

The nature of melon allergy in ragweed-allergic subjects: A study of 1000 patients

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ABSTRACT

Previous studies suggest cross-reactivity between specific ragweed pollen and melon allergens. This study was designed to clarify the origin of the cross-reactivity between ragweed pollen and the gourd family. One thousand ragweed-allergic subjects were interviewed about the presence of oral allergy syndrome (OAS) induced by melon or watermelon and were divided into reactive to ≤ 3 seasonal allergen sources or > 3 seasonal allergen sources. Patients reporting melon and/or watermelon allergy underwent a skin-prick test (SPT) with fresh melon and, after 2006, also with profilin-enriched date palm pollen extract. Because no IgE reactivity to melon extract was detected in vitro, ELISA was performed using date palm pollen extract, and inhibition experiments were performed using grass pollen, date palm profilin, and bovine serum albumin (BSA) as inhibitors. Six hundred forty-six and 354 subjects reacted to ≤ 3 seasonal allergens or > 3 seasonal allergens, respectively; 4/646 (1%) and 81/354 (23%) reported a history of melon/watermelon-induced OAS ($p < 0.0001$). Forty-three of 46 (93%) melon reactors scored positive on SPT with the profilin-enriched extract, which was positive in 0/2 (0%) versus 43/44 (98%) reactive to ≤ 3 or > 3 seasonal allergen sources, respectively ($p < 0.0001$). In vitro, serum from melon-allergic subjects showed a strong IgE reactivity to the profilin-enriched date palm pollen extract, which was abolished by preabsorption with both grass pollen extract and date palm pollen extract, but not by BSA. In ragweed pollen-allergic subjects, melon allergy is most likely associated with cross-sensitization to the plant pan-allergen profilin and not to specific ragweed pollen allergens. This study confirms the association between profilin sensitization and melon allergy.

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It is well known that hypersensitivity to certain seasonal airborne allergens may cause allergy to plant-derived foods because of the cross-reactivity between homologous proteins in pollen and food. Birch pollen allergy represents the best-studied model in this sense,¹ although also mugwort pollen allergy may be associated with food allergy in the so-called mugwort-celery-spice syndrome.² Other clinically relevant cross-reactions between pollen and food allergens are those involving the pan-allergen profilin, a minor pollen allergen. Things with ragweed pollen allergy are less clear. Nearly 40 years ago a study by Anderson *et al.* examining a possible cross-reactivity between ragweed and melon and banana appeared on the medical literature.³ This observation was confirmed years later by Enberg *et al.* who found that up to 50% of sera from ragweed-hypersensitive subjects reacted to members of the gourd family (melon, watermelon, zucchini, and cucumber) and/or banana.⁴ In both studies most patients with a history of adverse reactions induced by vegetable foods reported a classic oral allergy syndrome (OAS). At the beginning of the new century, one

study showed that melon-allergic subjects may also react to a number of botanically unrelated plant-derived foods including peaches, figs, and kiwi, and frequently show latex sensitization as well.⁵ More recently, the major melon allergen was identified as the pollen pan-allergen profilin,⁶ thus confirming previous indirect evidence of a good clinical response of allergy to members of the gourd family after injection allergen-specific immunotherapy with different pollen extracts.⁷ Nonetheless, although some reviews suggest that pan-allergens such as profilin or lipid transfer protein are the most likely candidates involved in the ragweed-melon association,^{8,9} the idea of a possible cross-reactivity between specific ragweed pollen and plant food allergens still appears in recent and authoritative reviews of food allergy.¹⁰ The present study aimed to clarify the origin of the possible cross-reactivity between specific ragweed pollen allergens and members of the gourd family.

METHODS

Patients and Skin Tests

One thousand adult (> 12 years of age) patients with ragweed pollen allergy randomly selected among those who presented at the Allergy Outpatient Department of the Clinica San Carlo from the beginning 2003 to the end of 2009 were studied. Ragweed pollen allergy was diagnosed in the pres-

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ence of a typical clinical history of rhinoconjunctivitis with or without asthma from mid-August to the end of September in the area of Milan, Italy, associated with clear-cut positive skin-prick test (SPT) with commercial ragweed pollen extract (5000 BU/mL; Allergopharma, Reinbeck, Germany). All patients were thoroughly interviewed about the presence of OAS after the ingestion of melon or watermelon and underwent SPT with commercial extracts of the main seasonal airborne allergens present in this geographic area including grass, ragweed, mugwort, pellitory, plantain, birch, plane, olive, and cypress (Allergopharma). To group patients based on their probable IgE reactivity to profilin, a plant pan-allergen present virtually in all pollen sources and causing cross-reactivity between pollen and vegetable foods, based on SPT results, patients were divided into two groups either reactive to ≤ 3 allergen sources or reactive to > 3 allergen sources. Patients reporting melon and/or watermelon allergy underwent SPT with fresh melon by the prick-prick technique to confirm the IgE-mediated origin of their disorder, and after 2006 (when it became available on the market) they underwent also SPT with commercial profilin-enriched date palm pollen extract (profilin, Pho d 2, 50 $\mu\text{g}/\text{mL}$; ALK-Abellò, Madrid, Spain). All SPTs were performed and read following established methods.¹¹ Because all *in vivo* tests were part of the routine evaluation of subjects presenting spontaneously at the clinic for diagnostic and therapeutic purposes, no formal informed consent was required. The study was approved by the local institutional review board.

In-Vitro Tests

Fresh melon was extracted for 1 hour in 0.1 M of phosphate-buffered saline (PBS), pH 7.4, at 4°C while stirring. After centrifuging at $30,000 \times g$ for 30 minutes, the supernatant was harvested and dialyzed against the same buffer as previously described.¹² Protein contents were assessed according to Bradford¹³ using a commercial BioRad Protein Assay Dye Reagent (BioRad, Milan, Italy) and bovine serum albumin (BSA) as reference standard. The protein concentration of melon extract was 613 $\mu\text{g}/\text{mL}$ (Fig. 1). The extract was used to perform ELISA assays, and it was our intention to use it also for the subsequent immunoblot analysis. However, after many unsuccessful *in vitro* ELISA experiments using several melon extracts and sera from different subjects, we had to conclude that the abstract lacked the relevant allergen or contained it in amounts that were below the sensitivity limit of the *in vitro* assays. Whether this has been caused by the degradation of the relevant allergen or by its extremely low concentration (in

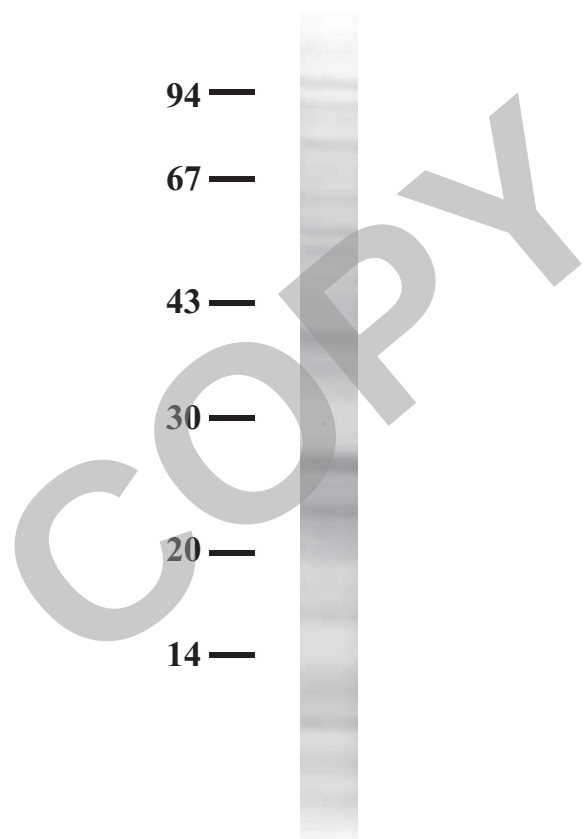


Figure 1. SDS-PAGE of melon extract (silver staining; Invitrogen, Milan, Italy). The electrophoresis shows the presence of several bands at different molecular weights including the molecular weight of profilin (12–16 kDa).

terms of w/v ratio) in the fresh fruit remains to be established. Thus, in view of the significant sequence identity ($> 74\%$) between the aligned profilins from melon, Cuc m 2, date palm pollen, Pho d 2, and grass pollen, Phl p 2, using BLASTp, both ELISA and ELISA-inhibition assays were performed using a commercial profilin-enriched date palm pollen extract (Pho d 2, 50 $\mu\text{g}/\text{mL}$; ALK-Abellò), prepared by affinity chromatography with a poly-L-proline-Sepharose.¹⁴ IgE reactivity to the date palm profilin extract of a pool of sera from three ragweed-allergic patients reporting OAS after the ingestion of melon and showing strong skin reactivity to melon on the prick-prick test was measured by ELISA both with and without preabsorption (overnight at 4°C) of sera with an independent profilin source such as *Phleum pratense* pollen extract (10 and 100 μg^7), with date palm profilin (1 μg), or with BSA (10 μg). Absorbance values read at 450-nm spectrophotometer were expressed as optical density units. Optical density values < 300 were considered negative.

Table 1 Study population

	<4 Positive SPT	>3 Positive SPT	<i>p</i>
No.	646	354	
Male/Female	282/364	135-219	NS
No. reporting melon allergy (%)	4 (1%)	81 (23%)	<0.0001
No. positive on SPT with date palm profilin	0/2	43/44 (98%)	<0.0001

SPT = skin-prick test.

Table 2 ELISA inhibition assays

IgE to Palm Profilin		Inhibition by			
		Phleum, 100 µg	Phleum, 10 µg	Date Palm Profilin	BSA
Serum pool	3279	685 (79%)	567 (83%)	168 (94%)	3187 (3%)
Negative control	266				

IgE values are expressed as optical density.

BSA = bovine serum albumin.

Statistics

Proportions were compared by chi-square test with Yates' correction. Values of $p < 5\%$ (*i.e.*, <0.05) were considered statistically significant.

RESULTS

Patients

Results are summarized in Table 1. Six hundred forty-six and 354 subjects showed skin reactivity to ≤ 3 seasonal allergen sources or >3 seasonal allergen sources, respectively. A history of melon/watermelon-induced OAS was reported by four (1%) patients reacting to ≤ 3 allergen sources versus 81 (23%) patients reacting to >3 allergen sources ($p < 0.0001$); no patient reported systemic symptoms after the ingestion of melon. Most melon reactors reported OAS also after the ingestion of other plant-derived foods, including watermelon, tomato, bananas, peaches, apples, oranges, apricots, *etc.* Forty-six of these 85 melon reactors underwent SPT with the profilin-enriched extract; 43/46 (93%) scored positive. Interestingly, among melon-allergic patients 0/2 (0%) reacting to ≤ 3 airborne allergen sources versus 43/44 (98%) reacting to >3 airborne allergen sources scored positive on SPTs with date palm profilin ($p < 0.0001$).

In Vitro Test

The results of ELISA and ELISA inhibition experiments are shown in Table 2. The uninhibited serum pool showed a strong IgE reactivity to the profilin-enriched date palm pollen extract. IgE reactivity to date palm pollen extract was nearly abolished by preabsorption of serum pool with both date palm pollen

extract and with a source of profilin other than ragweed pollen, such as grass pollen extract, but not by BSA. This result further confirmed the cross-reactive nature of the relevant allergen.

DISCUSSION

In this study, which was performed on ragweed-allergic subjects diagnosed during the routine clinical activity in an outpatient clinic, the diagnosis of melon allergy was based on clinical history and positive SPT, and no confirmative double-blind placebo-controlled food challenges were performed. However, it has to be considered that oral food challenges are not a routine diagnostic means^{9,15} and performing them in a large number of subjects would be practically infeasible. Nonetheless, the results of *in vivo* investigations were clear-cut: in ragweed pollen-allergic subjects melon allergy is mostly associated with hypersensitivity to the plant pan-allergen profilin. In other words, in these subjects, melon allergy is probably not the consequence of sensitization to specific ragweed allergens. This conclusion is indirectly supported by the association of melon profilin allergy with clinical allergy to cross-reacting profilins from a series of other sources including, among others, tomato, watermelon, bananas, citrus fruits, and peaches.¹⁶

Although we were not able to perform confirmative *in vitro* experiments using a melon extract, probably because of too little concentration of the relevant allergen in this fruit or, alternatively, to its loss during extraction procedures, the clinical observations were, nonetheless, confirmed by the experiments performed using the profilin-enriched date

palm pollen extract as a surrogate, which showed a strong inhibition of IgE reactivity to date palm pollen if the serum pool was preabsorbed with an unrelated source of profilin such as grass pollen extract.

Of course, ragweed may act as the primary sensitizer to profilin, but the same holds true for other relevant pollens. Respiratory allergy and food allergy represent the two main reasons for consultation in allergy/immunology¹⁶; this study confirms the association between airborne profilin sensitization and melon allergy.¹⁷

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