

Cross-reactivity Between nsLTPs From Cucurbitaceae Peels

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Cucurbitaceae (melon [*Cucumis melo*], watermelon [*Citrullus lanatus*], zucchini [*Cucurbita pepo*], pumpkin [*Cucurbita maxima*], and cucumber [*Cucumis sativus*]) have frequently been reported to cause food allergy. Melon is the most widely studied fruit of the Cucurbitaceae family. The most common clinical manifestation after intake is oral allergy syndrome caused by allergy to the pulp [1]. The main allergens in melon pulp are cucumisin (Cuc m 1), profilin (Cuc m 2), pathogenesis-related protein 1 (Cuc m 3), malate dehydrogenase, and phloem lectin (Lec 17-1) [2]. Our group recently reported on the role of melon peel in allergic reactions to melon in patients who developed urticaria and angioedema after ingestion. We showed that the proteins involved were a nonspecific lipid transfer protein (nsLTP) and a thaumatin [3]. In a subsequent multicenter study, we confirmed that melon peel nsLTP was the major allergen responsible for contact allergy (urticaria and angioedema) to the peel of this fruit [4]. The variation in homology reported for nsLTPs (35%-95%) indicates that not all patients sensitized to nsLTP in melon peel were sensitized to nsLTP from the peel of Rosaceae fruits (Pru p 3) [3-5]. The objective of the present study was to demonstrate cross-reactivity between nsLTP from melon peel and nsLTPs from the peel of other Cucurbitaceae, as previously described for the Rosaceae family.

We present 2 patients who experienced allergic reactions on eating different Cucurbitaceae without separating their peels. Patient 1 was a 36-year-old woman who, on several occasions, had presented oropharyngeal pruritus, edema of the lips and uvula, dyspnea, and nausea immediately after eating unpeeled melon or zucchini puree that contained peel. She tolerated both fruits peeled and reported occasional oral pruritus with the pulp of peeled watermelon. She also reported oral pruritus after eating kiwi. Patient 2 was a 30-year-old woman who always developed urticaria, facial angioedema, dyspnea, and dizziness after exercise when she had previously eaten melon, watermelon, cucumber, or zucchini puree (all without

removing the peel). Both patients experienced itching on touching the peel of peach but had never eaten the pulp of this fruit. Skin tests with commercial extracts (prick) (ALK-Abelló S.A.) were positive (wheal >3 mm) for melon and for peach extracts in patient 1 (6 mm and 12 mm) and in patient 2 (3 mm and 8 mm) and for profilin from palm tree (Pho d 2) in patient 1 (7 mm). Prick-prick testing was positive in both patients with melon peel and pulp. The wheals induced by melon peel were at least twice the size of those induced by melon pulp (patient 1, 15 mm and 3 mm; patient 2, 10 mm and 3 mm). The same was true for watermelon (patient 1, 10 mm and 5 mm; patient 2, 10 mm and 3 mm), zucchini (patient 1, 9 mm and 4 mm; patient 2, 8 mm and 3 mm), cucumber (patient 1, 10 mm and 3 mm; patient 2, 9 mm and 3 mm), and pumpkin (patient 1, 13 mm and 3 mm; patient 2, 10 mm and 3 mm). LTP from melon peel was purified following 2 chromatography steps in an AKTA Purifier System (GE Healthcare). First, melon peel extract was loaded onto a Superdex 75 10/300 GL column (GE Healthcare) and equilibrated in phosphate-buffered saline. A fraction containing nsLTP was dialyzed against 20 mmol of 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 [4]. SDS-PAGE immunoblotting was performed under nonreducing conditions (without 2-mercaptoethanol) and reducing conditions (with 2-mercaptoethanol) [6,7] with purified melon peel nsLTP and the patients' serum. IgE-binding bands of approximately 14 kDa (without 2-mercaptoethanol) and 10 kDa (with 2-mercaptoethanol) were revealed with both sera. SDS-PAGE immunoblotting-inhibition under nonreducing conditions (without 2-mercaptoethanol) with purified melon peel nsLTP in the solid phase and Cucurbitaceae peel extracts in the inhibitory phase revealed complete inhibition of the 14 kDa band for the following: melon and zucchini peel extracts with sera from both patients; cucumber and pumpkin peel extracts with serum from patient 1; and watermelon peel extract with serum from patient 2 (no inhibition with serum from patient 1). Inhibition was partial for cucumber and pumpkin peel extract with serum from patient 2 (Figure). SDS-PAGE immunoblotting-inhibition with purified melon peel nsLTP in the solid phase and purified Pru p 3 in the inhibitory phase revealed complete inhibition of IgE binding with serum from patient 1, but no inhibition with serum from patient 2 (Supplementary Figure).

nsLTPs, which belong to the PR-14 family, are proteins with variable homology between the sequences within the group. They have a molecular weight of approximately 8-9 kDa and are highly stable when subjected to heat treatment and pepsin digestion, with the result that they are potent allergens that produce systemic allergic reactions to vegetable foods. They were identified first in Rosaceae fruits, nuts, cereals, latex, and pollens. Our group recently showed nsLTP to be a relevant allergen in melon peel, but not in the pulp. Contact urticaria and angioedema had been reported to be typical clinical manifestations of peach allergy, although this had not been found with other vegetable foods [3,4]. We recently confirmed that melon peel nsLTP was responsible for contact allergy in sensitized patients [4]. There have been reports of cross-reactivity between pumpkin and other Cucurbitaceae (in vivo and in vitro), although nsLTP was not shown to be

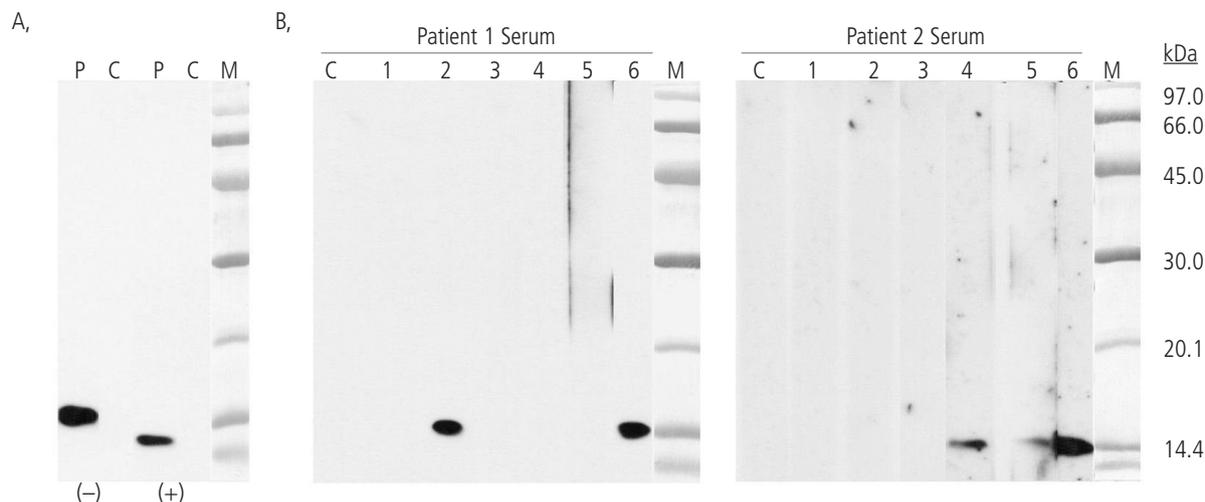


Figure. SDS-PAGE immunoblotting of purified melon peel LTP. Lane P, Serum from patient 1; Lane C, control serum (pool of sera from nonatopic subjects); Lane M, Molecular mass standard. (–) sample without 2-mercaptoethanol; (+) sample with 2-mercaptoethanol. B, SDS-PAGE immunoblotting-inhibition. Lane C, control serum (pool of sera from nonatopic subjects), Lanes 1-6, Patient serum previously incubated with melon peel extract (lane 1), watermelon peel extract (lane 2), zucchini peel extract (lane 3), cucumber peel extract (lane 4), pumpkin peel extract (lane 5), and lamb extract (lane 6). Purified melon peel nsLTP in the solid phase and Cucurbitaceae peels extracts in the inhibitory phase.

the protein involved and it was not specified whether the work-up was performed with or without peels [8,9]. As we had expected, the present study demonstrated cross-reactivity between nsLTP from melon peel and nsLTPs from the peel of other Cucurbitaceae. The homology between nsLTPs is very variable, as shown in the cases we report. Inhibition of melon peel nsLTP was not always complete or did not occur with all of the Cucurbitaceae peel extracts. Inhibition of melon peel nsLTP with Pru p 3 occurred in patient 1 but not in patient 2. Thus, some patients are allergic to the peel of all Cucurbitaceae and others are allergic to some peels and merely sensitized to others. Given that nsLTPs are very stable and frequently cause systemic and contact allergic reactions, it seems appropriate to follow the model described for the Rosaceae family and recommend patients who are allergic to a specific Cucurbitaceae peel not to eat or touch other Cucurbitaceae peels (even if they are cooked) unless their tolerance is confirmed.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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