

Different patterns of allergen recognition in children allergic to orange

To the Editor:

There are not many well-documented cases of allergy to orange (*Citrus sinensis*) fruit, despite its wide consumption, and orange allergens have not been studied. We describe 6 patients with allergy to orange, as well as a preliminary characterization of the allergens involved.

Patients were selected on the basis of clinical symptoms suggestive of a type I hypersensitivity reaction after the ingestion of orange fruit-juice and positive skin prick test responses with fresh orange (peel and pulp) on at least on 2 occasions. All patients were also tested with a battery of relevant inhalants (ALK-Abelló S. A., Madrid, Spain) in our area. Patients were challenged with increasing amounts of orange every half an hour until symptoms were elicited or a whole orange fruit was consumed. Specific IgE to orange and other citric fruits and rBet v 2 (birch pollen profilin) levels were determined by means of CAP-FEIA (Pharmacia Diagnostic, Uppsala, Sweden).

Patient's characteristics and the results of the in vivo tests and specific serum IgE determinations are shown in Table I. During orange challenges, all patients presented similar symptoms to those reported by the clinical history. The dose eliciting symptoms was between 20 and 100 g. Patients were allergic to other plant-derived foods and were sensitized to grass pollen, but only patients 1, 2, 3, and 6 had symptoms of seasonal rhinitis, asthma, or both. Patients 1, 3, and 6 tolerated small quantities of lemon

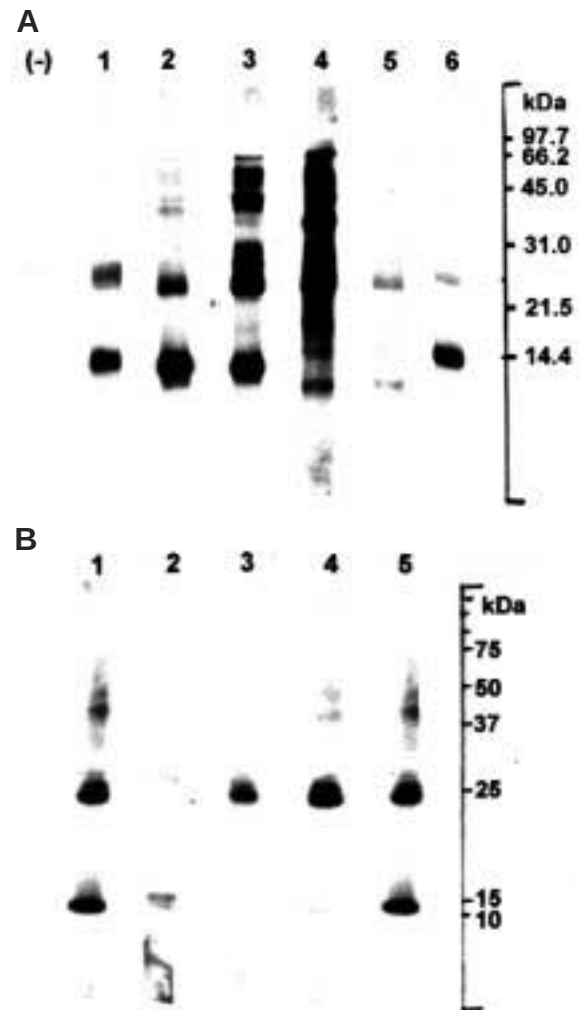


FIG 1. A, Orange IgE immunoblotting with individual sera: (-), negative control. B, IgE immunoblotting with a pool of sera from patients 1, 2, 3, and 6: 1, orange extract (50 μ g of protein); 2, rBet v 2 (2 μ g); 3 to 5, orange IgE immunoblotting inhibited with 100 μ g of *L perenne* protein, 4 μ g of rBet v 2, and 4 μ g of peach LTP, respectively.

juice, and the other patients did not remember having eaten lemon. Patient 5 tolerated tangerine, but patients 2 and 3 had oral allergy syndrome (OAS) with this fruit. The other 3 patients had never eaten tangerine. None of them had ever eaten grapefruit.

Freeze-dried, nonfiltered orange juice was extracted at 10% wt/vol in 1.8% NaCl for 90 minutes with magnetic stirring. After centrifuging, the supernatant was sterilized through a 0.22- μ m pore-diameter membrane, placed in vials, and stored at -30°C . Protein concentration was determined by using the Lowry method (1.2 mg/mL). Skin prick tests performed with the orange extract at 0.5 mg protein/mL was positive in all patients.

IgE immunoblotting with the individual sera performed under nonreducing conditions¹ revealed 2 main IgE-binding orange components (Fig 1, A). One predominant band had a molecular weight of approximately 25 kD and was detected by all patients but with low intensi-

TABLE I. Patient data, in vivo tests, and specific serum IgE results

Patient	Sex	Age (y)	Symptoms with pollen	Allergy to other foods	Orange p-p (mm)		Orange fruit challenge test	IgE, kU/L (class)				
					Peel	Pulp		Orange	Tangerine	Lemon	Grapefruit	Bet v 2
1	M	15	Yes	Tomato, melon, pomegranate, apple, plum, cherry	5	5	OAS	0.76 (2)	1.29 (2)	0.67 (1)	1.19 (2)	8.81 (3)
2	F	11	Yes	Peach, banana, melon, watermelon	5	6	OAS	1.74 (2)	1.91 (2)	1.25 (2)	2.92 (2)	23.5 (4)
3	M	13	Yes	Tomato, banana, melon, watermelon, pinenut	3	5	OAS	1.88 (2)	2.34 (2)	1.74 (2)	2.57 (2)	12.6 (3)
4	F	2	No	Milk, egg, fish, legumes	4	5	OAS, AD, GD	2.17 (2)	2.52 (2)	2.34 (2)	2.19 (2)	<0.35 (0)
5	F	7	No	Tomato, peach, fig, mulberry, mustard	6	4	OAS, U	1.21 (2)	<0.35 (0)	1.73 (2)	<0.35 (0)	<0.35 (0)
6	F	13	Yes	Maize, banana, peach, grape, almond, nut	6	5	OAS	6.04 (3)	6.04 (3)	5.37 (3)	9.42 (3)	39.2 (4)

p-p, Prick-prick skin test; AD, atopic dermatitis; GD, general discomfort; U, generalized urticaria.

ty in patients 5 and 6. The other main band, with approximately 13 kd, was detected very strongly by patients 1, 2, 3, and 6. These patients had a high IgE titer to birch profilin, were primarily pollen allergic, and had OAS as a sole manifestation of orange allergy (Table I).

Immunoblotting of the orange extract with a rabbit anti-serum against pollen profilin² or peach lipid transfer protein (LTP)³ detected mainly a band of approximately 13 kd in each case, indicating the presence of putative LTP and profilin with similar apparent molecular weights in SDS-PAGE (data not shown). By means of immunoblotting inhibition assay (Fig 1, B), it was determined that in patients 1, 2, 3, and 6 the IgE binding to the 13-kd orange allergen was inhibited by *Lolium perenne* pollen extract and by rBet v 2 (Biomay, Vienna, Austria) but not by natural Pru p 3 (peach LTP).¹ These data indicate that the 13-kd allergen corresponds to orange profilin. In contrast, IgE binding to the 25-kd orange band was not inhibited by *L perenne* extract (Fig 1, B). This component might correspond to another plant-derived food allergen with compatible molecular weights, such as β -1,3-glucanases or thaumatin-like proteins.⁴ Three patients (patients 2, 3, and 4) had serum IgE against high-molecular-weight orange bands (>25 kd). Their IgE binding was inhibited by *L perenne* extract (Fig 1, B), suggesting that these bands could correspond to cross-reactive carbohydrate determinants.⁵ Patients 4 and 5 had no specific IgE to profilin, but they had IgE against an orange band with a slightly lower molecular weight in SDS-PAGE (Fig 1, A). These patients had OAS plus systemic reactions and did not report clinical symptoms to grass pollens, characteristics that fit into the current model of LTP sensitization. However, no inhibition by peach LTP of IgE binding to the putative orange LTP was observed (data not shown). These results could be explained considering the relatively low sequence identity of LTPs from different sources⁶ compared with profilins.⁷

In summary, 2 different patterns of IgE allergen recognition were found in our patients allergic to orange: one group with clinical symptoms of isolated OAS and pollen allergy who were sensitized mainly to profilin and to a 25-kd band and another group of patients with more severe symptoms after orange intake with no allergic symptoms to pollens who were sensitized mainly to the 25-kd band and to a nonprofilin low-molecular-weight band. The determination of the patient's allergen profile could be a useful tool for the diagnosis of orange allergy and the evaluation of the clinical outcome.

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