

Olive allergen-specific IgE responses in patients with *Olea europaea* pollinosis

Background Olive tree (*Olea europaea*) pollen is an important cause of pollinosis in the countries of the Mediterranean area.

Objective This work aimed to study the IgE-binding frequency of Ole e 1, Ole e 2, Ole e 3, Ole e 6 and Ole e 7 from *O. europaea* pollen in a large population of olive pollen-allergic patients.

Methods We studied: 119 consecutive patients with seasonal rhinitis and/or asthma and a positive skin prick test to *O. europaea* pollen extract; 10 atopic patients without history of pollinosis and a negative skin prick test to *O. europaea*; and 10 healthy controls. Allergens were purified from *O. europaea* pollen extract by reverse phase HPLC and characterized by N-terminal amino acid sequencing, MALDI analysis, and specific IgE immunodetection. Skin prick tests and ELISA titration against above mentioned purified olive pollen allergens were performed in all pollinic patients and controls.

Results One-hundred and seven (90.7%) patients had a positive skin response to Ole e 1; 88 (74.6%) reacted to Ole e 2; 57 (47.9%) reacted to both Ole e 6 and Ole e 7; and 43 (37.8%) reacted to Ole e 3. The allergenic activity determined by ELISA to Ole e 1 was found in 84%; to Ole e 2 in 61.3%; to Ole e 3 in 31.9%; to Ole e 6 in 39.4%; and to Ole e 7 in 41.2% of patients. All patients had positive skin responses to at least one of the allergens tested. However, a combination of Ole e 1 and Ole e 2 together with a minor allergen Ole e 6 or Ole e 7, disclosed the same diagnostic value that was obtained with the use of crude olive pollen extract. The nonatopic and atopic control subjects did not react to any purified allergens on the skin prick test.

Conclusions These results indicate that Ole e 1 and Ole e 2 are major allergens in patients with *O. europaea* pollinosis in our population. A combination of a few olive pollen allergens can substitute the crude extract for *in vivo* as well as *in vitro* diagnostic purposes.

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Allergen products for allergy diagnosis and treatment are based on heterogeneous allergen extracts, which include a mixture of allergenic and nonallergenic proteins. Many efforts have been attempted in the last years to delineate the composition of these extremely complex allergen extracts and most of the major inhalant allergens have been now identified.

Olive pollen is one of the most important cause of the respiratory allergy in the Mediterranean area (1). Patients had rhinitis and/or bronchial asthma of an increased severity when they were exposed to high level pollen counts (more than 4000

grains/m³ in Jaén, Southern Spain) (2). Using different standard laboratory methods (SDS-PAGE, isoelectrofocusing, immunoblotting), the olive pollen extract has showed the presence of at least 20 protein bands with allergenic activity (3). To date, eight olive pollen allergens have been purified of *Olea europaea* pollen and they have been named Ole e 1–Ole e 8, according to the current International Nomenclature (4–10). But, some evidence has suggested a more complex IgE response against the olive pollen allergens in these pollinic patients. Furthermore, it has become evident in recent years the wide diversity of these allergograms among

Table 1. IgE prevalence of the olive pollen allergens (4–10)

Allergen	IgE prevalence (%)
Ole e 1	55–90
Ole e 2	24
Ole e 3	20
Ole e 4	80
Ole e 5	35
Ole e 6	15–55
Ole e 7	47
Ole e 8	5

patients with olive pollen allergy, with several IgE-reactive bands when each individual serum was analysed (11).

Therefore, it is not a surprise that some researchers have tried to set up the dissection of this specific IgE responses in several well-defined populations in order to better understand the pollen disease and hopefully find new ways for treatment and possible prevention. However, the patients' sera used for the allergen purification and characterization procedures were only selected on the basis on an increased RAST value mostly equal or more than class III in patients of different geographic areas (5–11) and therefore exposed to different level of pollen in the atmosphere. Also the patients have been poorly defined for the clinical characteristics, such as the type and the severity of the allergy syndrome and/or association with other atopic conditions.

With these restricted recruitment criteria, the allergenic prevalence of all known olive pollen allergens, evaluated throughout serological methods, have been reported (Table 1). Studies concerning the value of the use of purified allergens in the diagnosis of olive allergy are few, and involving only to Ole e 1 (12). For this reason, the induction of skin reactivity with the remaining purified olive allergens has not yet been analysed and therefore it has not been compared with other standard methods used such as an allergen-specific ELISA.

The aim of this study was to evaluate the immunological activity of some purified olive pollen allergens (Ole e 1, Ole e 2, Ole e 3, Ole e 6, and Ole e 7) in a population of patients suffering *O. europaea* pollinosis using skin tests as well as an allergen-specific ELISA.

Methods

Patients

This study included 119 consecutive patients who fulfilled the following criteria: (1) seasonal rhinitis

and/or bronchial asthma from late April to June; (2) a positive skin prick test to *O. europaea* pollen extract (Abelló Laboratory, Madrid, Spain) and (3) no previous *O. europaea* immunotherapy. Ten healthy subjects and 10 atopic patients (four mono-sensitized to mites and six to animal dander) were also included as a control group. Ethical approval for the skin testing with purified allergens in human subjects was obtained from the Ethical and Research Committee of the Hospital Ciudad de Jaén. A full explanation of the procedure was given to all subjects and a written consent was obtained.

Purification of olive allergens from *O. europaea* pollen extract

Olive tree pollen was obtained from Allergom AB, Sweden. The pollen (5% w/v) was extracted with 50 mM ammonium bicarbonate, pH 8.0, containing 1 mM phenylmethylsulfonyl fluoride, followed by centrifugation at 12 000 g for 20 min at 4°C. The lyophilized supernatant was stored at –20°C. The olive pollen allergens Ole e 1, Ole e 2, Ole e 3, Ole e 6 and Ole e 7 were isolated as previously described (4–6,8,9).

Skin testing

Skin prick test (SPT) with purified olive allergens were performed in duplicate by puncture with a Morrow-Brown needle (Bayer SA Laboratory, Madrid, Spain) on the volar side of the forearm. Histamine phosphate (10 mg/ml) was used as positive control and physiological saline solution was used as negative control. The test sites were placed 3 cm apart to avoid false-positive results. From an initial dilution of allergen of 1 mg/ml, four serial 10-fold dilutions (from 100 µg/ml to 0.1 µg/ml) of each allergen were made in sterile physiological saline solution. The patients were also tested with a panel of common aero-allergens, including *Dermatophagoides pteronyssinus*, *Blattella germanica*, dog and cat dander, *Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Lolium perenne*, *Cynodon dactylon*, *Cupressus sempervirens*, *Platanus acerifolia*, *Artemisia vulgaris*, *Chenopodium album*, *Salsola kali* and *Parietaria judaica* (Abelló Laboratory, Madrid, Spain). The reactions were recorded after 15 min by measuring the maximal longitudinal (D) and transversal (d) diameters of the wheal reaction. The surface area was calculated according the following formula (12): $[(D + d)/2]^2$. Patients were regarded as

being sensitized to specific pollen allergen if the wheal had a surface area of at least 9 mm².

Detection of allergen-specific IgE antibodies

IgE-binding measurements of allergic sera were performed by the titration of solid phase-fixed purified allergen as previously described (12). Briefly, ELISA plates were coated overnight at 4°C with 0.1 ml of allergen (1 µg/ml) in PBS and 0.5% (v/v) Tween 20, then saturated with PBS containing 3% (w/v) nonfat milk and 0.1% (v/v) Tween 20. Plates were incubated with the human sera diluted 1:10 followed by 100 µl of mouse antihuman IgE diluted 1:5000 in blocking solution. Afterwards, plates were incubated with horseradish peroxidase-labeled goat antimouse IgG (1:50 000 dilution). Finally the peroxidase reaction was developed using fresh enzyme substrate (0.03% v/v) H₂O₂ and 0.63 mg/ml of o-phenylenediamine in 0.1 mM sodium citrate, pH 5.0) and the optical density was measured at 492 nm. Values under 0.1 were considered as a negative response.

Data analysis

Chi square test was used for comparing the number of subjects with specific IgE for each olive allergen in the groups of patients with concomitant Chenopodiaceae and/or Poaceae pollinosis. Differences associated with probability values of $P = 0.05$ or less were considered as significant.

The diagnostic efficiency of the prick test with each allergen was evaluated in terms of sensitivity and specificity. The sensitivity of the diagnostic test was defined as the proportion of truly diseased persons in the population correctly identified by the prick test. The specificity was defined as the proportion of nondiseased persons correctly identified as negative by the test.

Results

Patients

The clinical features of patients included in the study are summarized in Table 2. The patients with *Olea europaea* pollinosis included 43 males and 76 females, ranging in age from 18 to 42 years (mean age 23 years). Eighty-nine patients had experienced seasonal bronchial asthma and 30 had rhinitis before being included in the study.

Table 2. Clinical features of the population studied

Clinical features		
Sex (male/female)		43/76
Mean age in years (SD)		22.9 (7.1)
Atopic diseases	Bronchial asthma	89 (74.8%)
	Rhinitis	30 (25.2%)
Only olive pollinosis		11 (9.2%)
Concomitant pollinosis	<i>Chenopodium album</i>	86 (72.3%)
	<i>Lolium perenne</i>	84 (70.6%)
	<i>Plantago lanceolata</i>	72 (60.5%)
	<i>Salsola kali</i>	65 (54.6%)
	<i>Cynodon dactylon</i>	50 (42.0%)
	<i>Artemisia vulgaris</i>	49 (41.2%)
	<i>Platanus acerifolia</i>	49 (41.2%)
	<i>Cupressus sempervirens</i>	28 (23.5%)
	<i>Parietaria judaica</i>	25 (21.0%)

SD, standard deviation.

Table 3. Distribution of specific IgE responses for each olive allergen in patients with *Olea europaea* pollinosis

Allergen	Prick-test (%)	ELISA (%)
Ole e 1	108 (90.7)	100 (84.0)
Ole e 2	89 (74.6)	73 (61.3)
Ole e 3	45 (37.8)	38 (31.9)
Ole e 6	57 (47.9)	47 (39.4)
Ole e 7	57 (47.9)	49 (41.1)

IgE-prevalence of olive pollen allergens

The distribution of allergen-specific IgE responses obtained by both prick test and ELISA method are summarized in Table 3. Different rates of sensitization have been observed for the distinctive allergens. Ole e 1 and Ole e 2 may be considered as major allergens, because more than 50% of unselected sensitized patients had specific antibodies for each allergen. We have also identified a response against Ole e 6 and Ole e 7 in 47.9% of our patient-group. In contrast, purified Ole e 3 showed a positive skin responses for only 45 (37.8%) of the patients.

One-hundred eight patients (90.7%) with olive allergy had a concomitant pollinosis (see Table 2). Using skin prick test and ELISA measurements, olive pollinic patients with concomitant pollinosis had significant levels of specific IgE for Ole e 2, Ole e 3, Ole e 6 and Ole e 7 (Table 4). In contrast, Ole e 1 was lacking this association when compared with the same pollen sources.

Table 4. Association of olive allergen specific IgE responses with other concomitant pollinosis

Olive allergen	Chenopodiaceae	Poaceae
Ole e 1		
Ole e 2	$P = 0.003$	
Ole e 3		$P = 0.02$
Ole e 6	$P = 0.003$	$P = 0.01$
Ole e 7	$P = 0.003$	$P = 0.01$

Blank spaces denotes absence of association.

Table 5. Olive allergen-specific IgE profiles in the population studied

Allergen pattern*	Frequency	Cumulated percentage
1, 2, 3, 6, 7	28	23.5
1, 2	27	46.1
1	17	60.3
1, 2, 6, 7	12	70.3
1, 2, 3	8	77.0
1, 6, 7	6	82.0
6, 7	5	86.2
2	4	89.5
1, 2, 7	2	91.2
1, 3, 6, 7	2	93.0
2, 3, 6, 7	2	94.6
2, 3	1	95.6
1, 2, 6	1	96.4
3, 6, 7	1	97.3
1, 2, 3, 6	1	98.2
1, 3, 6	1	99.1
1, 2, 3, 7	1	100
Total	119	

*The number denotes each specific olive allergen tested.

Olive allergen-specific IgE profile

The olive allergen-specific IgE profiles showed a great variability (Table 5). Seventeen different combinations of specific IgE antibodies against olive pollen allergens, ranged from 1 to 5 allergens (mean $\pm$ SD, 3.0 ± 1.1), were described in our patient-group. Sixty-five patients (54.6%) presented an IgE profile involving three or more allergens. About 17.6% of olive allergic patients were sensitive to unique allergen: 17 patients only reacted to Ole 1 and four reacted to Ole e 2; the 33 remaining patients (27.7%) reacted to two olive allergens.

Diagnostic value of skin test with olive pollen allergens

All of the 119 patients with a positive skin test responses to a commercial olive pollen extract reacted to at least one of the five olive pollen allergens used in this study. In 10 nonatopic and 10

Table 6. The diagnostic value of some defined combinations of olive allergens

Allergens	Sensitivity (%)	Specificity (%)
Ole e 1, Ole e 3	94.1	100
Ole e 1, Ole e 2	94.9	100
Ole e 1, Ole e 7	94.9	100
Ole e 1, Ole e 6	95.7	100
Ole e 1, Ole e 3, Ole e 6	96.6	100
Ole e 1, Ole e 3, Ole e 7	96.6	100
Ole e 1, Ole e 2, Ole e 3	97.4	100
Ole e 1, Ole e 2, Ole e 6	100	100
Ole e 1, Ole e 2, Ole e 7	100	100

atopic control subjects with negative olive skin test results, there were no positive reaction to olive pollen allergens, even at the highest concentrations, confirming the specificity of these allergen skin tests. Therefore, the sensitivity in establishing olive sensitization was 100% when these allergens were used. The diagnostic value of these specific combinations are summarized in Table 6. A combination of Ole e 1 and Ole e 2 together with one of the minor allergens Ole e 6 or Ole e 7, disclosed the same diagnostic value that was obtained with the crude olive pollen extract.

Discussion

We have firstly provided the allergen-specific IgE profile in a population with *O. europaea* pollinosis. Several concerns could be made about the recruitment criteria in this population. First, the clinical syndrome was the primary inclusion criteria for the study, rather than other previously reported criteria (4–10) such as the increased specific IgE values against the crude olive pollen extract. By using a combination of regular symptom-recording and peak expiratory flow measurements during the spring season, the course of asthma and/or rhinitis was effectively monitored in all patients. Secondly, our patient-group belonged to the same geographic area, in Jaén, where more than 4000 grain/m³ are recorded from late April to June (2).

According to our results, the major allergens identified (more than 50% of frequency) were Ole e 1 and Ole e 2. The IgE binding frequency to the olive allergens were assessed using skin prick test and ELISA methods. The percentage of agreement between IgE test and skin test ranges between 73 and 85% depending on the allergen. Thus, the skin test appear to be more sensitive than IgE test, since the levels of specific IgE antibodies in the sera of a number of patients with positive skin tests was undetectable.

Our results also demonstrated that the sensitivity in establishing the diagnosis of olive pollinosis was 100% using skin prick test when certain olive allergen mixture was utilized. Moreover, in the control patients with negative olive pollen skin results, there was no positive reaction to olive allergens confirming the 100% specificity for each one of them. Thus, a defined combination of purified major allergens Ole e 1 and Ole e 2, when is associated with one of the following minor allergens Ole e 6 or Ole e 7, may provided a useful tool to diagnose all patients with olive allergy. To this respect, the inclusion of Ole e 6 or Ole e 7 into this allergen mixture displayed a similar diagnostic value in terms of sensitivity. Other minor allergens such as Ole e 3, belonging to the calcium-binding protein family, did not influenced the diagnostic efficiency rate when they were added to the above mentioned panel of allergens. However, our preliminary data indicated that this protein could be a useful marker for characterizing a patient with sensitivity to multiple pollen sources. Ole e 3 exhibits amino acid sequences similar to others contained in the pollen of unrelated species and cross-reactions have been previously demonstrated between Ole e 3 and other protein members of the polcalcin family from *Gramineae*, cypress and mugwort (6,13).

The IgE prevalence of most allergens tested in our patient group agreed with other previous observations, except for Ole e 2, the olive profilin. The prevalence of specific IgE for profilin among different populations ranged from 15 to 24% as detected after SDS-PAGE of the purified protein and immunoblotting (5). In our study, we have demonstrated an Ole e 2-specific IgE response in 74% of the patients. Therefore, it may be considered as major allergen. The reason why we found a higher value is not clear, but could be related to the population studied, which constituted mainly of asthmatic patients heavily exposed to olive pollen. Thus, genetic and environmental factors could be involved in the different sensitivity of the individuals to a specific allergen. However, the prevalence rates may also be influenced by the tests used in the diagnosis (2,11). Previous studies

have indicated the existence of conformational epitopes in olive profilin which are denatured during SDS-PAGE (2,5) and, therefore the frequency of specific IgE for Ole e 2 on immunoblot may be drastically reduced. In any case serological methods for detecting specific IgE for olive profilin, appear to be less sensitive than skin tests with the purified protein.

Profilins are widely distributed protein family involved in cytoskeleton interactions of eukaryotic cells and due to ubiquitous presence were considered as panallergens (14). Possibly, most of the patients sensitized to olive profilin could be allergic to other pollen sources. Both, its increased prevalence and its high cross-reaction degree among the different pollen profilins (15), could explain the low incidence of the olive monosensitized patient in our population (lower than 10%).

In summary, the IgE responses have been studied with purified allergens in patients with *O. europaea* pollinosis. Our results confirm the sensitivity and specificity of the purified allergens in the diagnosis of this disease. Moreover, olive proteins could be useful to understand the cross-reactivity with other nontaxonically related pollens.

Lastly, an important challenge for the future will be the correlation of the increasing laboratory data on allergens and clinical information. For this purpose, the studies executing adequate clinical follow-up in well-defined diseased patient populations, such as those with seasonal rhinitis and bronchial asthma, will be indispensable for understanding the impact of allergen biology on this specific pollinosis.

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