS and identified as being an LTP according to the sequence of internal peptides obtained. The IgE-binding activity of the purified melon-peel LTP was maintained throughout the entire process. To prove that its IgE-binding activity was the same as the natural counterpart contained in the whole extract, an immunoblotting inhibition assay was performed using melon-peel extract in the solid phase with a pool of patient sera that recognized the LTP in the immunoblotting studies. Purified LTP inhibited the IgE-binding capacity of its counterpart from the melon-peel extract (Fig. 2b).

Contact challenge test

A contact challenge test (rubbing test) with this purified melon-peel LTP was performed in five patients from the group, all of whom showed a positive result, developing itchy erythema and hives in the area of contact (Fig. 3). The rubbing test was negative in the five control subjects who had developed no symptoms after coming into contact with melon peel.

Discussion

The results of this report provide evidence that melon LTP is a major allergen with a capacity to elicit contact urticaria in patients who are allergic to melon peel.

Lipid transfer proteins are conserved proteins and have a molecular weight of ~8–9 kDa with high stability when sub-

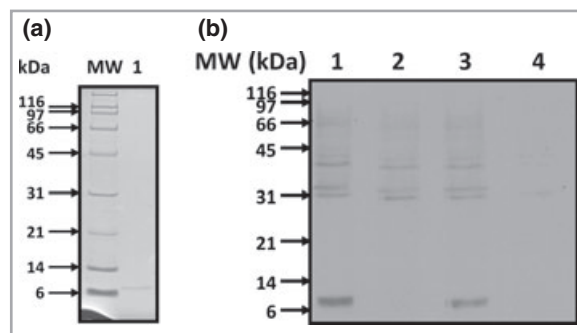


Fig 2. (a) Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis of purified protein under reducing conditions. Lane 1: Purified lipid transfer protein (LTP) from melon peel. (b) SDS-PAGE immunoblotting inhibition results; melon extract in solid phase. Lane 1, pool of sera from melon-allergic patients (positive control); lane 2, serum pool from melon-allergic patients after preincubation with purified LTP; lane 3, serum pool from melon-allergic patients after preincubation with bovine serum albumin (negative inhibition control); lane 4, pool of sera from nonatopic subjects (negative control); MW, molecular weight markers (kDa).

jected to thermal processing and pepsin digestion. These properties make them potent food allergens and explain why they typically induce systemic symptomatology after ingestion. They are polypeptides that are widely found in the plant kingdom and are implicated in the defence against pathogens (PR-14). LTPs have been identified in Rosaceae foods, other vegetable foods and in pollens. The high rate of sensitization to vegetables in the Mediterranean area can be explained by sensitization to panallergens (profilin and LTP) and the high degree of cross-reactivity between each of these proteins.¹⁹ Homology between LTP sequences from botanically unrelated vegetable foods varies from 35% to 95%.¹³ For example, Tri a 14, the LTP from wheat, which has been described as the major allergen associated with baker's asthma caused by wheat-flour sensitization, has shown poor cross-reactivity with peach LTP in tests performed using sera from some of the patients.²⁰

Contact urticaria in conjunction with angio-oedema has been described as a typical clinical manifestation of peach-peel allergy; the same is not found with other Rosaceae fruits. It has been reported that peach-induced contact urticaria might be associated with sensitization to LTP (Pru p 3) and occurs rarely in peach-allergic patients sensitized to Pru p 1 (Bet v 1

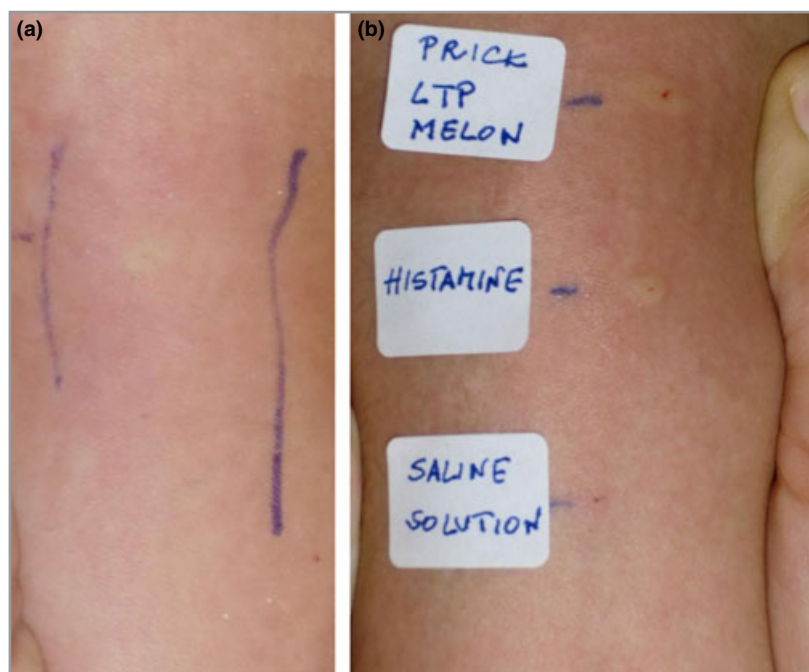


Fig 3. (a) Positive result of the rubbing test with purified melon-peel lipid transfer protein (LTP). (b) Positive result of the skin prick test with purified melon-peel LTP.

homologous protein) and Pru p 4 (peach profilin). This conclusion was in keeping with the observation that patients with contact urticaria had a positive peach-peel rubbing test, and it is well known that the concentration of Pru p 3 is much higher in the peel.⁶ It has also recently been reported in a population of Spanish children, that more than 90% of the subjects who were allergic to peach peel tolerated peeled peach intake.²¹

Until recently, melon allergy was mainly considered to be a marker of profilin hypersensitivity, as the only reactions described were related to melon-pulp ingestion, and almost all the reactions were mild.^{22–24} Until now, the possibility of allergic reactions to melon peel has not been taken into account, perhaps because this part of the fruit is not usually eaten. A study on a number of safe plant-derived foods described the association of clinical tolerance and a negative SPT, suggesting the absence of LTP in the edible part of melon and therefore concluding that melon seemed safe for LTP-allergic patients.²⁵

The first investigation of the allergic properties of melon-peel LTP was recently performed by our group in a case of contact urticaria.¹¹ The present study proves the clinical relevance of sensitization to peel LTP in a large group of patients with contact allergy to melon peel. In one of our previous reports on melon allergy, the absence or scarce presence of this protein in melon pulp was pointed out.¹⁰ LTP is a panallergen showing frequent cross-reactivity among LTPs from different sources, mainly Rosaceae fruit and nuts. Nonetheless, this cross-reactivity is variable among different sources and therefore neither Ole e 7 nor Par j 1 are included in this group.²⁶ In this work, we have been unable to prove cross-reactivity between peel LTP from peach and melon, although other studies should confirm this matter.¹¹

In conclusion, this study confirmed our previous findings that melon-peel LTP is a major allergen and is responsible for contact urticaria in patients sensitized to this protein. In some cases, allergic reactions attributed to melon-pulp ingestion can actually be caused by contact with the peel of this fruit while eating it. This type of patient could tolerate melon-pulp ingestion, if they are not sensitized to the proteins present in this part of the fruit (such as profilin) but rather only to the LTP present in melon peel. This finding has clinical relevance in that it may improve the diagnosis and management of these patients.

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