

The Major Allergen of Olive Pollen Ole e 1 Is a Diagnostic Marker for Sensitization to Oleaceae

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Key Words

Tree pollen allergy · Oleaceae · Cross-reactivity · Ole e 1

Abstract

Background: Trees of the family Oleaceae are important allergen sources, with a strongly varying geographic distribution. For example, olive pollen is an important allergen source in Mediterranean countries, whereas ash pollen dominates in Northern and Central Europe and North America. The aim of this study was to compare the profiles of olive and ash pollen allergens and to study the degree of cross-reactivity using populations of allergic patients selectively exposed to olive or ash pollen. **Methods:** Olive and ash pollen extracts were analyzed by IgE immunoblotting using sera from Spanish patients highly exposed to olive pollen and Austrian patients without olive but ash pollen exposure. IgE cross-reactivity was studied by qualitative immunoblot inhibition assays and semiquantitative ELISA inhibitions using olive, ash, birch, mugwort, timothy grass pollen extracts and the major olive pollen allergen, Ole e 1. **Results:** Spanish and Austrian patients exhibited an almost identical IgE-binding profile to olive and ash pollen allergens, with major reactiv-

ity directed against Ole e 1, and its homologous ash counterpart, Fra e 1. IgE inhibition experiments demonstrated extensive cross-reactivity between olive and ash pollen allergens. However, whereas cross-reactions between profilins and calcium-binding allergens also occurred between unrelated plant species, cross-reactivity to Ole e 1 was confined to plants belonging to the Oleaceae. **Conclusions:** Ole e 1 is a marker allergen for the diagnosis of olive and ash pollen allergy.

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Introduction

The ash tree (*Fraxinus excelsior*) is a wind-pollinated tree, widely distributed in the temperate zones of Europe and America [1, 2], where it is frequently grown as a source for hardwood timber. Together with the closely related olive tree (*Olea europaea*), which is recognized as an important cause of pollinosis in Mediterranean countries and in some parts of America [3, 4], and with the insect-pollinated genera jasmine (*Jasminum*), lilac (*Syringa*), privet (*Ligustrum*) and forsythia (*Forsythia*), ash

belongs to the plant family Oleaceae. For a long time the importance of ash pollen in eliciting type I allergic reactions has been overlooked. This might have been partially due to the fact that ash blossoms at the same time (March to May) as other unrelated trees, whose importance in eliciting spring pollinosis is well documented [4, 5]. However, several publications documented heavy loads of ash pollen for some European and American locations during springtime and recognized sensitization to ash pollen as an important health problem [1, 2, 6–8].

Although plants belonging to the family Oleaceae are distributed selectively in different geographic areas, there is evidence for allergenic cross-reactivity [9–13]. This might predominantly be due to the significant amino acid sequence homology between Ole e 1, the major olive pollen allergen which is recognized by more than 70% of olive pollen-allergic patients [3, 14], and the corresponding proteins in taxonomically related plants. Ole e 1, an acidic, polymorphic glycoprotein of 145 amino acids, exhibits 88% sequence identity to Fra e 1 from ash pollen, 89% identity to Syr v 1 from lilac and 87% identity to Lig v 1 from privet [14–17]. However, several clinical observations and in vitro inhibition studies revealed also partial cross-reactivity between Oleaceae pollen and pollen of unrelated plants, such as grasses [18] and birch [19]. Evidence has been provided that at least some of this cross-reactivity is due to the presence of very conserved, highly cross-reactive pollen panallergens (e.g. profilins and two EF-hand calcium-binding proteins) [20, 21]. Furthermore, high-molecular-weight components, such as pectinesterase-like molecules and proteins sharing epitopes with group 4 grass pollen allergens have been suggested as sources of the observed cross-reactivity [20, 21]. It was the aim of our work to search for cross-reactive allergens in pollens from two Oleaceae species with different geographic distribution (i.e., olive and ash) using sera from patients who are exposed exclusively to one of the two species. For this purpose we compared ash and olive pollen allergen profiles using sera from Spanish allergic individuals highly exposed to olive pollen and sera from Austrian patients without olive pollen but ash pollen exposure. Our data showed that allergic individuals from both areas exhibited very similar IgE reactivity patterns to ash and olive pollen allergens and almost complete IgE cross-reactivity between olive and ash pollen allergens was observed. Cross-reactive allergens such as profilin and calcium-binding allergens were also found in grasses, weeds and unrelated trees but the major allergen of olive pollen, Ole e 1, was identified as a diagnostic marker for genuine Oleaceae sensitization.

Table 1. Clinical and serological characterization of Spanish (1–10) and Austrian (11–20) patients

Pa-tients	Pollen allergies	Symp-toms	Specific IgE		
			olive kUA/l	ash kUA/l	Ole e 1 OD _{492 nm}
1	t, w, g	r	6.96	0.87	0.788 ± 0.01
2	t, w, g	r, as	83.6	61.3	2.608 ± 0.07
3	t, g	r, as	2.79	0.65	0.582 ± 0.01
4	t, w, g	r, as	4.93	0.76	0.349 ± 0.01
5	t, w	r, as	95.4	71.6	2.753 ± 0.08
6	t, w, g	r, as	42.4	32.9	1.134 ± 0.09
7	t	r, c	16.7	1.57	2.570 ± 0.09
8	t, g	r, c, as	14.5	1.32	2.917 ± 0.06
9	t, g	r	8.21	9.79	0.068 ± 0.01
10	t, w, g	r, c	18.8	18.5	0.069 ± 0.01
11	t, w, g	r, c, as	7.78	1.59	1.667 ± 0.04
12	t, w, g	ad, r, c, as	43.8	38.4	0.519 ± 0.01
13	t, w, g	r, c	7.83	6.62	0.558 ± 0.01
14	t, g	r, c	29.7	23.4	0.728 ± 0.06
15	t, w, g	r, c	4.34	0.92	0.675 ± 0.02
16	t, w	r, c, as	5.09	2.75	0.239 ± 0.02
17	t, g	r, c	10.9	8.81	1.453 ± 0.03
18	t, w, g	r, c	31.1	7.93	1.936 ± 0.02
19	w, g	r, c	1.26	1.93	0.059 ± 0.02
20	t, w, g	r	13.3	12.6	0.063 ± 0.02

Specific IgE levels to olive and ash pollen were determined with the CAP-FEIA system and specific IgE levels to Ole e 1 were measured in an ELISA. t = Trees; w = weeds; g = grasses; ad = atopic dermatitis; as = asthma; r = rhinitis; c = conjunctivitis.

Material and Methods

Characterization of Patients

The diagnosis of olive pollen allergy in the 8 Spanish patients was based on a case history, positive skin prick test reactions to olive pollen extract and specific IgE antibodies to olive pollen, as determined using the CAP-FEIA System (Pharmacia, Uppsala, Sweden; positive cutoff 0.7 kUA/l). Among 354 sera from pollen-allergic Austrian patients tested by ELISA, 58 sera contained IgE antibodies to olive pollen extract. As determined by ELISA and IgE immunoblot analysis, 36 of them reacted with the major olive pollen allergen Ole e 1. Eight Ole e 1-positive Austrian patients were randomly included in the detailed serological analysis. Two patients sensitized to profilin (No. 9: Spain; No. 19: Austria) and two sensitized to EF-hand calcium-binding proteins (No. 10: Spain; No. 20: Austria) were included in the analysis. Clinical features of the patients as well as specific IgE levels to olive and ash pollen (determined by CAP-FEIA) and specific IgE levels to Ole e 1 (measured by ELISA) are given in table 1.

Allergens and Pollen Protein Extracts

Ole e 1 was purified from olive pollen (Allergon, Ängelholm, Sweden) as previously described [14]. Pollen from olive (*O. euro-*

paea), ash (*F. excelsior*), birch (*Betula verrucosa*), mugwort (*Artemisia vulgaris*) and timothy grass (*Phleum pratense*) were purchased from Allergon. Pollen proteins were extracted overnight at 4°C in phosphate-buffered saline (PBS), pH 7.4 (1 g pollen/10 ml). After centrifugation at 20,000 g for 30 min at 4°C, supernatants were stored at -20°C. The quality of the protein extracts was analyzed by SDS-PAGE performed according to Laemmli [22] using 15% polyacrylamide gels under nonreducing conditions. Proteins were visualized by Coomassie brilliant blue R-250 staining (BioRad, Richmond, Calif., USA) and the molecular masses of the protein bands were estimated by comparison with standard protein markers (MW-SDS-70L) from Sigma-Aldrich (St. Louis, Mo., USA). The protein concentration was determined using the method of Lowry et al. [23] with bovine serum albumin (BSA; Sigma-Aldrich) as a standard.

IgE Immunoblot and IgE Immunoblot Inhibition Experiments

Olive and ash pollen extracts (approximately 80 µg protein/cm gel) and Ole e 1 (2 µg protein/cm gel) were separated by 15% preparative SDS-PAGE under nonreducing conditions and blotted onto nitrocellulose membranes (Amersham Biosciences, Buckinghamshire, UK). After blocking with 3% w/v nonfat milk, 0.1% v/v Tween 20 in PBS (pH 7.4; blocking buffer), the membranes were probed with patients' sera (diluted 1:10 in blocking buffer) at room temperature for 2 h. Bound IgE antibodies were detected by incubating first with mouse anti-human IgE antibodies (diluted 1:5,000; kindly provided by M. Lombardero, ALK-Abelló, Madrid, Spain) and then with an HRP-coupled goat anti-mouse antiserum (diluted 1:5,000; Pierce, Rockford, Ill., USA). The peroxidase reaction was developed using the ECL Western blotting reagent (Amersham Biosciences, Little Chalfont, UK) as described previously [24].

For IgE immunoblot inhibition experiments, sera were diluted 1:10 in blocking buffer and preincubated for 2 h at room temperature either with olive, ash, birch, mugwort or timothy grass pollen extracts (500 µg/ml of total protein), or with purified Ole e 1 (10 µg/ml) or, for control purposes, with BSA (10 µg/ml). Thereafter, the preadsorbed sera (1 ml serum dilution per strip) were incubated with nitrocellulose-blotted proteins (olive and ash pollen extracts, Ole e 1) and bound IgE antibodies were detected as described above.

ELISA and ELISA Inhibition Experiments

Specific IgE antibodies against Ole e 1 were measured by ELISA [25]. ELISA plates were coated with 1 µg/ml of Ole e 1 and exposed to Spanish or Austrian sera (100 µl/well; diluted 1:5). Binding of human IgE was detected with 1:5,000 diluted mouse anti-human IgE antibodies (described above) and 1:5,000 diluted HRP-coupled goat anti-mouse antiserum (Pierce). The peroxidase reaction was developed using fresh enzyme substrate (0.03% H₂O₂, and 0.63 mg/ml of *o*-phenylenediamine in 0.1 M sodium citrate, pH 5.0) and the reaction was stopped with 3 N H₂SO₄. Optical density (OD) was measured at 492 nm in an ELISA reader (ASYS Hitech, Eugendorf, Austria). All measurements were carried out in duplicates.

ELISA inhibition assays were performed as previously described [25]; ELISA plates were coated with approximately 500 µg/ml of olive or ash pollen protein extract. Then they were incubated with the Spanish patients' sera (1:5 diluted) or the Austrian patients' sera (1:4 diluted) that had been preincubated with

10 µg/ml of purified Ole e 1, with 500 µg/ml of olive or ash pollen protein extract and, for control purposes, with 10 µg/ml of BSA. Bound IgE antibodies were detected as described above. The percentage of inhibition of IgE binding to olive or ash pollen extracts after preincubation with the different inhibitors was calculated as $[(OD_{BSA} - OD_{inh})/OD_{BSA}] \times 100$, where OD_{BSA} and OD_{inh} indicate the absorbance values after preincubation with BSA and the inhibitors, respectively.

Results

Spanish and Austrian Patients Recognize Similar Allergen Profiles in Olive and Ash Pollen Extracts

Nitrocellulose-blotted pollen extracts of the two Oleaceae plants (fig. 1) were exposed to sera from Spanish patients with high olive pollen exposure and Austrian patients without olive pollen exposure. Overall the IgE-binding patterns to olive and to ash pollen extracts of Spanish and Austrian patients were not different from each other. The following groups of IgE-reactive moieties with the following molecular masses could be distinguished in both extracts: 19–22 kDa, 14–16 kDa, 8–10 kDa and high-molecular-weight protein clusters of 35–80 kDa (fig. 1).

In both pollen extracts the most frequently recognized allergens had molecular masses of 19–22 kDa. All Spanish sera and seven of the eight Austrian sera showed IgE binding to proteins of this size in olive pollen extract. Only 1 Austrian patient who reacted with Ole e 1 by ELISA (table 1) did not react with nitrocellulose-blotted Ole e 1, presumably due to recognition of conformational epitopes. The high frequency of recognition and the binding pattern typical for Ole e 1 strongly suggested that patients of both populations had recognized Ole e 1, the major olive pollen allergen. Six of the Spanish (patients 1, 4, 5, 6, 7 and 8) and all eight Austrian sera also displayed IgE reactivity to bands of 19–22 kDa in ash pollen extract, which most likely represent Fra e 1, the Ole e 1-homologous major ash pollen allergen (fig. 1).

Nitrocellulose-blotted olive and ash pollen extracts were also exposed to sera from patients monosensitized to profilins (patients 9 and 19), and to sera from patients monosensitized to the two EF-hand calcium-binding proteins (patients 10 and 20). Profilin-sensitized patients recognized in both protein extracts proteins of 14–16 kDa (fig. 1, lanes 9, 19), whereas patients sensitized to the calcium-binding allergens recognized proteins of 8–10 kDa (fig. 1, lanes 10, 20).

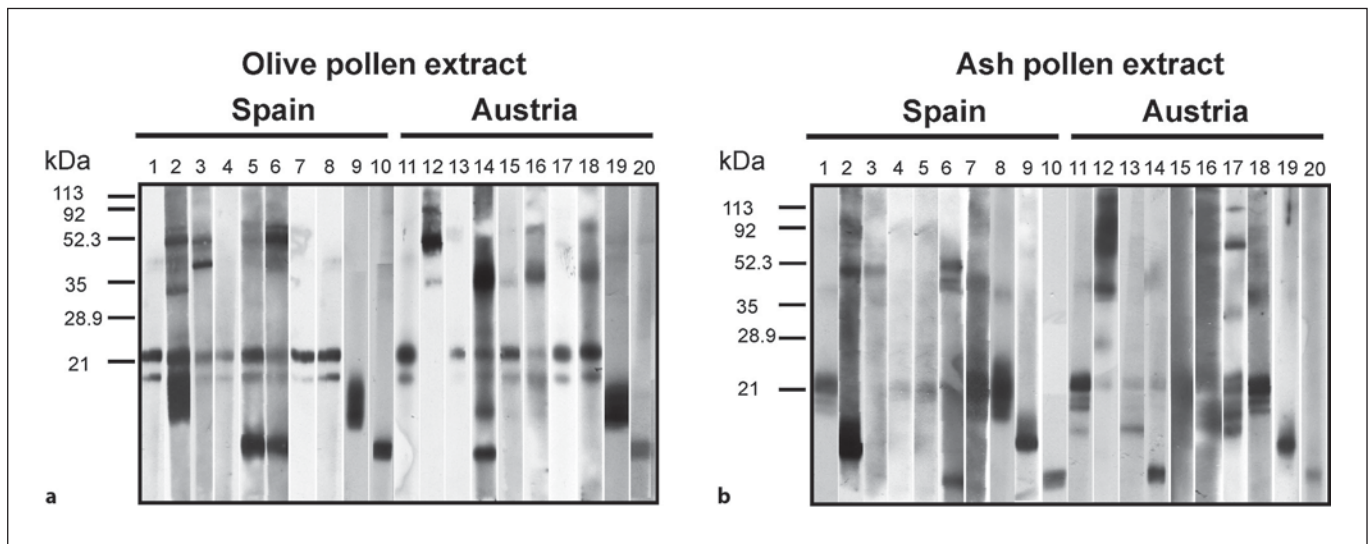


Fig. 1. IgE reactivity of sera from Spanish (lanes 1–10) and Austrian patients (lanes 11–20) to nitrocellulose-blotted olive (**a**) and ash (**b**) pollen extracts. Molecular masses are indicated in the left margins.

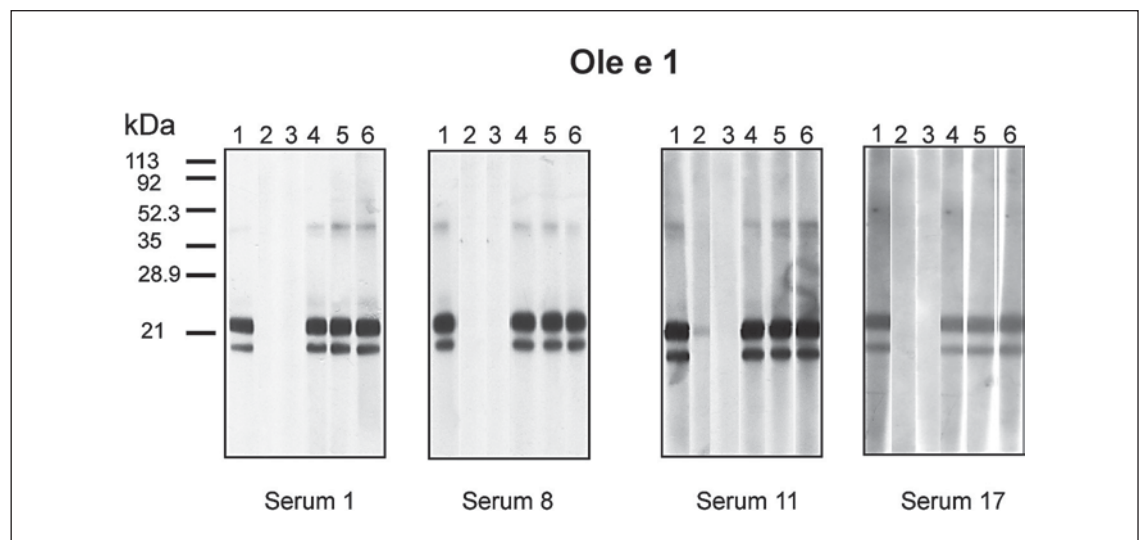


Fig. 2. IgE inhibition experiments to detect Ole e 1 cross-reactive allergens in various pollen extracts. Nitrocellulose-blotted Ole e 1 was exposed to Ole e 1-reactive sera from 2 Spanish (sera 1 and 8) and 2 Austrian patients (sera 11 and 17) that had been preincubated with pollen extracts from ash (lanes 3), birch (lanes 4), mugwort (lanes 5), timothy grass (lanes 6), with an olive pollen extract (lanes 2) and with BSA (lanes 1). Molecular masses are indicated in the left margin.

Ole e 1 Is a Highly Cross-Reactive Allergen in Plant Species Belonging to the Oleaceae

To investigate whether the 19- to 22-kDa proteins recognized by the Spanish and Austrian patients in olive and ash pollen extracts are indeed Ole e 1 and its homologous

ash pollen counterpart (Fra e 1), respectively, IgE immunoblot inhibition experiments were performed. Sera from 2 Spanish patients (patients 1 and 8) and from 2 Austrian patients (patients 11 and 17), exhibiting IgE reactivity to the 19- to 22-kDa proteins in both pollen extracts, were

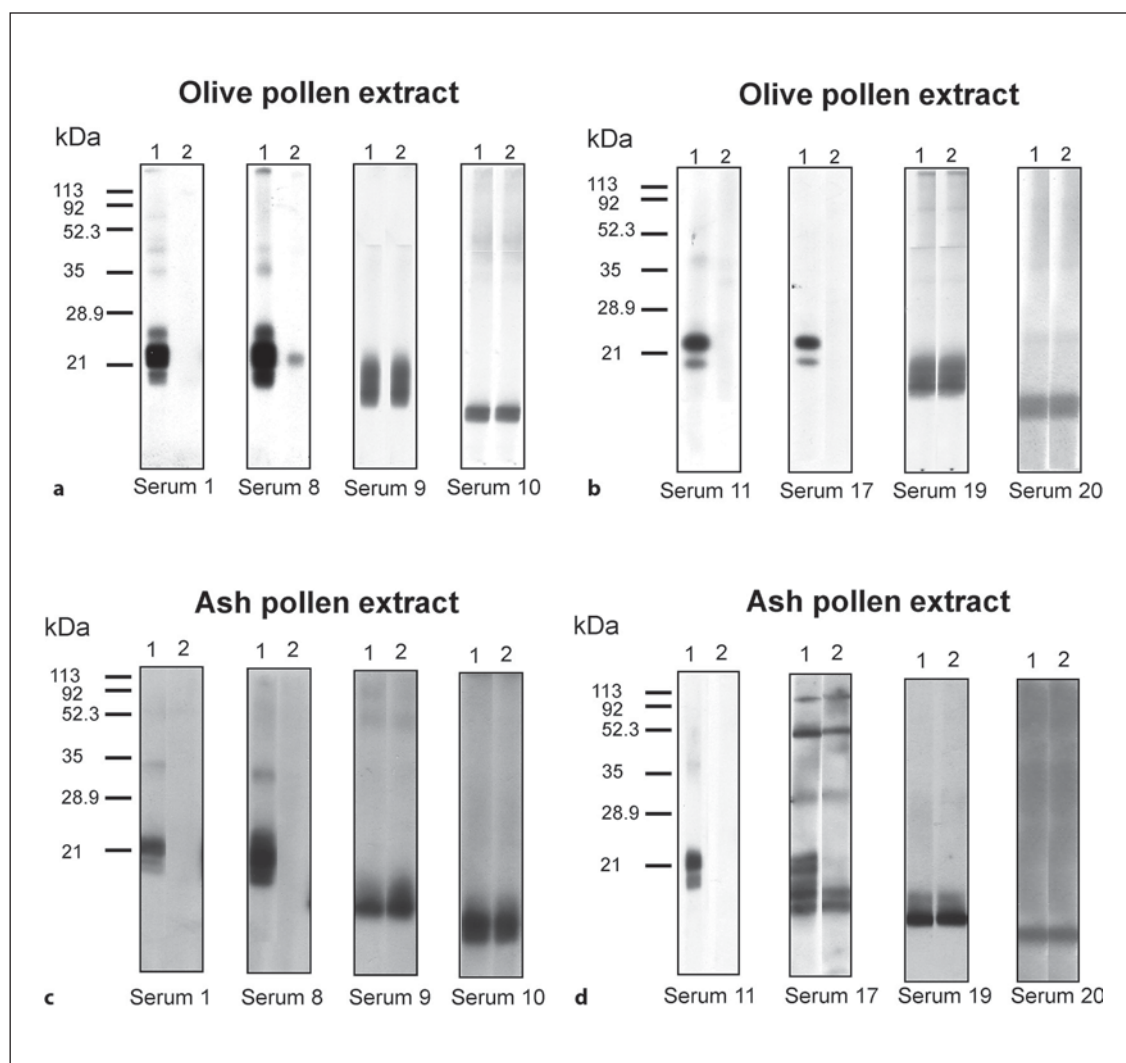


Fig. 3. Inhibition of IgE binding to nitrocellulose-blotted pollen extracts from olive and ash with purified natural Ole e 1. Sera from 4 Spanish (sera 1, 8, 9 and 10) and 4 Austrian patients (sera 11, 17, 19 and 20) were preadsorbed with purified Ole e 1 (lanes 2) or with BSA (lanes 1) and subsequently exposed to nitrocellulose-blotted olive (a, b) and ash (c, d) pollen extracts. Molecular masses are indicated in the left margin of each panel.

preadsorbed with pollen extracts from olive and ash and subsequently exposed to nitrocellulose-blotted purified Ole e 1 (fig. 2, lanes 2, 3). After separation by SDS-PAGE, Ole e 1 showed the typical profile with two main components, which has previously been described [14]: a band of 19 kDa, representing the nonglycosylated protein, a band of 21 kDa, representing the glycosylated form and a fainter band of 40 kDa, representing an Ole e 1 dimer. Spanish and Austrian sera displayed IgE reactivity to these three bands of Ole e 1. The IgE reactivity of sera from both populations to Ole e 1 was totally abolished after preincubation with olive and with ash pollen ex-

tracts, indicating that the 19- to 22-kDa proteins were Ole e 1 and its homologous ash allergen, Fra e 1. These data demonstrated that IgE-binding epitopes of Ole e 1, recognized by Spanish and Austrian sera, were also contained in Fra e 1, and thus present in ash pollen.

For a further examination of the cross-reactive potential of Ole e 1, sera from Spanish and Austrian patients were preincubated with pollen extracts from common pollen allergen sources in Austria (i.e. birch, mugwort and timothy grass), before exposure to nitrocellulose-blotted Ole e 1 (fig. 2, lanes 4–6). The allergen sources causing pollinosis in Austria did not affect the IgE bind-

Table 2. IgE binding to olive pollen extract and percentage inhibition of IgE binding to olive pollen extract after preadsorption of sera with Ole e 1, olive pollen and ash pollen extract

Patients		IgE reactivity to olive pollen ¹	Inhibitors, %		
			Ole e 1	olive pollen	ash pollen
Spanish	1	0.939	95.63	97.66	97.98
	2	0.674	83.83	99.70	97.33
	3	0.128	77.34	99.22	96.09
	4	0.550	93.45	99.82	99.45
	5	0.241	92.12	99.59	99.59
	6	0.193	63.21	99.48	99.48
	7	0.272	70.59	72.79	62.87
	8	2.605	97.39	99.50	99.54
Mean		0.700 ± 0.818	84.20 ± 12.68	95.97 ± 9.39	94.04 ± 12.66
Austrian	11	0.561	80.57	94.65	88.59
	12	1.270	89.84	98.50	96.30
	13	0.196	82.65	89.29	85.71
	14	0.439	25.74	96.36	92.03
	15	0.448	79.02	95.31	84.60
	16	0.309	56.96	90.29	77.02
	17	1.217	70.58	94.08	89.73
	18	0.704	65.06	80.97	90.63
Mean		0.643 ± 0.401	68.80 ± 20.32	92.43 ± 5.53	88.08 ± 5.76

¹ Determined as OD at 492 nm by ELISA.

ing to purified Ole e 1, suggesting that Ole e 1 cross-reactive epitopes do not occur in pollen of trees of the order Fagales, in weed pollen and in grass pollen.

Spanish and Austrian Patients Exhibit IgE Cross-Reactivity to Ole e 1 and Fra e 1

To examine the immunological relationship between Ole e 1 and cross-reactive components present in ash pollen extract, four Spanish sera (patients 1, 8, 9 and 10) and four Austrian sera (patients 11, 17, 19 and 20) were preincubated with purified Ole e 1 and then exposed to nitrocellulose-blotted olive (fig. 3a, b) and ash (fig. 3c, d) pollen extracts. Preadsorption of sera 1 and 8 from Spain and sera 11 and 17 from Austria with Ole e 1 resulted in an almost complete inhibition of IgE binding to olive pollen allergens. Preincubation of sera 1, 8 and 11 with Ole e 1 also abolished the IgE reactivity to ash pollen extract (fig. 3c, d). Serum 17 (from Austria) displayed IgE reactivity to several ash pollen allergens and only IgE recognition of the 19- to 22-kDa components could be reduced after preincubation with Ole e 1 (fig. 3d). Preadsorption of sera from patients exclusively sensitized to profilins (patients 9 and 19) or calcium-binding allergens (patients 10 and 20) with Ole e 1 had no effect on the IgE reactivity to the olive and ash pollen extracts.

Almost Complete Cross-Reactivity between Olive and Ash Pollen Is Mainly Mediated by Ole e 1

To estimate the overall cross-reactivity between olive and ash pollen, cross-inhibition ELISA experiments were performed with protein extracts of olive and ash pollen and, for control purposes, with BSA in Spanish and Austrian patients. Preincubation of Spanish and Austrian sera with olive and ash pollen extracts led to a strong reduction of IgE binding to ELISA plate-bound olive and ash pollen allergens: mean inhibitions of IgE binding to ash pollen allergens with olive pollen extract were 96.62% for Austrian sera and 94.62% for Spanish sera and mean inhibitions of IgE binding to olive pollen allergens with ash pollen extract were 88.08% for Austrian sera and 94.04% for Spanish sera (tables 2, 3). These findings indicate an extensive degree of cross-reactivity between the allergens of the two Oleaceae species.

The degree of cross-reactivity between Ole e 1 and its homologous counterpart in ash pollen was studied in Spanish and Austrian patients using ELISA competition experiments (tables 2, 3). Preincubation of the sera with purified Ole e 1 resulted in strong inhibitions of IgE binding to ELISA plate-bound protein extracts of both plants. Percent inhibition of IgE binding ranged for Spanish sera between 63.21 and 97.39% (mean inhibition 84.20%) to

Table 3. IgE binding to ash pollen extract and percentage inhibition of IgE binding to ash pollen extract after preadsorption of sera with Ole e 1, olive pollen and ash pollen extract

Patients		IgE reactivity to ash pollen ¹	Inhibitors, %		
			Ole e 1	olive pollen	ash pollen
Spanish	1	0.433	97.00	96.54	96.07
	2	0.292	86.99	99.66	99.66
	4	0.219	99.54	99.54	99.54
	5	0.167	99.40	99.40	99.40
	7	0.258	72.09	75.19	76.74
	8	0.502	91.43	97.41	96.02
	Mean	0.312 ± 0.118	91.08 ± 9.60	94.62 ± 8.77	94.57 ± 8.13
Austrian	11	0.632	94.78	96.52	99.68
	12	1.060	95.28	99.91	99.91
	13	0.161	99.38	99.38	99.38
	14	0.114	71.93	99.12	99.12
	15	0.766	99.87	99.87	99.87
	16	0.199	80.90	83.42	79.90
	17	1.498	38.72	96.60	97.53
	18	0.818	78.61	98.17	96.21
	Mean	0.656 ± 0.487	82.43 ± 20.52	96.62 ± 5.50	96.45 ± 6.81

¹ Determined as OD at 492 nm by ELISA.

olive pollen and between 72.09 and 99.54% (mean inhibition 91.07%) to ash pollen allergens. For Austrian sera Ole e 1 inhibited IgE binding to olive pollen extract between 25.74 and 89.84% (mean inhibition 68.80%) and to ash pollen extract between 38.72 and 99.87% (mean inhibition 82.43%). The fact that Ole e 1 was able to strongly inhibit the IgE binding to total olive and ash pollen extracts using sera from both populations emphasizes the major role of Ole e 1 and the homologous allergen Fra e 1 for olive pollen and ash pollen allergy. In addition, these data also provide evidence that Ole e 1 contains most of the relevant IgE-binding epitopes of Fra e 1. Only in polysensitized patients, which displayed IgE reactivity to several proteins (e.g., patient 14 to olive pollen or patient 17 to ash pollen; fig. 1), the extent of IgE inhibition caused by Ole e 1 was below 50% (25.74% for patient 14 to olive pollen, table 2, and 38.72% for patient 17 to ash pollen, table 3). In these patients cross-inhibition with whole olive and ash pollen extracts almost completely abolished IgE binding to the coated extracts (patient 14: 99.12% inhibition with olive pollen to ash pollen extract, 92.03% inhibition with ash pollen to olive pollen extract; patient 17: 96.60% inhibition with olive pollen to ash pollen extract, 89.73% inhibition with ash pollen to olive pollen extract). These results provided evidence for the high degrees of cross-reactivity between other allergens from olive and ash pollen.

Discussion

Plants belonging to the Oleaceae represent important allergen sources worldwide but exhibit selective geographic distributions. For example, olive trees (*O. europaea*) represent a major allergen source in southern countries such as the Mediterranean area of Europe, where its intensive cultivation causes highly elevated airborne concentrations of olive pollen [26]. In Spain, olive pollen is after grass pollen the second most important cause of pollinosis, responsible for clinical symptoms, such as rhinoconjunctivitis and/or asthma [27]. In contrast, in countries from the northern and central parts of Europe and America, where olive trees are rarely grown, another species belonging to the Oleaceae family, namely the ash tree (*F. excelsior*), is widely distributed and has been suggested as a potent allergen source during spring time [1, 2, 6–8]. In a paradigmatic approach, we investigated the presence of cross-reactive allergens and epitopes in the two differently distributed members of the Oleaceae family, olive and ash, using sera from two populations who are selectively exposed either to olive or to ash pollen. Although olive is not common in Austria, we found that approximately 16.4% of 354 Austrian pollinosis patients showed IgE reactivity to olive pollen.

Using ELISA and immunoblot inhibition experiments (data not shown) we found that Spanish as well as Aus-

trian patients showed almost complete IgE cross-reactivity to olive and ash pollen allergens both in terms of the profile of recognized allergens and regarding the extent of IgE cross-reactivity despite apparent exposure to one of the two allergen sources. Comparable to other studies, which were either focused on olive pollen-sensitized patients [12] or ash pollen-sensitized patients [20, 21], we found that IgE cross-reactivity between olive and ash pollen was attributed to several different allergens of which profilin, calcium-binding allergens and Ole e 1 were identified. While profilins, calcium-binding allergens and other highly conserved allergens showed cross-reactivity with the corresponding allergens present in a variety of plant species also unrelated to the Oleaceae (e.g., birch, weeds, grasses), Ole e 1 cross-reacted only with Fra e 1, the major allergen of ash. The cross-reactivity between Ole e 1 and Fra e 1 is based on the extensive sequence homology of the two allergens, which may also explain the cross-reactivity of Ole e 1 with major allergens of other members of the Oleaceae, such as privet and lilac. Also grass pollens contain allergens which exhibit some degree of sequence similarity to Ole e 1, but, in accordance with our results, no significant cross-reactivity has been reported between Ole e 1 and the group 11 allergens from rye grass, Lol p 11, and timothy grass, Phl p 11 [28, 29].

The almost complete IgE cross-reactivity between Ole e 1 and Fra e 1 from ash has been demonstrated by several experiments: IgE immunoblot and ELISA inhibition assays revealed that preincubation of sera of both populations with ash and olive pollen extract could completely abolish the IgE binding to purified natural Ole e 1. These data provided evidence that the 19- to 22-kDa protein bands, recognized by Spanish and Austrian patients, represented Ole e 1 and its homologous ash pollen allergen Fra e 1. Our data further revealed the high degree of cross-reactivity between Ole e 1 and Fra e 1, and demonstrated that cross-reactive IgE-binding epitopes were recognized by both populations of patients. In fact approximately 10% of the Austrian pollinosis patients tested by us ($n = 354$) showed IgE reactivity to Ole e 1. Additional ELISA inhibition experiments, in which Spanish and Austrian sera were preincubated with Ole e 1, showed that the purified allergen could significantly reduce patients' IgE binding to olive as well as to ash pollen extract. This indicated that Ole e 1 and Fra e 1 contained the majority of IgE-binding epitopes present in olive and ash pollen extract. The fact that preincubation with extracts from birch, mugwort or timothy grass pollen, the major sources of pollen allergens in Austria, could not affect patients' IgE binding to Ole e 1 demonstrated that IgE-

binding epitopes of Ole e 1 and Fra e 1 are only present in Oleaceae.

Finally we showed by crosswise ELISA inhibition experiments using olive and ash pollen extracts as inhibitors that olive pollen extract inhibited IgE binding of both populations to coated ash pollen extract as efficiently as ash pollen. The latter finding demonstrates that Ole e 1 contained most of the potent IgE-binding epitopes present in ash pollen, and thus on Fra e 1.

Pajarón et al. [12] previously showed that olive pollen contained all the relevant IgE-binding epitopes of Oleaceae pollen and therefore suggested the use of olive pollen extract for diagnosis of patients sensitized to all Oleaceae species. Our results now allowed us to identify Ole e 1 as a marker allergen for the diagnosis of patients who are genuinely sensitized to Oleaceae. Ole e 1 may thus be used as a diagnostic marker in populations who are exposed to a mixed vegetation of various trees, grasses and weeds to discriminate those patients who are genuinely sensitized to Oleaceae from those who appear positive due to cross-reactivity to panallergens such as profilin and/or calcium-binding allergens. Using allergen extracts for diagnosis it is not possible to discriminate these two groups of patients because allergen extracts contain both species-specific (e.g., Ole e 1) as well as highly cross-reactive allergens (e.g., profilin, calcium-binding allergens). Ole e 1 may, therefore, be used as a diagnostic marker allergen to determine the suitability of patients for immunotherapy with olive pollen allergens more accurately and to monitor the success of treatment. Due to the extensive cross-reactivity between Ole e 1 and Fra e 1, it seems possible to develop vaccines based on Ole e 1 which may be suitable for the treatment of allergies to various members of the Oleaceae, including ash.

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