

Antigenicity and Immunocrossreactivity of Orange Tree Pollen and Orange Fruit Allergenic Extracts

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Key Words

Allergenic extracts · Orange tree pollen · Orange fruit · Crossreactivity

Abstract

Background: It is important to study the crossreactivity between orange tree pollen (OTPE) and orange fruit (OFE) due to the high incidence of pollen/food-related allergies worldwide. The aim of the present study was to determine the antigenic relationship between OTPE and OFE. **Methods:** OTPE and OFE rabbit antisera as well as sera from patients allergic to OTPE and OFE were comparatively applied in IgG- and/or IgE-specific ELISA inhibition, crossover or inhibition immunoblotting assays using OTPE and OFE allergenic extracts as solid phase. Periodate and proteinase K treatments were used for carbohydrate and protein depletion, respectively. **Results:** The antigenicity of OTPE and the presence of common structures between OTPE and OFE extracts were demonstrated by rabbit IgG-specific ELISA inhibition and crossover immunoblotting assays. A 30-kDa protein, common target of the IgE response on OTPE, OFE and mandarin extract, but absent in lemon extract, was identified by ELISA and immunoblot inhibition assays in patients suffering from primary sensitization to OTPE in the context of occupational exposure. Moreover, biochemical treat-

ments showed that antigenic epitopes on the 30-kDa protein contain polypeptidic but no carbohydrate moieties.

Conclusions: The antigenicity of OTPE, the presence of common antigenic determinants between pollen and citrus fruits and an IgE-specific crossreactive protein band of 30 kDa sharing carbohydrate-free epitopes were described. After isolation and purification, this common antigen might be useful for allergen immunotherapy in pollen/fruit-related allergic patients.

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Introduction

It has been well documented that pollen causes human respiratory allergic disorders such as hayfever and seasonal asthma. Although primary treatment for asthma and allergic rhinitis is pharmacological, allergen avoidance and immunotherapy with appropriate allergen constitute the tripod for treatment of chronic and severe allergic diseases [1]. Antigenic crossreactivity among different allergens has been extensively studied, so that pollen-allergic subjects are sensitive to a number of different pollen types at a time, but rarely sensitive to a single species [2]. Moreover, shared allergenicity has also been demonstrated among pollens of taxonomically distant plant kingdoms and foods [3, 4], and pollen-derived

allergens were indicated as the sensitizing agents responsible for many forms of plant food allergy [5, 6].

In Argentina, pollens derived from grasses, weeds and trees are the main allergens causing allergic rhinitis. In particular, pollen of orange tree (OTPE) belonging to the Rutaceae family, producer of a fruit which is consumed by masses of people, will be considered in this study.

Orange (*Citrus sinensis*) and other citrus fruits have been implicated in allergic reactions as well as in oral allergy symptoms, such as itching and swelling of the lips, tongue and throat [7]. The majority of pollen-related reactions to fruits is associated with birch pollen allergy [8]. Birch pollen and some rare fruit and vegetable extracts share crossreactive allergen structures, including a 35-kDa protein named Bet v 6 [9, 10]. IgE-binding proteins related to the birch pollen minor allergen Bet v 6 have been found in many vegetable foods such as apple, peach, orange, lychee fruit, strawberry, persimmon, zucchini and carrot. A common allergenicity between birch pollen and fruits including orange was demonstrated by radioallergosorbent test (RAST) inhibition assays [11]. However, no reports regarding either the antigenicity of OTPE or the possible antigenic and allergenic relationship between the OTPE and the orange fruit (OFE) or other citrus-related allergenic extracts have been presented to date.

Previous studies of our laboratory demonstrated that allergenic extracts from different types of oranges were able to elicit a specific immune response in rabbits [12]. Moreover, we found that these antigens were immunogenic in children allergic to oranges *Citrus aurantium* and *C. sinensis* [13].

The present study demonstrates that OTPE as well as OFE and mandarin fruit (MFE) extracts are antigenic in patients suffering from primary sensitization to OTPE in the context of occupational exposure. Common antigenic determinants between these allergens, capable to produce human seasonal rhinitis, are also described.

Material and Methods

Preparation of Allergenic Extracts

The OTPE powder (Bayer Corporation RM:9610599, USA) was extracted in phosphate-buffered saline (PBS; 0.1 M Na-phosphate containing 0.15 M NaCl, pH 7.3) by stirring for 24 h at 4°C in a 1:50 (w/v) ratio. After centrifugation at 12,500 g for 40 min, the clarified extract was dialysed in PBS. Oranges (*C. sinensis*) were carefully peeled, and the bark and all other fibrous structures including the seeds were discarded. The remaining pulp was submitted to the Frugoni Hansen's method [14] to obtain the crude extract (OFE). MFE and lemon (LFE) extracts were obtained according to

the same method. *Blattaria sudamericana* extract (BE) used as control was obtained as previously described [15].

Extracts were sterilized by filtration through 0.22-µm Millipore filters. Sterility controls were performed, and the filtrated extract was frozen and stored at -20°C.

The Bradford's method was applied to determine the protein concentration of each extract, using bovine serum albumin as standard solution [16].

Chemical Modification of Allergenic Extracts

For carbohydrate moieties oxidation assays, lyophilized protein extracts were incubated in 50 mM sodium metaperiodate in the dark with gentle mixing for 72 h at 4°C. The reaction was stopped by intensive dialysis against PBS, according to the method of Owhashi et al. [17]. Periodic acid oxidation was also performed on protein extracts separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose sheets by adding 0.1 M periodic acid in sodium acetate buffer, pH 4.5, for 18 h at 4°C under shaking. Strips soaked in sodium acetate buffer served as controls. After treatment and intensive washing, Western blotting assays were performed as previously described [18]. Proteinase treatment was done by incubation of allergenic extracts with proteinase K (1 µg/ml) for 1 h at 4°C.

Production of Antisera Specific to Allergenic Extracts

Experimental antisera to OTPE or OFE were obtained by immunizing rabbits with each whole allergen extract diluted in PBS emulsified with Freund's complete adjuvant (1:1 v/v) mixture, as it was described elsewhere [12]. Ouchterlony, immunoelectrophoresis and passive hemagglutination tests [19, 20] were used to determine the antigenicity of allergenic extracts as well as the IgG subtype of the polyclonal sera obtained.

Patient Eligibility

Atopic patients suffering from primary sensitization to OTPE were selected among subjects working in an orange plantation in San Pedro, Buenos Aires, Argentina.

Atopy was determined by a clinical history of allergic symptoms related to OTPE and OFE allergies, by positive skin prick test responses to OFE and OTPE and by positive RAST to both allergenic extracts.

Patients were divided in two groups: (1) 4 patients showing positive skin prick test and positive RAST against OTPE and OFE, and (2) 2 patients with positive skin prick test and positive RAST against OTPE but negative against OFE, who had been receiving immunotherapy for 2 years (table 1). Patient symptoms included eyes itching, seasonal rhinitis, rhinoconjunctivitis and asthma. However, none of them showed urticaria, lip pruritus, facial angioedema, glossitis or shock after fruit ingestion. Patients allergic to other tree pollens present in the same area (plane or linden trees) as well as to other foods (tomato, rice, cacao, milk) were excluded from this study.

Healthy individuals with no history of allergy and with negative responses to pollens and food allergenic extracts were included as nonatopic controls.

Patients' Sera

Blood used for serum component analysis was allowed to coagulate at 4°C and centrifugated at 1,000 g for 15 min. Nonhemolyzed serum was separated, and aliquots were stored at -70°C until use.

Table 1. Characteristics of study population

Pa-tients	Sex	Age years	Symp-toms		Skin test	Rast score PRU/ml serum
1	M	30	RC/I	OTPE	++++	4.8
				OFE	++	2.7
				PTE	–	<0.35
				LTE	–	<0.35
				BE	–	<0.35
2	M	27	RC/I	OTPE	+++	3.9
				OFE	++	0.60
				PTE	–	<0.35
				LTE	–	<0.35
				BE	–	<0.35
3	M	42	RC/A	OTPE	++	0.7
				OFE	++	0.42
				PTE	–	<0.35
				LTE	–	<0.35
				BE	–	<0.35
4	F	54	R	OTPE	+++	1.3
				OFE	++	0.69
				PTE	–	<0.35
				LTE	–	<0.35
				BE	–	<0.35
5	F	63	RC/A	OTPE	+++	3.4
				OFE	–	<0.35
				PTE	–	<0.35
				LTE	–	<0.35
				BE	–	<0.35
6	M	38	RC/I	OTPE	++	0.70
				OFE	–	<0.35
				PTE	–	<0.35
				LTE	–	<0.35
				BE	–	<0.35

Results of skin test are given as follows: + = below half positive reference; ++ = above half positive reference; +++ = above positive reference; ++++ = above twice positive reference. PRU = Phadebas RAST units; M = male; F = female; R = rhinitis; RC = rhinoconjunctivitis; A = asthma; I = itching. The extracts tested for skin test and RAST are orange tree pollen (OTPE), orange fruit (OFE), plane tree (PTE), linden tree (LTE) and *B. sudamericana* (BE). Symptoms: no glossitis, shock, lip pruritus, angioedema was detected after fruit ingestion

Skin Testing

Skin prick tests were carried out to measure reactivity to allergenic extracts according to Aas and Belin [21]. Skin tests were performed in the morning and graded after 15 min based on weal size of histamine control, as it was conventionally established [21]. On the day the skin test was performed, none of the allergic patients or controls were taking steroids, antihistamines or immunosuppressive drugs.

RAST

Determination of specific IgE in human sera was done using cyanogen bromide-activated cellulose 5-mm disks coupled to 10 µg of allergenic extract proteins in 0.1 M carbonate/bicarbonate buffer, pH 8.5, according to the method of Ceska et al. [22]. After blocking with 0.05 M methanolamine, the disks were incubated with 50 µl of serum overnight at room temperature. Specific IgE antibodies were developed using Phadebas RAST (Pharmacia, Uppsala, Sweden). Results are expressed in Phadebas RAST units (PRU/ml) considering positive responses when IgE values are higher than 0.35 PRU/ml [23, 24].

ELISA and Inhibition ELISA

ELISA was performed as described by Sridahara et al. [25]. Briefly, the wells were coated with 100 µl of each extract (10 µg/ml in sodium carbonate buffer, pH 9.6) for 2 h at 37°C and blocked with 3% defatted milk in 0.1 M PBS. Plates were then washed four times with PBS containing 0.05% Tween-20 and incubated with 100 µl of a 1:10 dilution of human pooled sera for 2 h at 37°C. After additional washing with PBS containing 0.05% Tween-20, 100 µl of a 1:2,000 dilution of peroxidase-conjugated goat antihuman IgE was added to each well for 2 h at 37°C. The reaction was developed with *o*-phenyldiamine in citrate buffer, pH 4.6, and hydrogen peroxide and stopped with 50 µl of 2.0 M sulfuric acid. The absorbance was read at 492 nm utilizing an automated ELISA reader (MR 700, Dynatech Laboratory, USA).

IgE inhibition ELISA assays were carried out as mentioned above, but pooled sera were adsorbed with the different allergenic extracts. Inhibition assay was optimized testing different concentrations of protein extracts and antisera. A 1:10 dilution of human pooled sera was added to separate wells coated with 5 µg of each protein extract and incubated for 2 h at room temperature. Then, the adsorbed pooled sera were collected and used to detect IgE specific to the different allergenic extracts. Sera from nonatopic subjects and nonrelated allergenic extracts were used as negative controls for both ELISA and inhibition ELISA assays.

IgG inhibition ELISA assay was performed by incubating 100 µl of rabbit antisera with 100 µl of increasing concentrations of OTPE or OFE as inhibitor for 2 h at room temperature. The adsorbed serum was then added to the wells previously coated with antigen. Normal rabbit preimmune serum and immune serum incubated with PBS were used as negative controls. The antibodies bound to the plate were detected after addition of a peroxidase-labeled goat antirabbit IgG conjugate. The percentage of inhibition was calculated as total reactivity – remaining reactivity after adsorption × 100/total reactivity.

SDS-PAGE

Fifty micrograms of allergenic extract samples and standard molecular weight markers were electrophoresed in 12% polyacrylamide mini-gels in the presence of sodium dodecyl sulfate, according to Laemmli [26].

Western Blotting and Crossinhibition Western Blotting

OTPE and OFE protein samples were electrophoresed as described above and electrotransferred to nitrocellulose membranes, according to Towbin et al. [27]. The membranes were soaked for 90 min in blocking solution (TBS, 50 mM Tris, 150 mM NaCl, pH 7.6, containing 3% nonfat dry milk) and tested with a 1:100 dilution of rabbit anti-OTPE or rabbit anti-OFE sera at a 1:10 dilution

Fig. 1. Antigenic relationship between OTPE and OFE allergenic extracts. For IgG, inhibition ELISA assays, anti-OTPE (a) and anti-OFE (b) were adsorbed with different concentrations of OTPE or OFE and added to OTPE or OFE as the solid-phase antigen, respectively.

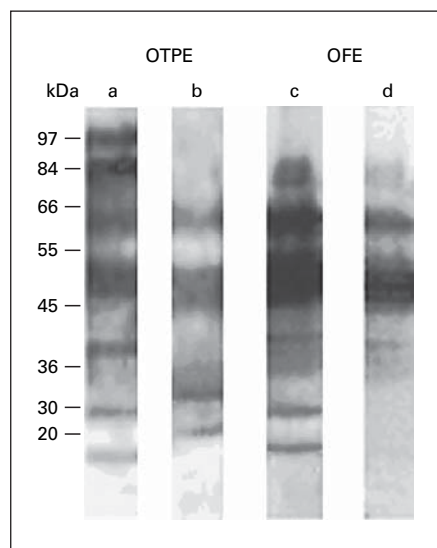
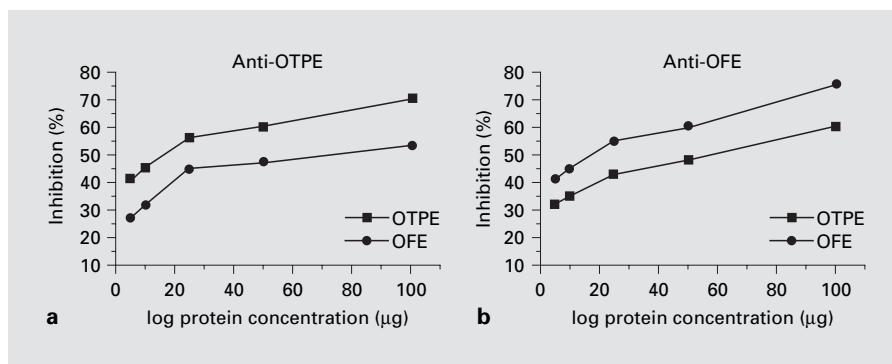


Fig. 2. Immune crossreactivity between OTPE and OFE extracts. Fifty micrograms of OTPE or OFE were separated by SDS-PAGE, electrotransferred to nitrocellulose sheets and probed with an anti-OTPE (lanes a and d) or anti-OFE (lanes b and c) polyclonal serum.

of human pooled sera. After a 90-min incubation with rabbit anti-sera, strips were washed and treated with a goat antirabbit IgG antibody (1:2,000), coupled to phosphatase alkaline for 90 min at room temperature. Individual and pooled human sera were incubated overnight and strips were then treated with peroxidase-labeled goat antihuman IgE for 90 min at room temperature [28]. Sera from nonatopic subjects and normal rabbit sera served as negative controls.

For inhibition of IgE binding, the optimal dilution of human pooled sera (1/10) was preincubated with 100 μg of each allergenic extract under continuous shaking, overnight at 4°C. Afterwards, adsorbed sera were added to individual strips containing the different allergenic extracts and tested by immunoblotting as described above. One hundred micrograms of extract protein were required for complete IgE inhibition.

Results

Immunocrossreactivity of Rabbit Anti-OTPE and Anti-OFE Sera to Allergenic Extracts

We explored the antigenic relationship between the OTPE and OFE allergenic extracts. A clear inhibition of the binding between anti-OTPE serum and its cognate antigen was observed when adding increasing concentrations of OFE (fig. 1a). Likewise, the addition of OTPE inhibited the immune recognition of anti-OFE serum to OFE protein extract (fig. 1b). As expected, at the same antigen concentration, the percentage of inhibition was higher when the cognate antigen was used as inhibitor in comparison with the nonhomologous antigen (fig. 1a, b). Interestingly, a 5- or 3-fold concentration of OFE and OTPE, respectively, as inhibitor antigen was required to obtain a 50% inhibition in the IgG binding of each anti-sera to its cognate antigen.

The antigenic relationship between OTPE and OFE was also analyzed by immunoblotting using rabbit antibodies raised to individual extracts. Duplicate strips loaded with OTPE or OFE (fig. 2) were confronted with anti-OTPE or anti-OFE antisera. Anti-OTPE rabbit serum recognized antigenic components with molecular weight ranging from 10 to 100 kDa (fig. 2, OTPE, lane a) on OTPE. A similar pattern of recognition was revealed with anti-OFE on OTPE-loaded antigen, with no reactivity to either protein bands between 80 and 90 kDa or to a 30-kDa protein (fig. 2, OTPE, lane b). Similarly, anti-OFE serum recognized bands of a broad range of apparent molecular weight when probed to OFE, without reactivity at molecular weights higher than 80 kDa (fig. 2, OFE, lane c). On the other hand, anti-OTPE revealed crossreactive proteins on OFE only in a narrow range of apparent molecular weight between 45 and 60 kDa (fig. 2, OFE, lane d).

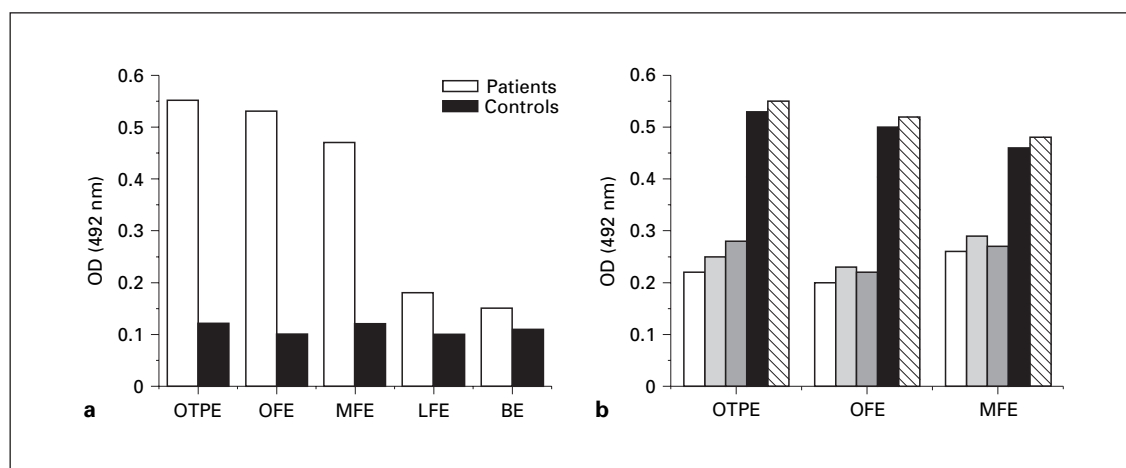


Fig. 3. Identification of IgE epitopes shared between OTPE and citrus fruit extracts. Levels of IgE antibodies specific to allergenic extracts were measured in sera from atopic patients. **a** Pool I (patients), nonallergic control pooled sera (controls). **b** After adsorption with OTPE (white bars), OFE (light gray bars), MFE (dark gray bars) or 3-BE (black bars). Nonadsorbed controls are also shown (hatched bars). Data represent mean OD $\pm$ SD of three different experiments.

Altogether, these results not only indicate that both allergenic extracts are antigenic but that these extracts share antigenic structures as well.

Evaluation of Allergenicity of Pollinic Patient Sera

Considering the immune crossreactivity found between OTPE and OFE, it is possible that patients suffering from primary sensitization to OTPE in the context of occupational exposure might develop IgE responses specific to OFE and other related citrus fruit extracts. To test this hypothesis, we measured IgE responses to OTPE, OFE, MFE, LFE and BE in patients allergic to OTPE and OFE, utilizing ELISA, Western blot and inhibition immunoblotting assays. According to the clinical findings and laboratory data, sera patients were pooled as follows: pool I, sera from patients with positive allergenic tests for OTPE and OFE; pool II, sera from patients with positive tests for OTPE but negative for OFE.

We found that patients allergic to OTPE and OFE have significantly raised levels of IgE antibodies to OTPE, OFE and MFE compared with nonallergic subjects (fig. 3a). However, no differences were recorded among the three extracts evaluated. By contrast, in these patients, levels of IgE specific to LFE did not differ from those recorded in control subjects. Likewise, no IgE responses to the nonrelated antigen BE were observed.

The presence of common IgE epitopes between OTPE and OFE and the related MFE extract was further investigated by ELISA crossover studies, measuring the re-

maining IgE levels after adsorption of sera with the different protein extracts. The levels of IgE significantly decreased in comparison with nonadsorbed sera, irrespective of the citrus fruit extract employed for adsorption (fig. 3b). Conversely, adsorption of pool I sera with BE had no effect on the ability of this sera to recognize either OTPE, OFE or MFE.

These results corroborate the findings of the skin and RAST tests, showing that the patients evaluated have IgE levels specific to OTPE and OFE. Common targets of the IgE response on OTPE, OFE and MFE were also revealed in the natural course of allergic disorders.

In order to identify the antigenic structures of the protein extracts under analysis, immunoblotting and immunoblotting inhibition assays were conducted. Sera from pool I, assayed individually, recognized 3 protein bands of 30, 60 and 70 kDa on OTPE extract, whereas on OFE and MFE, a 30-kDa protein was observed. No reactivity was detected on either LFE or BE. Likewise, pooled sera from controls failed to recognize any band on all the protein extracts tested. Interestingly, individual sera from patients allergic to OTPE but negative for OFE (patients 5 and 6, table 1) did not recognize the 30-kDa band in any of the citrus fruit extracts assayed (table 2).

The 30-kDa protein band detected on OTPE, OFE and MFE focused our attention to this protein to ascertain whether sera patients recognize common epitopes on this protein. No IgE binding could be detected against this band on OTPE or OFE after adsorption of atopic pool I

Table 2. Detection of specific IgE to allergenic extract proteins in patients allergic to OTPE and citrus fruit extracts, measured by immunoblotting assays

Serum sample	OTPE			OFE	MFE	LFE	BE
	30 kDa	60 kDa	70 kDa	30 kDa	30 kDa	30 kDa	30 kDa
1	+	+	+	+	+	–	–
2	+	+	+	+	+	–	–
3	+	+	+	+	+	–	–
4	+	+	+	+	+	–	–
5	+	+	+	–	–	–	–
6	+	+	+	–	–	–	–
PC	–	–	–	–	–	–	–

One hundred micrograms of OTPE, OFE, MFE, LFE and BE were separated by SDS-PAGE, electrotransferred and tested by immunoblotting with four serum samples obtained from patients 1–4 suffering from allergy to OTPE associated to OFE and patients 5 and 6 allergic only to OTPE. Pooled control sera (PC) were obtained from 5 nonallergic subjects.

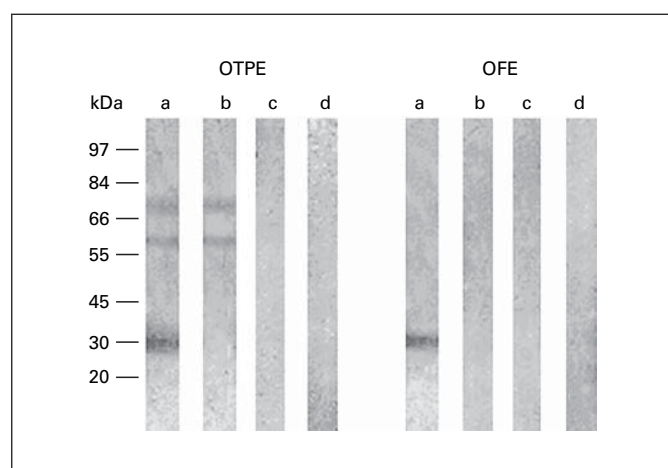


Fig. 4. Presence of common epitopes target of IgE response specific to OTPE extract and OFE extract in atopic patients. Fifty micrograms of OTPE or OFE were separated by SDS-PAGE, electrotransferred to nitrocellulose sheets and probed with pool I sera before (lanes a) and after adsorption with OFE (OTPE, lane b) or OTPE (OFE, lane b). Pooled sera from nonatopic patients (lanes c) and nonrelated allergenic extract (lanes d) were also assayed as controls.

sera with OFE or OTPE, respectively (fig. 4, lanes b), suggesting that the 30-kDa protein is an allergen bearing common epitopes.

Partial Characterization of the Antigenic Components Recognized by IgE Antibodies

To further characterize the antigenic components recognized by IgE antibodies in atopic patients, OTPE and OFE extracts were treated with proteinase K or periodic

Table 3. Characterization of antigenic components recognized by sera from atopic patients

Extract	OTPE	OFE
Without treatment	0.505 ± 0.04	0.485 ± 0.04
Protein depleted	0.120 ± 0.20	0.100 ± 0.20
Carbohydrate depleted	0.490 ± 0.07	0.470 ± 0.07

IgE antibodies were determined by ELISA in pool I sera before and after chemical modification of OTPE and OFE. Values are given as OD 492 nm $\pm$ SD obtained as mean of three different experiments.

acid. Abolishment of carbohydrate moieties did not modify the immune recognition to either allergenic extracts, whereas proteinase K treatment significantly decreased the IgE response to OTPE and OFE (table 3). These data show the protein nature of IgE epitopes with a very low content of carbohydrate moieties.

Discussion

The association between pollinosis to birch, ragweed, mugwort, grass or plantain and certain fruits, nuts or vegetables was extensively described. Despite crossreactivity between unrelated allergens previously reported [11], including a variety of nuts, fruits and vegetables, there are no reports relating pollen and citric fruits. Patients with hypersensitivity to fruits or vegetables often show allergenic reactions to various plant pollens [6, 8].

Therefore, it is unusual to find patients pollinic to a single species.

In the present study, we investigated the antigenic relationship between OTPE and OFE extracts in patients suffering from primary sensitization to OTPE in the context of occupational exposure. For the first time, we show that OTPE is antigenic and that OTPE, OFE and MFE extracts share antigenic structures as well. Furthermore, a 30-kDa protein appeared to be a common target of the IgE response on OTPE, OFE and MFE extracts, but not on LFE extract, in patients with allergy restricted to OTPE and OFE extracts.

The association between orange tree pollinosis and citric fruit sensitization might be explained by the presence of common epitopes on either homologous or different proteins in these extracts. However, in our study, these crossreactive epitopes do not seem to exist in all citrus fruits. No IgE levels specific to BE were found, confirming that the immune recognition is specific to the allergenic extracts tested. In this regard, pollen allergen moieties, specifically recognized for idiotypes of IgE antibodies, have been shown to be conserved in the plant kingdom, and a high level of sequence homology between various taxa and related pollen food allergens was also described [29, 30]. Interestingly, experiments performed using rabbit antisera raised against OTPE and OFE revealed that the common structures are represented in OTPE protein extract rather than in OFE. Therefore, OFE allergy is more likely to occur in patients primary sensitized to OTPE. In addition, mastocytes of the respiratory tract mucosa are more easily exposed to the inhaled allergen than those belonging to the oral mucosa and thus being activated via the specific IgE antibodies.

Herein, crossreactivity of OTPE and OFE was shown to be mainly dependent on the presence of a 30-kDa protein. Interestingly, sera from patients hypersensitive to OTPE but negative for OFE recognized the 30-kDa protein only on OTPE. The absence of recognition was not due to low concentration of OFE extract because overloading with a 10-fold concentration of antigen did not change the result. These data suggest the presence of distinct epitopes on the 30-kDa protein in OTPE capable of eliciting different IgE responses in pollen-related food allergy and pollen allergy. In contrast to the Western blot analysis with human sera, no crossreactivity of a 30-kDa protein from pollen extract and fruit extract could be seen with rabbit antibodies raised against OTPE and OFE in crossover immunoblotting assays. These findings might indicate the existence of different IgE and IgG crossreactive epitopes on these extracts. In agreement

with these observations, atopic patients who had been receiving immunotherapy with OTPE for 2 years increased the levels of IgG capable of recognizing the 30-kDa protein on OTPE. Conversely, no IgG reactivity on the 30-kDa protein was recorded on OFE (data not shown).

Some reductases of 35 kDa of apparent molecular weight which might represent a new family of pollen-related food allergens present in rarely allergenic fruits and vegetables, named Bet v 6 or Bet v 6-related proteins, were described [7, 8]. A common allergen of 30 kDa was also reported in a variety of related fruits, but in this study, patients were hypersensitive to fruits with no allergic responses to pollen [31]. In addition, a crossreactive allergen of 30 kDa was related to allergy to latex and some fruits [32]. Different proteins of 30–35 kDa might comigrate in SDS-PAGE and present immunoreactivity to IgE. Hence, we cannot rule out the 30-kDa crossreactive allergen to be the same protein described by other authors.

Generally, allergens causing immediate hypersensitivity are proteins, and most studies show that they are usually more than one [33]. Preliminary results obtained after depletion of protein or carbohydrates revealed the presence of protein epitopes on OTPE and OFE extracts, suggesting that the antigenicity of the 30-kDa protein would not be related to carbohydrate moieties. Epitope mapping analysis will further confirm these findings.

Patients evaluated in the present report displayed allergic symptoms, but not one had oral allergy symptoms, indicating that the 30-kDa protein might not be involved in the development of severe clinical manifestations in fruit-related allergic episodes.

In conclusion, the antigenic relationship between OTPE and related citrus fruits has been established. The presence of common epitopes between OTPE and OFE might explain the development of OFE allergy in patients sensitized to OTPE. These results point out that the isolation, purification and molecular characterization of crossreactive allergens might be useful for diagnosis and therapy of pollen fruit-related allergy.

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