

Food allergy: identification of the major IgE-binding component of peach (*Prunus persica*)

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Ramon Leonart, MD*; Ana Cisteró, MD*; José Carreira, PhD†; Alejandro Batista, MD*; and Jaime Moscoso del Prado, PhD†

Since peaches are a relatively common cause of food allergy, we set out to identify the allergens involved. With the use of a panel of 48 sera from patients allergic to peach, we demonstrate that most of the allergenicity of that fruit is confined to the skin, rather than to the flesh of peaches, and corresponds to a protein doublet with an estimated molecular weight of 8–10 kD.

INTRODUCTION

Although different immunologic mechanisms have been implied in food allergy, IgE-mediated hypersensitivity has been clearly established.¹ In particular, a variety of fruits have been described as causative agents of allergic reactions,^{2–5} although very little is known about the nature of the offending allergens.⁶ Diagnosis of fruit allergy is usually based on the patient's history, exclusion diet or food challenge, and occasionally, on skin prick testing or in vitro methods.⁷

In the Mediterranean area, peach is a relatively common cause of allergic reactions,⁸ sometimes leading to severe symptoms. As is the case with the majority of fruits, the allergens involved in these reactions have not been yet identified. A patient with allergic reactions after the ingestion of different fruits has been reported recently. This patient had serum IgE specific to a 30-kD component present in various fruit extracts including peach.⁹

In order to develop a practical

approach for diagnosis and to identify the allergens involved in peach allergy, we studied 48 patients with a history of peach allergy. We show that the most important allergen is an 8–10-kD protein doublet present only in the peach's skin. There are also other minor allergens, common to skin and flesh, with higher molecular weights.

MATERIALS AND METHODS

Serum Samples

Sera from 48 patients with peach allergy (35 women and 13 men) were collected. Their mean age was 21 years (range 16–27 years). The initial diagnosis of peach allergy was made based on clinical history and positive scratch tests using fresh fruit. Clinical manifestations varied from local cutaneous to generalized reactions after ingestion, contact, or even inhalation of peach dust.

Extracts

Fresh local peaches were hand-peeled, and two different extracts were prepared: either skin or flesh was crushed at 4 °C with a commercial blender in the presence of 50 mM Tris-HCl, 0.7% NaCl, pH 7 (TBS) with the addition of 1 mM PMSF as a protease inhibitor, in a 1:1.5 (weight:volume) ratio. The resulting puree was filtered through a muslin cloth, centrifuged at 10,000 g (4 °C, 20 minutes) and the supernatant was extensively dialyzed

against TBS, 1 mM PMSF, and finally against TBS with no additives, using a dialysis tubing with a nominal cutoff of 6–8 kD (Spectrapor, Los Angeles, CA). The extracts were then centrifuged, sterile filtered, and frozen until use. An identical procedure was employed to prepare plum and apricot extracts.

Specific IgE Determination

Immunolon II (Dynatech, Cambridge MA) wells were coated with 100 µL of either flesh or skin extracts. Optimal coating conditions for each extract were determined with fresh extracts by checkerboard titration. Thereafter frozen extracts were employed with similar results. After blocking the plates with 1% bovine serum albumin in PBS, the plates were washed with PBS, 0.05% Tween-20, air-dried, sealed, and kept at 4 °C until use. Specific IgE was determined by ELISA using the Hamlet^(R) (Alergia e Inmunología Abelló, S. A., Madrid) procedure and reagents.

Immunoblotting

Proteins from either flesh or skin extracts were separated on an SDS-PAGE gel (polyacrylamide gradient 8% to 18%), according to Laemmli.¹⁰ After running, part of the gel was cut for Coomassie blue staining, and the rest was electrotransferred to nitrocellulose.¹¹ The nitrocellulose was cut into strips, blocked with PBS, 0.05% Tween-20, 0.5% de-

* Unitat d'Al·lergia, Hospital Santa Creu i San Pau, San Antonio M^a Claret 167, Barcelona, Spain.

† Departamento de Investigación, Alergia e Inmunología Abelló SA, Miguel Fleta 19, 28037 Madrid, Spain.

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fated dry milk, and incubated with either a pool of positive sera or a pool of sera from healthy subjects, diluted 1:5 in the same buffer. Specific IgE was detected by autoradiography after a second incubation with ¹²⁵I-labeled monoclonal anti-human IgE antibody (Hamlet[®], Alergia e Inmunología Abelló, S. A., Madrid).

RESULTS AND DISCUSSION

The levels of specific IgE in the patients' sera were determined by ELISA both to skin and flesh extracts, and the results are shown in Table 1. The specificity of the test was checked with a panel of 20 sera from subjects with no history of fruit allergy, all of which proved negative against both extracts. The majority of the patients (81%) had specific IgE to peach.

There is, however, a considerable difference between the reactivity to flesh and skin extracts. Whereas 79% of the patients had specific IgE to skin, only 52% of them were positive to flesh extract. If only those sera with IgE levels of class 2 or above are considered positive, then that difference is even more impressive; 65% are positive to skin compared with only 17% positive to flesh. Even in those cases in which the patients were positive to both extracts, the levels of specific IgE to flesh was always much lower than to skin.

Only one patient (2%) was negative to skin but positive to flesh, and in this case with a very low titer (class 1). A comparison of the results for each individual serum is shown in Figure 1. These results indicate that, although both the skin and the flesh of the peach may induce hy-

persensitivity, the allergenic activity of peaches is largely concentrated in the skin.

It is noteworthy to mention that when we tried to prepare the extracts at room temperature rather than at 4 °C and/or in the absence of protease inhibitor, we obtained very low levels of activity. This suggests the presence of proteases in the extracts whose activity has to be maintained at minimal levels during the preparation of the extracts to prevent protein degradation and the consequent loss of activity.

The allergenic difference detected by ELISA between flesh and skin could be due to the presence of the same allergens in different concentrations in both extracts, or to the existence of one or more allergens exclusive to the skin. In order to discriminate between these two alternatives, and to identify the allergens involved in peach allergy, we analyzed both extracts by immunoblotting. Samples of skin and flesh extracts were electrophoresed in the

Flesh	4					
	3					1
	2			5	2	
	1	1	1	5	9	1
	0	9	6	6	2	
		0	1	2	3	4
		Skin				

Figure 1. Comparison of specific IgE levels of the individual sera to skin and flesh peach extracts.

Table 1. Specific IgE to Peach Extracts*

Extract	0	1	2	3	4
Skin	10 (21%)	7 (15%)	11 (23%)	16 (33%)	4 (8%)
Flesh	23 (48%)	17 (35%)	7 (15%)	1 (2%)	0 (0%)

* The results are expressed as the number of sera of each specific IgE class. Numbers in parentheses indicate the percentage of the total for each class.

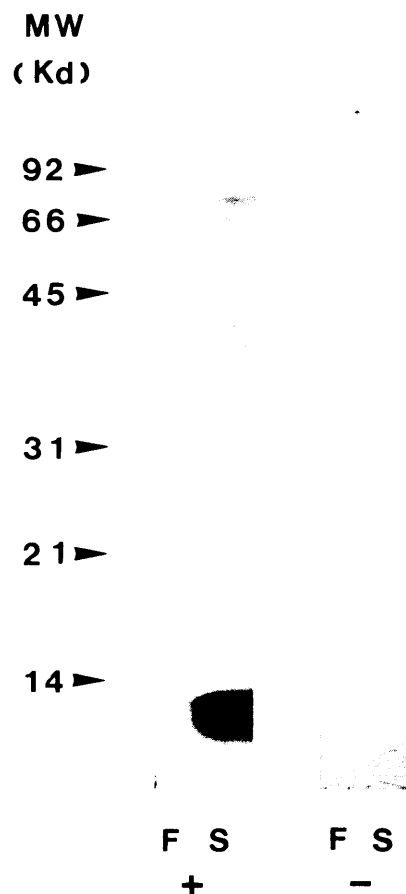


Figure 2. Identification of peach allergens by immunoblotting. On the left (+), autoradiography of a blot of flesh (F), and skin (S) proteins probed with a pool of positive sera. On the right (-) the same extracts probed with a pool of normal sera.

presence of SDS and 2-mercaptoethanol, blotted onto nitrocellulose, and probed with a pool of sera with the highest titers of specific IgE to both extracts (classes 3-4 and 2-3 to skin and flesh, respectively). The results are shown in Figure 2. Coomassie blue staining of the gels was difficult to interpret due to the high content in carbohydrates in both extracts, which resulted in a far from optimal staining; however, at least five very weak bands could be identified common to both extracts with apparent molecular weights of 70, 66, 60, 58, and 30 kD, respectively. In addition, the skin extract showed an exclusive band, much more in-

tense than the rest, with an estimated molecular weight of 10 kD. In the immunoblots, weak IgE-binding components, common to both extracts, can be identified corresponding to 70-, 66-, and 58-kD bands, plus an additional 43-40-kD component that cannot be identified in the Coomassie blue stained gel. Again, a strong IgE-binding component, exclusive to skin, can be seen. It corresponds to the 10-kD band, although it appears to be resolved in a doublet of approximately 10 and 8 kD. It seems that the different allergenicity of peach skin and flesh is not due to differences in the relative concentrations of allergens, but rather, it is due to the existence of an allergen exclusive to skin.

The result of this work contrasts with a recent report of a patient with allergic reactions after the ingestion of various fruits, who had serum IgE specific to a 30-kD component present in different fruits, among them, flesh and skin peach extracts.⁹ Although we find a 30-kD band common to both extracts, we have not detected specific IgE to it. We can think of several explanations to account for that discrepancy. (1) In our work, allergens have been identified using a pool of highly positive sera. It is possible that some individual sera may recognize a 30-kD component present in both extracts. That would represent, however, the exception rather than the rule, as we have shown that 50% of the sera are positive only to skin, and in the rest, the levels of specific IgE are always much higher to skin than to flesh. (2) The authors of that report do not state whether the 30-kD band was estimated under reducing or nonreducing conditions. If their samples were run under nonreducing conditions, then the 30-kD band could correspond to one of the larger weak allergenic bands that we detect under reducing conditions, provided that protein has intramolecular disulphide bonds.¹² (3) Following the same argument, the allergenicity of

the 30-kD component might have been abolished upon reduction, as it has been described with other allergens.¹³ (4) Finally, the allergen described by the author is common to a variety of different fruits. We have performed ELISA against two very closely related fruits, plum and apricot, with a selection of 24 sera and found no evidence of crossreactivity among them. A total of 18 (75%) of the sera were positive to peach skin, but only eight (33%) proved positive to plum skin, all of them being also positive to apricot skin. Most significantly, the two sera with the highest titer of specific IgE against plum and apricot were negative against peach skin. This result strongly suggests that the 8-10-kD allergen that we describe is exclusive to peach skin. The remaining six sera were positive both to peach skin and flesh thus opening the possibility of a certain degree of crossreactivity limited to the minor bands detected in peach skin and flesh.

Crossreactivity between food and pollen allergens, notably in the case of apple and birch, have been found.¹ A similar situation could occur in the case of peach. Although we cannot exclude that possibility, the clinical history of the patients studied fails to show any correlation between allergy to peach and any other inhalant or food allergen.

In summary, we have demonstrated the existence of a 8-10-kD protein doublet, exclusive to peach skin, which largely accounts for the allergenicity of that fruit, for which we tentatively propose the name of *Pru p I*. The extreme difference in allergenicity found between the fruit's skin and flesh should be taken into consideration in the preparation of peach extracts for in vivo and in vitro tests, and should be explored in the case of other fruits. Results similar to the ones we describe could, in some instances, explain the poor correlation frequently found between symptoms and in vivo (prick test) or in vitro fruit allergy diagnosis.

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Request for reprints should be addressed to:
Jaime Moscoso del Prado
Departamento de Investigación
Alergia e Immunología Abelló S. A.
Miguel Fleita 19
28037 Madrid
Spain