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Allergenic cross-reactivity among peach, apricot, plum, and cherry in patients with oral allergy syndrome: An in vivo and in vitro study

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Abstract

Background: Oral allergy syndrome in response to fruits and vegetables frequently occurs as clusters of hypersensitivity to members of the same botanical family, for which the immunologic basis lies in a number of common allergens, most of them still unidentified.
Objective: This study was designed to assess the in vivo and in vitro cross-reactivity between fruits of the Prunoideae subfamily (i.e., peach, cherry, apricot, and plum) and to identify their major allergens and the cross-reactivity of the peach extract with grass and birch pollen.
Methods: The in vivo study was conducted by skin prick tests and open food challenges with fresh fuits in 23 patients with oral allergy syndrome for peach and positive skin prick test and RAST results for the other Prunoideae. In vitro sodium dodecylsulfate–polyacrylamide gel electrophoresis was followed by immunoblotting and immunoblotting inhibition.
Results: A 13 kd component was identified as the only major allergen common to all the Prunoideae; the other major allergens were found at 14 kd in peach and at 30 kd in cherry. Immunoblotting inhibition showed wide cross-reactivity within the Prunoideae, whereas grass and birch pollen partially inhibited the peach blotting.
Conclusions: Clinical cross-reactivity to Prunoideae is essentially due to a common 13 kd IgE-binding component, which seems to be the most important major allergen of this subfamily, not shared with grass and birch pollen. (J ALLERGY CLIN IMMUNOL 1994;94:699-707.)

Abbreviations used	
MW	Molecular weight
OAS	Oral allergy syndrome
OFC	Open food challenge
SDS-PAGE	Sodium dodecylsulfate–polyacrylamide gel electrophoresis
SPT	Skin prick test

Key words: Oral allergy syndrome, cross-allergenicity, Prunoideae, immunoblotting

Eating certain fruits and vegetables may elicit allergic symptoms localized to the oral mucosa in sensitized subjects, giving rise to the so-called oral allergy syndrome (OAS). [1] Clusters of hypersensitivity have been described in response to fruits and vegetables belonging to the same botanical family, such as apple and pear, stone fruit, and nut fruits. [2-5]

In a recent study on OAS, [6] we found that one of the most frequent clusters of associations in Italy was hypersensitivity to fruits of the Prunoideae subfamily, of which the main members are peach, apricot, plum, and cherry. [3-5] We found no associations between allergy to peach, apricot, and plum and any kind of pollinosis, even though allergies to fruits and vegetables are often reported to be clinically associated with hay fever caused by pollens such as grasses, mugwort, and especially birch. [1, 4-6]

The aim of this study was to identify the allergenic components in these fruits and assess their cross-reactivity by analyzing the sera of a group of patients with allergic symptoms in response to peach and one or more of the other three main components of the Prunoideae subfamily (plum, apricot and cherry), as demonstrated by both history and open food challenge (OFC). The presence of cross-reactivity between peach extract and pollen of grasses and birch was also investigated.

Methods

Study population

We selected our study population from outpatients referred to the First Department of Internal Medicine of the University of Milan and the Bizzozzero Division of Niguarda Ca' Granda Hospital from 1989 to 1991 because of OAS after eating fruits or vegetables.

To be admitted to the study, patients had to have a clear history of OAS to peach, with symptoms occurring within a few minutes after eating the offending fruit (about 5 to 15 minutes). Symptoms required were those used for defining OAS [1, 7] : the contact of the food with orolabial mucosa had to elicit oral irritation, local itching, tingling, and angioedema, throat tightness, lip tightness or swelling, oral mucosal papulas or blisters; contact with the gastrointestinal mucosa had to elicit abdominal pain, vomiting, and diarrhea.

Patients also had to have a positive skin prick test (SPT) response and RAST result for this fruit and for the other three main Prunoideae (i.e., cherry, apricot, and plum); the patient's history of allergy to these last three could be positive or negative. Patients could have hay fever symptoms, but such symptoms were not required for admission to the study.

We asked all these patients to undergo an OFC for peach, cherry, apricot, and plum and a negative control test (with a fruit of another botanical family to which patients were definitely not sensitive) in order to select only those who showed a positive correlation between the results of the OFC and clinical history. Only

patients whose OFC results agreed with their history either positive or negative were admitted to the in vitro study to detect any correlation between positive history and presence of given IgE-binding components.

We also admitted two patients to the in vitro study who had OAS for several vegetables or fruits but not for Prunoideae. They did, however, have positive RAST and SPT responses to the four Prunoideae under evaluation.

In vivo methods

OFC

For the OFC, patients had to be free of symptoms and fasting since the previous evening and had to have discontinued use of any antihistamines or corticosteroids at least 1 week before challenge (for astemizole, at least 4 weeks). Patients were given increasing amounts of the fresh fruit, starting with a small portion (250 mg). Each patient was asked to chew the fruit for 1 minute and then spit it out. If no symptoms appeared within 15 minutes, the patient was asked to repeat the test, with double the amount of fruit. The challenge lasted until the onset of objective symptoms (as described above) or up to the maximum single dose of about 64 gm, that is, the approximate mean weight of a fresh peach without the stone. Patients were asked not to spit out the last two doses (32 and 64 gm) but to swallow the fruit instead.

Symptoms were recorded by a physician member of the Allergy Unit Staff not involved in the study.

When the patient had only subjective symptoms (itching or tingling of the lips or oral mucosa), the test result was considered positive only when confirmed by two other positive challenges performed on separate days.

For the negative control challenge, an irrelevant fruit, as determined by a negative history and positive SPT response, was used according to the method described above.

SPTs

All patients underwent SPTs with commercial food extracts 1:20 wt/vol (DHS, Slough, U.K.); a 1 mm tip standard lancet (Allergy Pricker, DHS) was used with the standard panel of allergens.

SPTs with fresh fruits (peach, cherry, apricot, and plum) were done with the technique described by Dreborg and Foucard. [8] The same lancet was used for pricking first the fruit and then the volar surface of the subject's forearm.

SPTs with pollen extracts were performed with commercial material (Phazets; Pharmacia, Uppsala, Sweden). All the pollens typical of this area, (i.e., birch, grass, mugwort, and *Parietaria* species) were tested. To be considered positive, an SPT had to produce a wheal and flare reaction graded at least 2+, (i.e., 50% of the one elicited by a solution of histamine hydrochloride 10 mg/ml). [9]

In vitro methods

Extracts

Extracts of peach, cherry, plum, and apricot were prepared inhouse by extraction of antigen according to the method for apple described by Bjorksten et al. [10] The peel of each fruit, which has a higher protein content and allergenicity than the pulp (unpublished data), was used. The peel was homogenized (wt/vol 1:5) by a high-speed spinning-blade homogenizer in potassium phosphate buffer 10 mmol/L, pH 7, containing, in 500 ml, 10 gm of polyvinylpolypyrrolidone (Carlo Erba, Milan, Italy), 0.8 gm of diethyldithiocarbamate (Sigma Chemical Company, St. Louis, Mo.), 0.327 gm of ethylenediaminetetraacetic acid disodium salt (Sigma Chemical Company), and 0.09 gm of sodium azide (Carlo Erba). The homogenate was centrifuged at 40,000 *g* for 30 minutes, and the supernatant was dialyzed for 20 hours at 4° C against phosphate buffer 10 mol/L, pH 7. It was then centrifuged again at 3000 rpm for 10 minutes to separate any precipitate.

For grass pollen, we used a commercial extract of timothy pollen (wt/vol 1:20) (3M Diagnostic Systems, Santa Clara, Calif.), because it cross-reacts with the other grass pollens and has all the relevant allergens of the grass pollen, which are well known on the basis of World Health Organization grass standardization. [11]

For birch pollen, we used an extract obtained from Pharmacia (Uppsala, Sweden). The protein concentration of each extract was determined with Folin reagent, according to the method of Lowry et al. [12] All extracts were stored at –20° C.

Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

The extracts were mixed with Tris(hydroxymethyl)aminomethane, adjusted to pH 6.1 with concentrated sulfuric acid, Tris-SO_4 4 ×, containing 4% sodium dodecylsulfate (SDS) and 6% β-mercaptoethanol (BIO-RAD, Richmond, Calif.). The samples, together with low molecular weight (MW) markers (Pharmacia-LKB, Bromma, Sweden), were denatured by heating at 100° C for 5 minutes, then centrifuged at 10,000 *g* for 5 minutes. The extracts were separated in a discontinuous buffer system in an SDS-polyacrylamide gradient gel with a 6% stacking gel and a 7.5% to 20% separation gradient gel at 6 mA for 16 to 20 hours in a BIO-RAD Protein IIxi vertical electrophoresis slab cell, essentially as described by Neville. [13] After separation, a part of the gel was fixed in a solution of 30% methanol and 7% acetic acid (Carlo Erba) in distilled water for 2 hours on a horizontal shaker, immersed in staining solution for 1 hour with Coomassie Brilliant Blue R-250 (Pharmacia-LKB) and then in a destaining solution (30% methanol, 7% acetic acid in distilled water), which was changed until the background was clear.

Immunoblotting

After electrophoresis, the allergenic protein components were electroblotted to a nitrocellulose membrane, pore size 0.2 to 0.45 µm (Amersham, Buckinhshire, U.K.) in 25 mmol/L Tris, 192 mmol/L glycine, 20% methanol (using a Trans-Blot Cell from BIO-RAD) in a cold room with an aluminium coil with water circulating at 4 to 8° C at 0.4 Å for 4 hours. The unoccupied protein binding sites on the nitrocellulose membrane were then saturated by placing the membrane in blocking solution, phosphate-buffered saline pH 7.4 with 0.5% Tween 20 for 30 minutes at room temperature.

The nitrocellulose blot was cut into strips and incubated with the investigated sera, diluted 1:4 in blocking solution, overnight. Each strip was washed three times with washing solution (0.9% NaCl, 0.5% Tween 20) on a shaker. After washing, the IgE-binding components were detected by incubating each strip with iodine 125–labeled anti-human IgE antiserum (Phadebas RAST; Pharmacia Diagnostics, Uppsala, Sweden) diluted 1:4 in blocking solution for 4 hours.

Autoradiography

Blotted nitrocellulose strips were washed three times with washing solution, dried, and exposed on autoradiography film (Amersham, Hyperfilm) in exposure cassettes at –70° C. Exposure time ranged between 4 and 60 hours, depending on the amount of labeled antiserum bound.

The MW of a protein under investigation was determined by comparing its electrophoretic mobility with the protein standards of known MW, ranging from 14,400 to 94,000 d.

The migration values of the IgE-binding components were located on a calibration curve plotted with the protein standards' migration values against the logarithms of their MW.

We identified as major allergens the components (bands) against which at least 50% of the tested sera presented specific IgE; minor allergens were the components against which less than 30% of the sera presented specific IgE, and all other components were considered intermediate allergens.

Immunoblotting inhibition

Immunoblotting inhibition was only done for peach, with the sera of four patients after preabsorption for 1 hour on a shaker with each of the other allergenic extracts investigated.

The blot inhibition was done for patient 11, who had symptoms in response to all the fruits investigated; patient 12, who had symptoms in response to peach, cherry, and plum; patient 14, who had symptoms only in response to peach; patient 20 who did not have any symptoms in response to the fruits investigated (control serum).

After electrophoresis, the peach components were electroblotted onto nitrocellulose strips, and each strip was incubated with the preabsorbed serum overnight. Immunodetection was done as usual.

Control sera

Five healthy volunteers with no history of atopy, members of the staff of our department, provided serum samples, which were pooled. SDS-PAGE and immunoblotting were done for peach, cherry, plum, apricot, birch pollen, and grass pollen to assess nonspecific binding.

Results

Among the patients first seen with positive history, SPT, and RAST results for peach, we selected 23 who also had a positive RAST result of at least class 2 for cherry, apricot, and plum. A total of 115 OFCs were performed in these 23 patients.

OFCs

Nineteen (82.6%) of the 23 patients showed an OFC concordant with history for the four members of the Prunoideae subfamily and for the negative control food challenge. The two control patients were completely free of symptoms for all four items.

Among the 19 patients with OAS for peach, 12 (63.15%) had clinically relevant hypersensitivity to more than one component of the Prunoideae subfamily. Five (26.3%) had clinical symptoms for two fruits (3 peach-cherry, 1 peach-apricot, 1 peach-plum); two (10.5%) for three fruits (peach-cherry-plum); and five (26.3%) for all four fruits. The most frequent clinical cross-reactivity was between peach and cherry (10 patients, 52.6%), then between peach and plum (8 patients, 42.1%), and peach and apricot (6 patients, 31.5%).

All the patients reported subjective symptoms after chewing the dose of 500 mg. The majority of the patients presented objective symptoms at the dose of 4 to 8 gm. Only two patients had gastrointestinal symptoms: one had abdominal pain and vomiting after the dose of 32 gm, and one had abdominal pain after the dose of 64 gm.

Table I shows the clinical characteristics of the 21 patients admitted to the in vitro part of the study, 19 with a concordance between symptoms and OFC results for the four Prunoideae and two who were free of symptoms for these foods but had a positive RAST result.

TABLE I. OFC findings, history (pollens), SPTs, and RAST results in the 21 patients admitted to the in vitro part of the study

	Symptoms						SPT						RAST					
	OFC5																	
Patient No.	P	C	A	PL	G	B	P	C	A	PL	G	B	P	C	A	PL	G	B
1	OP	–	–	OP	–	–	++	+++	++	++	–	–	2	1	2	2	0	0
2	OP	–	–	–	+	–	++++	++	++	++	++++	–	3	2	3	3	3	0

3	OP	–	–	–	–	–	++	++	++++	+++	–	–	3	2	3	2	0	0
4	OP	–	–	–	–	–	+++	+++	+++	++	–	–	3	2	2	2	0	0
5	OP	OP	–	–	–	–	++++	++	++	++	–	–	3	2	3	3	0	0
6	OP	OP	OP	OP	+	–	++	++	++	++	+++	–	3	1	3	3	3	0
7	OP	OP	OP	OP	–	–	++	++	++	+++	–	–	4	3	4	4	0	0
8	GI	OP	–	–	+	+	++	++	++	++	+++	++++	3	2	3	3	4	4
9	OP	–	OP	–	+	–	+++	+++	++	+++	++	++	3	2	3	3	4	3
10	OP	–	–	–	+	–	++	++	++	++	++	+++	2	3	3	2	2	3
11	OP	OP	OP	OP	+	+	++	++	++	++	++++	++++	2	2	2	2	3	2
12	OP	OP	–	OP	+	+	++	+++	++	++	++++	++++	3	2	3	3	4	4
13	OP	GI	–	OP	–	–	++	++	++	++	++	++	2	2	2	2	3	3
14	OP	–	–	–	+	+	++	++	++	+++	++++	+++	4	3	4	4	4	2
15	GI	GI	–	–	+	–	++++	++	+++	+++	++++	++	2	1	2	2	4	2
16	OP	OP	OP	OP	+	+	++	+++	+++	+++	+++	+++	3	2	3	3	3	4
17	OP	–	–	–	+	–	+++	++	+++	++	+++	+++	3	2	3	3	4	4
18	OP	OP	OP	OP	+	+	++++	+++	+++	++	+++	+++	4	3	3	3	3	3
19	OP	–	–	–	+	+	+++	++	++	++	+++	++	3	3	3	3	4	2
20	–	–	–	–	+	+	+++	+++	++	++	+++	+++	2	3	3	3	3	4
21	–	–	–	–	–	+	+++	++	++	++	++	++	2	2	2	2	2	4

SPT results are expressed in number of pluses compared with the wheal flare elicited by histamine 10 mg/ml (considered +++).
RAST results are expressed as classes of positivity: 0, < 0.35 PRU; 1, 0.35-0.7 PRU; 2, 0.7-3.5 PRU; 3, 3.5-17.5 PRU; 4, > 17.5 PRU.
P, peach; *C,* cherry; *A,* apricot; *PL,* plum; *G,* grass; *B,* birch; *OP,* oropharyngeal symptoms (oral irritation, local itching and tingling, angioedema, throat tightness, lip tightness/swelling, oral mucosal papules or blisters); *GI,* gastrointestinal symptoms (abdominal pain, vomiting); *PRU,* Phadebas RAST units

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Sixteen of the 21 patients (76%) had a positive RAST result for grasses, and 14 (66.6%) had a positive RAST result for birch.

Protein content of the extracts

Our “in-house” extracts had the following protein concentrations: peach, 3.56 mg/ml; cherry, 7.34 mg/ml; apricot, 2.87 mg/ml; and plum, 3.92 mg/ml. Commercial grass pollen extract had a protein concentration of 9.37 mg/ml, and birch pollen extract had a concentration of 5 mg/ml.

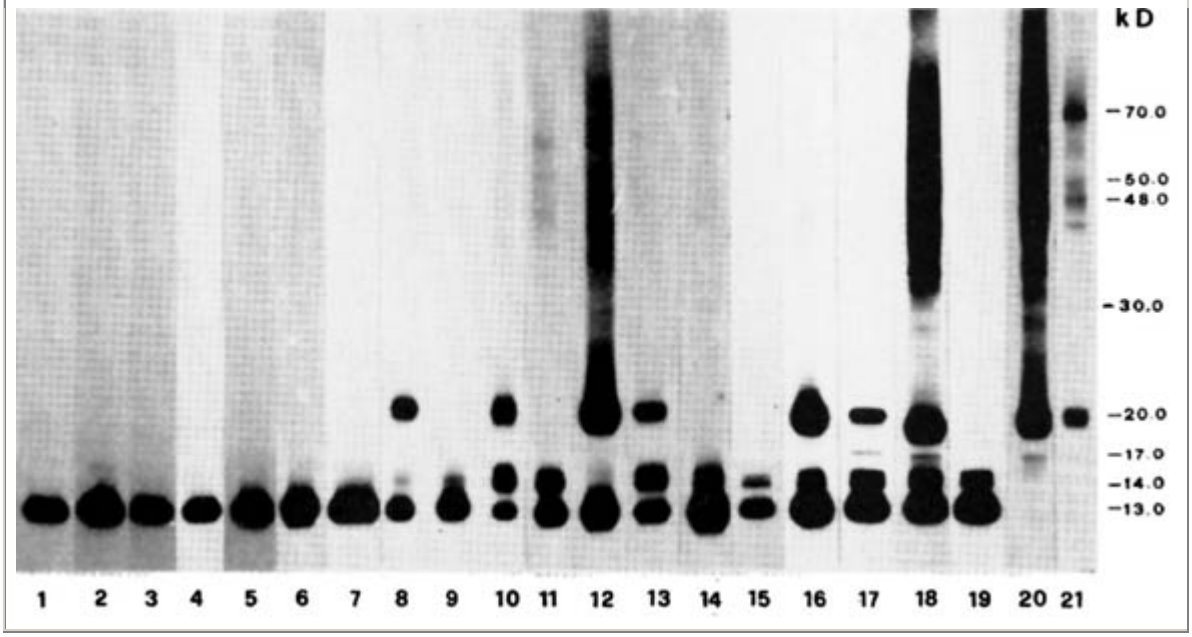
IgE-binding patterns of allergen components in fruits of the Prunoideae family

To identify the IgE-binding components of peach, cherry, plum, and apricot and of birch and timothy pollens, in house extracts for fruits and purified commercial extracts for pollens were analyzed by SDS-PAGE, transferred to nitrocellulose, and immunoprobed with patients' sera. SDS-PAGE and immunoblotting for peach were done for all 21 patients. For the other Prunoideae fruits and for birch and grass pollen, immunoblotting was not done for all patients, because there was too little serum. Thus for each extract we report the number of patients for whom the test was done. The patient's reference number remains the same in each figure.

Peach

Immunoblotting was performed for 21 patients, as shown in Fig. 1. At least 10 allergenic components could be identified in our in-house extract of peach, ranging from a MW of 12 to 80 kd. A component with MW of 13 kd was detectable in 19 of 21 patients (90.4%). Other allergenic components were located at 14 kd (12 of 21, 57.1%), 20 kd (9 of 21, 42.8%), 48 kd (6 of 21, 28.5%), 70 kd (6 of 21, 28.5%), 50 kd (5 of 21, 23.8%), and 17 kd (4 of 21, 19%).

FIG. 1. Autoradiographs of IgE-immunoblot analysis of peach extract with sera from 21 patients presenting specific IgE to peach. The MW of some of the main allergens are given. Patients' identification numbers are reported in the lower part of the figure. Patients 1 to 19 presented OAS to peach.



On the basis of the presence or absence of specific IgE to birch pollen, we divided the study population into two subgroups. In the birch-negative subgroup (seven patients) we identified only an IgE-binding component at 13 kd. In the birch-positive subgroup (14 patients) we detected all the other IgE-binding components described above.

Cherry

Immunoblotting was performed for 18 patients. Eight allergenic components were detectable. Two were major allergens: 13 kd (16 of 18, 88%) and 30 kd (14 of 18, 77%). The other allergenic components were: 20 kd (8 of 18, 44%), 70 kd (7 of 18, 38%), 14 kd (6 of 18, 33%), 48 kd (5 of 18, 27%), 17 kd (3 of 18, 16%), and 50 kd (3 of 18, 16%).

Of the seven patients with negative responses to birch pollen, five showed only one IgE-binding component at 13 kd, and two (patients 5 and 6) also had a second band at 30 kd.

Apricot

Immunoblotting was performed for 16 patients. Only the IgE-binding component with a MW of 13 kd was identified as the major allergen, being present in 14 of 16 patients (87.5%). Other IgE-binding components were: 70 kd (6 of 16, 37.5%), 20 kd (5 of 16, 31%), 14 kd (4 of 16, 25%), 40 kd (4 of 16, 25%), and 30 kd (2 of 16, 12.5%).

Only the allergenic component at 13 kd was detectable in patients with negative responses to birch pollen, as with the other fruit extracts.

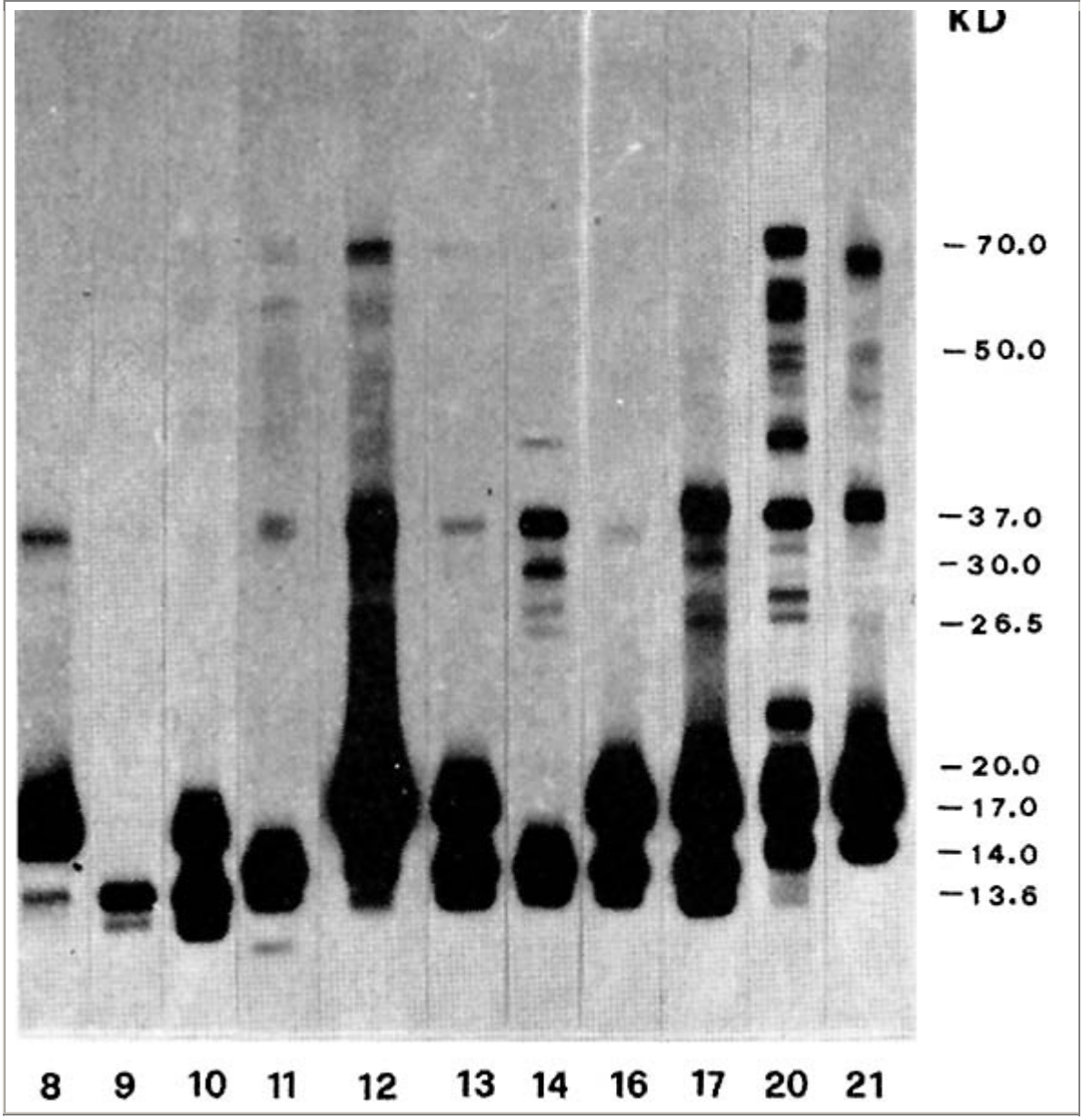
Plum

Immunoblotting was performed for 16 patients. The IgE-binding component at 13 kd acts as major allergen, being present in 14 patients (87%). Other bands were detectable at 14 kd (6 of 16, 37%), 20 kd (6 of 16, 37%), 30 kd (3 of 16, 18.7%), 40 kd (6 of 16, 37%), 48 kd (3 of 16, 18.7%), and 70 kd (5 of 16, 31%). These bands were detectable in all but one patient with positive responses to birch pollen (patient 6).

Birch

Immunoblotting was performed for 11 patients (■Fig. 2). IgE-binding components were detecting at 13.5 kd, 14 kd (probably two bands of the same allergen), 17 kd, and 20 kd. Other bands were detectable at 26.5 kd (6 of 11, 54.5%), 33 kd (7 of 11, 63.6%), 37 (11 of 11, 100%), and 70 kd (6 of 11, 54.5%).

FIG. 2. Representative IgE antibody pattern against a birch pollen extract obtained from Pharmacia (Uppsala, Sweden): reactivity of sera from 11 patients.



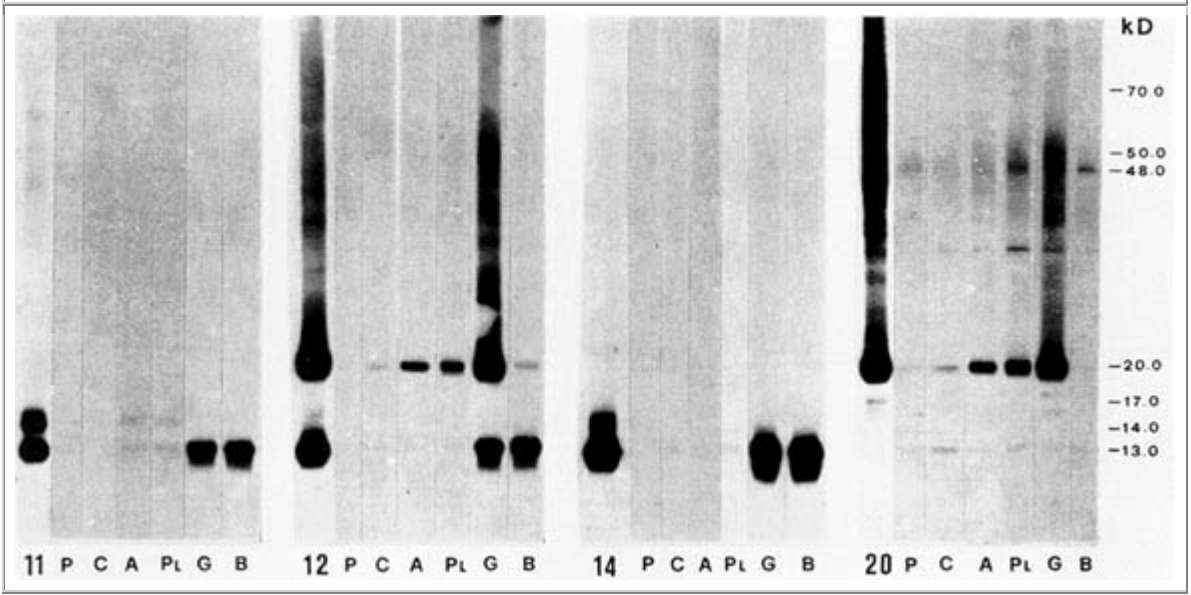
Timothy

Immunoblotting was performed for 11 patients. Two main groups of IgE-binding components were detectable, approximately between 14 kd and 22 kd and between 26 kd and 30 kd. Another band was clear at 50 kd (9 of 11 patients, 75%).

Immunoblotting inhibition

Peach immunoblotting inhibition was done for three patients hypersensitive to one or more of the fruit allergens (patients 11, 12, and 14) and for one of the two control subjects (No. 20) (Fig. 3). Peach immunoblotting was inhibited with the following extracts: peach, cherry, apricot, plum, grass pollen, and birch pollen. In all the patients the peach extracts caused complete peach blot inhibition.

FIG. 3. Immunoblotting inhibition in the sera of four patients. *P,* Peach; *C,* cherry; *A,* apple; *PL,* plum; *G,* grass; B, birch.



In patient 11 apricot and plum extracts almost completely inhibited IgE binding at 13 and 14 kd, whereas grass and birch pollen extracts completely inhibited the 14 kd component, but not the one at 13 kd.

In patient 12 cherry extract almost completely inhibited the peach blot, whereas apricot and plum extracts completely inhibited the band at 13 kd but only lowered the background at 20 kd. Grass pollen extract only inhibited the band at 14 kd, whereas birch pollen extract inhibited all the peach blot except the bands at 20 kd (which was lowered) and 13 kd.

In patient 14 cherry, apricot, and plum extracts completely inhibited the peach blot, whereas grass and birch pollen extracts only inhibited the 14 kd component.

In patient 20, cherry extract completely inhibited the peach blot except for the band at 20 kd, which was only lowered. This 20 kd band was partially inhibited by apricot and plum extract, not affected by grass pollen extract, and completely inhibited by birch pollen extract. The peach blot of this patient, who was free of symptoms for peach, did not present the band at 13 kd.

Discussion

In this study we found evidence of in vivo and in vitro cross-allergenicity of the fruits belonging to the botanical subfamily of Prunoideae (peach, apricot, plum, and cherry). This evidence was obtained from patients selected on the basis of an OAS in response to peach and positive SPT and RAST results for the other Prunoideae. In the in vivo study the patients' clinical reactivity was verified by OFC with all the Prunoideae. Of 23 patients, 19 (82.6%) showed a concordance between history and food challenge ($p < 0.001$, chi square test), and 12 of them (63%) had symptoms for more than one Prunoideae fruit. Ten (52.6%) had symptoms for cherry, eight (42.1%) for plum, and six (31.7%) for apricot.

The food challenge findings, of which concordance with history was not unexpected because the allergic symptoms to fresh fruit are immediate and localized in the oral mucosa, provide evidence of an in vivo cross-

reactivity among these members of the same botanical subfamily. Different observations were reported with other foods belonging to taxonomically related groups: in the study by Bernhisel-Broadbent and Sampson [14] only two (5%) of 41 patients with positive SPT results for extracts of Leguminosae had clinical symptoms for more than one legume. The same authors reported a lack of in vivo cross-reactivity among different fish in patients with fish allergy. [15] These different findings may be explained by differences in the characteristics of the studies. For instance, in the Leguminosae study the patients had variable rates of SPT positivity for the various components of the family (ranging from 87% for peanut to 22% for green bean), whereas in our study the rate of skin (and RAST) positivity was 100% for all the Prunoideae, thus making an in vivo cross-reactivity more conceivable. In the other studies mentioned, reactivity to foods was assessed by double-blind placebo-controlled food challenge, considered the “gold standard” for diagnosis of food allergy. [16] However, in OAS a series of factors make it difficult to perform double-blind placebo-controlled food challenge so that no standardized procedure is yet available. The main causes of difficulty are: (1) the lability of allergens in fresh fruits, [10, 11] which do not resist the lyophilization necessary to give foods in capsules; (2) the difficulty of ensuring oral contact with adequate concentrations of the offending food if it is disguised, after squeezing and handling, in a masking solution; (3) the risk of giving increasing doses of food with oropharyngeal bypass, and therefore without local symptoms, may elicit severe systemic reactions.

The in vitro part of the study provided an immunologic basis for the clinical findings by identification of cross-reactive allergens in Prunoideae. All patients with OAS in response to peach showed an IgE-reactive band at 13 kd and 88%, 87.5% and 87% of those with positive SPT and RAST results for cherry, apricot, and plum, respectively.

This observation seems remarkable because the presence of specific IgE toward the 13 kd component accounts for sensitization in about 90% of patients allergic to Prunoideae, making this component an extremely important major allergen, comparable to *Bet v* I in birch-sensitive patients and *Fel d* I in cat-sensitive patients. [17, 18] IgE to the 13 kd allergen seems to govern the allergic symptoms to peach, because patients 20 and 21, who had positive RAST results but no symptoms, had no IgE to this allergen. However, many more patients need to be studied before it can be assumed that this band is always related to clinical symptoms.

On the other hand, only about half of patients with IgE binding to the 13 kd component in cherry, apricot, and plum had symptoms in response to these fruits. Thus sensitization to this allergen alone may well not be enough to trigger symptoms for the other Prunoideae. Possibly, the fact that fewer of these fruits are consumed than peach, because they are not available so long — especially cherry and apricot — may play a role in whether symptoms develop. The sole presence of specific IgE with no symptoms is not sufficient for prescribing avoidance of a fresh fruit because several subjects in our study population had no symptoms in response to the challenge with cherry, apricot, and plum, despite positive RAST results.

Other important IgE-binding components, with MWs 14 kd and 20 kd, were detectable with high frequency in our fruit extracts. In cherry extract, IgE in 77% of patients recognized an allergenic band at 30 kd, which seems peculiar to this fruit, because it was otherwise detectable at a lower percentage (18.7%) only in plum extract. Other bands of high MW (from 40 to 70 kd) were recognized in the different fruits, with frequencies ranging from 16% to 38%.

Few immunoblot studies have been published for such a large number of patients allergic to peach, and none deal with all the Prunoideae. The only study on a worthwhile number of patients is an in vitro study in which a major IgE-binding component of peach was identified in 48 patients and was described as an 8 to 10 kd protein. [19] Technical differences in performing the blotting might account for the different molecular size compared with our 13 kd component. In the same study, high MW components were identified in the range between 45 and 70 kd, comparable to the bands of 48, 50, and 70 kd we found to be intermediate allergens. In the study mentioned, no definition of major, intermediate, or minor allergens was possible because the sera of peach-sensitive patients were pooled for the blotting, so the intensity of staining was measured, but not the frequency of IgE-binding to the different bands of peach in single serum samples.

Wadee et al. [20] identified IgE antibodies to a 30 kd protein in peach and other botanically unrelated fruits. However, only one patient allergic to peach was investigated. Of the 19 peach-sensitive patients we studied, only in patients 12 and 18 IgE recognized this band, which can thus be considered a minor allergen.

We can instead confirm the importance of the 20 kd component identified by Calkhoven et al. [21] in one patient with OAS in response to fresh fruits, who had IgE directed toward this component present in peach and cherry as well as in apple (which belongs to the same family of Rosaceae).

Our blot-inhibition experiments showed complete inhibition of three bands of peach extract (13, 14, and 20 kd) by cherry extract and complete inhibition of the 13 and 14 kd bands with partial inhibition of the 20 kd band by apricot and plum extracts. The different protein content in the fruit extract may explain why the 20 kd band was only partially inhibited by apricot and plum extracts. These extracts had a lower protein concentration than cherry extract, which produced complete inhibition. These findings support the concept that clinical symptoms induced by a cluster of hypersensitivity reactions to fruits belonging to the Prunoideae subfamily are due to identical allergens present in all these fruits.

An interesting observation was the different IgE-binding patterns shown by patients with negative or positive RAST results for birch pollen. Although IgE of patients with negative responses to birch pollen recognized only the 13 kd band, IgE of patients with positive responses to birch pollen recognized many allergenic bands to all the Prunoideae (particularly 14, 17, and 20 kd for peach extract, 14, 20, and 30 kd for cherry extract, and 20 kd for apricot and plum extracts). Thus sensitization to components other than the 13 kd allergen seems to depend on hypersensitivity to birch. Considering the recent literature, this is not unexpected because allergenic structures in the range of 14 kd and 17 to 20 kd cross-react between birch and some fruits and vegetables such as celery (14 kd), [22] apple, and nuts (17 to 20 kd). [5]

The association between allergy to foods and pollens is well known. In 1942 [23] it was reported that hypersensitivity to fruits and vegetables mainly occurs in patients with pollinosis. The first reported association concerned birch pollinosis and apple allergy, [4] and later other associations were found. [1, 6, 24] Ebner et al. [5] have recently demonstrated by the Western blot technique that allergens in birch pollen and apple share allergenic epitopes, and this might explain the clinical symptoms caused by these different allergenic sources.

We selected patients with allergy to peach, which in a previous study [1] did not show clinical cross-reactivity with any pollinosis, though some cases of concomitant sensitivity to peach and birch pollen have been reported. [21] We obtained in vitro confirmation of this in vivo observation, because the 13 kd allergen was not inhibited by grass and birch pollen extracts, in which we detected all the IgE-binding components described for these two allergenic sources. Thus this protein seems specific to the Prunoideae fruits and is not shared by these pollens. Conversely, grass and birch pollen extracts inhibited the binding to 14 kd and 20 kd bands of peach. This is very interesting because birch pollen contains a 14 kd component, identified as birch *profilin*. [25] A component of similar MW has also been detected in grass pollen and could be present in vegetable foods as a cross-reactive allergen. [26] In our study the 14 kd allergen appeared as a two-band component in birch, as Vallier et al. [22] reported for profilin.

The 14 kd component of the peach extract was recognized by 57% of sera, and this band was completely inhibited by both grass and birch extracts. This component, which is likely to be the profilin mentioned above, seems to be an important allergen in Prunoideae. On the basis of the lack of peach blotting inhibition by grass and birch pollen extracts, it seems possible to exclude that our 13 kd component is profilin. However, more studies are needed to clarify this point, because we did not use profilin in inhibition experiments. Moreover, the 13 kd band, despite the low MW, does not seem to represent any of the low MW allergens of groups II, III, and X recently described in grass pollen [27, 28] because, as already mentioned, it did not disappear after blotting inhibition by the grass pollen extract.

In contrast, the 20 kd band was completely inhibited by birch and grass pollen extracts, partially confirming the findings of Calkhoven et al. [21] who used SDS-PAGE and immunoblotting to identify two bands at 18 and 20 kd responsible for the co-occurrence of IgE-binding protein in birch pollen and fruit extracts. This fact, together with the recognition of the 20 kd allergen in 9 of 14 patients with positive RAST results for birch, suggests that this allergen might be the major allergen of birch, *Bet v* I.

In conclusion, the results of this study show that the clinical observation of a cluster of hypersensitivity to fruits such as peach, cherry, apricot, and plum has a solid immunochemical basis represented by the presence of specific IgE to a 13 kd component common to all the Prunoideae. This seems to be an important major allergen of this botanical subfamily.

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