

Cross-reactivity between olive and other species. Role of Ole e 1-related proteins

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Olive (*Olea europaea*) pollen is considered as one of the most important causes of respiratory allergic disease in the Mediterranean region. In Spain, it is the second cause of pollinosis after grass pollen (Table 1) (1). However, the epidemiological and clinical impact of olive pollinosis varies markedly from one area to the next since the pollen source, the olive tree, is a cultivated species and its distribution changes from one region to the next (2). Another relevant characteristic of the aerobiology of the olive pollen is the relative shortness and severity of the pollen seasons, although these follow a pattern of alternation due to a phenomenon called 'Alternance of Production' (2).

The pioneering work on olive allergens started in the 1980s. In these studies the main allergen

(currently known as Ole e 1) was found to be an acidic protein which appeared as a double band of about 17 and 19 kDa on SDS-PAGE (3,4). However, it was not until the 90s when the application of monoclonal antibodies and molecular biology techniques allowed to characterize up to nine different olive allergens.

Some of the olive allergens identified have counterparts (homologue proteins) in other pollen sources: Ole e 1, Ole e 2 (profilin), Ole e 3 (polcalcin) and Ole e 7 (LTP) (Table 2).

Table 1. Pollens causing allergic rhinoconjunctivitis and/or asthma in Spain (1)

Pollen	Allergic disease	
	Rhinoconjunctivitis*	Asthma*
Grasses	77.5	83.5
Olive	46.7	50.5
Parietaria	17.6	15.4
Artemisia	14.2	14.1
Chenopodium	11.4	10.9
Weeds	4	3.5
Other pollens	12.7	14.6

*Percentage of patients sensitized to each pollen.

Table 2. Olive pollen allergens

Name	Molecular mass (kDa)	Biological function	IgE-prevalence (%)	Pollen allergens with homology
Ole e 1	18/20	Homology to LAT52, Zmc13, OSPG	60–90	Lig v 1, Syr v 1, Fra e 1 Lol p 11 Pla l 1 Che a 1, Sal k?
Ole e 2	15	Profilin	20	Bet v 2 Cyn d 12, Phl p 12 Hel a 2, Mer a 1
Ole e 3	9	Ca ²⁺ -binding protein	20–25	Cyn d 7, Phl p 7 Bet v 4, Aln g 4 Syr v 3, Fra e 3
Ole e 4	32/36	—	80	—
Ole e 5	16	Superoxide Dismutase	35	—
Ole e 6	6	—	15	—
Ole e 7	10	LTP	45	Parj 1, Art v 3
Ole e 8	19	Calmodulin	15	Jun o 2
Ole e 9	45	β-glucanase	—	—

Ole e 1 homologues in *Oleaceae* pollens

The main allergen of olive pollen, Ole e 1, is a polymorphic protein of 145 amino acids (5–7). The allergen has an N-glycosylation site located at Asn-111 (5,6) which explains the typical two-band pattern obtained on SDS-PAGE, corresponding to the glycosylated form (≈ 20 kDa), which is the main band, and the nonglycosylated one (≈ 18 kDa). Ole e 1 has six Cys residues involved in three disulphide bonds whose topology has been determined (8). These bonds are probably responsible for the high stability of the allergen (9).

The first Ole e 1 homologues with clear clinical relevance were detected in *Oleaceae* pollens. A high degree of IgE cross-reactivity in the *Oleaceae* family was determined in several clinical observations (10–12). Probably, apart from olive, only ash (*Fraxinus*) and privet (*Ligustrum*) are primary causes of sensitization in some patients, other *Oleaceae* species being involved through cross-sensitization.

The first clue on the nature of the cross-reactive allergens in the *Oleaceae* family comes from the work of Baldo et al. (13) and, mainly, from that of Obispo et al. (14).

This last work revealed the presence of Ole e 1 homologs in pollen extracts of ash, privet and lilac at concentrations in the range detected in olive pollen extracts. The homologues had a similar SDS-PAGE behaviour and N-terminal amino acid sequence. It was also shown the presence of common rabbit and mouse IgG and human IgE epitopes on the four proteins, and the names of Fra e 1, Lig v 1 and Syr v 1 were proposed for the new characterized allergens. These results were confirmed by Batanero et al. (15,16), who cloned the lilac and privet allergens. They showed that Syr v 1 and Lig v 1 had a 145-residues polypeptide chain with an N-glycosylation site at Asn-111 and six Cys residues. Several isoforms were detected in each allergen, but they presented a high degree of similarity with Ole e 1 isoforms (85–95% identity).

The natural allergens were shown to be N-glycosylated, with a similar behaviour as Ole e 1 as regards the enzyme PNGase F (9).

Table 3 shows the results of skin tests carried out with natural Ole e 1, Fra e 1, Lig v 1 and Syr v 1 in 15 olive-monosensitized patients. All patients but one (number 9) had a positive response to the four allergens. From a quantitative point of view, the intensity of the response was clearly similar, with the possible exception of

Table 3. Skin reactivity of group 1 allergens from *Oleaceae*

Patient	Olive IgE (KU/l)	Wheal area (mm ²)				Histamine 10 mg/ml
		Ole e 1	Lig v 1	Fra e 1	Syr v 1	
1	7.9	121	93	60	154	34
2	8.1	248	107	142	136	61
3	86.4	102	44	162	91	32
4	1.3	158	90	161	180	44
5	38.7	155	86	130	101	40
6	0.7	42	20	25	22	25
7	5.4	120	32	96	62	48
8	9.5	126	53	127	178	84
9*	3.5	3	2	—	2	47
10	39.5	64	33	40	55	36
11	8.6	69	22	47	76	28
12	5.2	73	29	100	70	34
13	7.2	101	34	87	106	60
14	2.7	79	38	41	47	40
15	1.8	35	24	44	43	38
Mean		107	51	90	94	43

*Positive skin prick test with whole olive extract.

Table 4. Histamine release test with group 1 allergens from *Oleaceae*

Patient	Olive IgE (KU/l)	% maximum histamine release			
		Ole e 1	Lig v 1	Fra e 1	Syr v 1
1	7.9	26	33	26	26
2	8.1	40	43	44	41
3	86.4	0	0	0	0
4	1.3	35	27	30	33
5	38.7	32	29	41	33
6	0.7	0	0	0	0
7	5.4	52	26	52	48
8	9.5	30	28	40	44
9*	3.5	0	0	0	0
10	39.5	80	42	87	77
11	8.6	ND#	26	33	27
12	5.2	0	0	0	0
13	7.2	46	46	43	51
14	2.7	0	0	0	0
15	1.8	0	0	0	0
Mean		42.6	33.3	44	42.2

* 22% HR with whole olive extract.

Not determined.

Lig v 1, in which a lower reaction was obtained. The results obtained in skin prick test were confirmed by histamine release test (Table 4). Six of the 15 patients studied were nonreleasers for any preparation, including antihuman IgE antibody. Of the remaining nine patients, all but one (number 9) were positive in histamine release test, with a clear quantitative correlation except in the case of Lig v 1, in which again a lower response was obtained.

All these results seem to imply that the presence in *Oleaceae* pollens of an 'Ole e 1-like' allergen is

mainly responsible for the IgE cross-reactivity observed. However, the existence of cross-reactivities at the level of other allergens is also possible. At least three pan-allergens have been identified in olive pollen extracts: profilin (Ole e 2), Ca^{2+} -binding proteins (Ole e 3) and lipid-transfer protein (LTP, Ole e 7). Ole e 3 homologues have been detected in ash and lilac (17), and profilin has been detected in ash (18).

Our current knowledge of cross-reactivity within Oleaceae species comes mainly from subjects with primary allergy to olive pollen, whereas patients with primary allergy to other Oleaceae species have hardly been investigated. In a recent study carried out by Hemmer et al. (19) in central Europe, an area without olive trees, it was shown that sensitization to ash pollen is not all that uncommon among the atopic population ($\approx 18\%$). About 80% of ash pollen-sensitized individuals have Fra e 1-specific IgE, which is clearly the most important allergen. However, although Fra e 1 sensitization always occurs in mono- and oligo-sensitized patients, its frequency is below 50% in subjects with multiple pollen allergies. On the other hand, sensitization to pollen pan-allergens (profilin and Ca^{2+} -binding proteins) was significantly more frequent in polysensitized individuals than in mono- and oligo-sensitized patients.

All these results indicate that Ole e 1 homologues are very relevant in Oleaceae pollen-sensitized patients although their importance decreases in patients with multiple pollen allergies.

Ole e 1 homologues in nonOleaceae pollens

Shortly after the determination of the primary structure of Ole e 1, it was found that this allergen had homology ($\approx 30\%$) to putative proteins encoded by the pollen-specific genes LAT52 from tomato, Zmc13 from maize and OSPG from rice (5,6). This homology is probably not very relevant from the clinical point of view, but it may have implications on the biological function of Ole e 1.

Apart from the olive family, other Ole e 1 homologs with allergenic activity have been found in *Lolium* (Lol p 11), *Plantago* (Pla l 1) and *Chenopodium* (Che a 1).

Lol p 11 is a 134-amino acid protein with a calculated molecular mass of 14.9 kDa (20). The sequence contains an N-glycosylation site at position 24 and six Cys residues. The allergen is a glycoprotein with a molecular mass of 16 kDa. Its IgE-prevalence in 270 sera with IgE against grass pollen is $> 65\%$. A 44% similarity was found with

Ole e 1. Lol p 11 shares 32% sequence identity with soybean trypsin inhibitor (Kunitz), and it has been proposed that Ole e 1-related proteins are members of this family of inhibitors (20). However, the allergens lack the active site of the enzyme and the disulphide bond topology differs, which suggests that the proposed homology between the Kunitz family and Ole e 1 homologues should be revised (8).

There is no direct data on IgE cross-reactivity between Lol p 11 and Ole e 1, but it seems to be low. Cross-reactivity between grass and olive pollen is probably mediated by profilins and polcalcins.

Very recently (21) a homologue of Ole e 1 has been described in *Plantago lanceolata*. The genus *Plantago* belongs to the family Plantaginaceae and comprises about 250 species, *Plantago lanceolata* (English plantain or ribwort) being one of the most common species. This weed is distributed in temperate zones of Europe, North America and Australia, and has been considered as one of the most important dicotyledons that cause allergic diseases.

The allergogram of *P. lanceolata* pollen extract, carried out by IgE-immunoblotting after SDS-PAGE, showed that a group of about four proteins, with a molecular mass between 16 and 20 kDa, reacted with most of the sera assayed (21). This group of proteins was purified by HPLC, and the N-terminal amino acid sequence revealed out that they were isoforms or variants of the same basic protein, which was called Pla l 1. The allergenic significance of Pla l 1 was studied *in vitro* with sera from allergic patients sensitized to plantain (21). Of the 22 sera analysed, 19 had IgE antibodies to Pla l 1. In addition, Pla l 1 accounted for 80% of the total IgE-binding capacity of the whole extract. All these results indicate that Pla l 1 is probably the most important plantain allergen.

The N-terminal amino acid sequence determined for Pla l 1 showed a partial identity with Ole e 1 for the sequence stretch between positions 9 and 16 (21). This result has been confirmed after the determination of the whole sequence of Pla l 1 (22). It has six Cys residues, a potential site of N-glycosylation and a homology with Ole e 1, depending on the isoform considered, of about 30–40%.

About 50% of natural Pla l 1 are glycosylated (23). The glycoprotein is resistant to deglycosylation by PNGase F (23), in contrast to Ole e 1, but sensitive to treatment by PNGase A, suggesting the presence of $\alpha 1,3$ -fucose at the innermost N-acetyl glucosamine residue. The N-glycan moiety

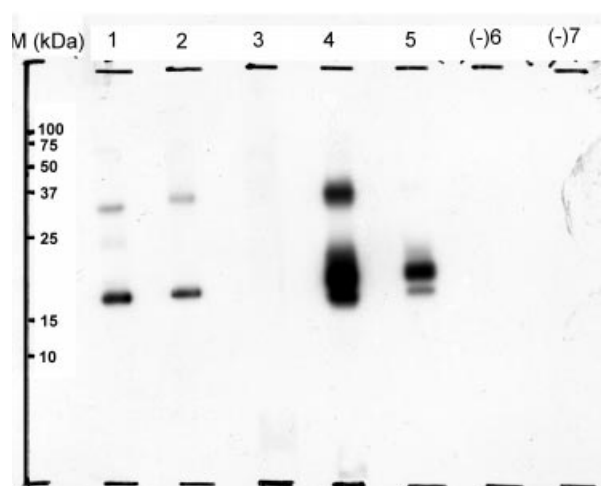


Figure 1. Immunodetection by Ole e 1-specific mAb of Ole e 1-related proteins in some weeds. 1. *C. album* pollen extract + mAb OL-3. 2. *S. kali* pollen extract + mAb OL-3. 3. *P. lanceolata* pollen extract + mAb OL-3. 4. *O. europaea* pollen extract + mAb OL-3. 5. *O. europaea* pollen extract + mAb OL-2. 6 and 7. Negative controls.

of Pla l 1 does not seem to constitute an important IgE-binding epitope (23).

Although preliminary data indicate a limited antigenic and allergenic similarity (23), the sequence homology of Pla l 1 and Ole e 1 discloses a source of potential cross-allergenicity that should be further investigated, mainly taking into account that both allergens are very important in each corresponding species.

Finally, Ole e 1 homologs have also been detected in chenopod pollens. Figure 1 shows an immunodetection experiment carried out with the Ole e 1-specific mAb OL-3 (24), after SDS-PAGE of *Chenopodium album*, *Salsola kali*, *P. lanceolata* and *O. europaea* pollen extracts. A main band of about 18 kDa is detected in *Chenopodium* and *Salsola* extracts (lanes 1 and 2), suggesting the presence of Ole e 1 homologues in these pollens. Interestingly, the mAb OL-3 did not recognize the Ole e 1-related protein (Pla l 1) in *Plantago* pollen. This mAb recognizes a continuous epitope on the Ole e 1 molecule (25), which is not present in Pla l 1 (F. Polo, personal communication).

These 18 kDa proteins of *Chenopodium* and *Salsola* have IgE-binding activity, as demonstrated by IgE-immunodetection with a serum pool from chenopod-allergic patients (M. Lombardero, unpublished). Barderas et al. (26) demonstrated the existence of the Ole e 1 homologue in *Chenopodium* pollen. The isolated allergen, called Che a 1, is glycosylated and has a molecular mass

slightly lower than that of Ole e 1. Analyses of the individual IgE responses to Che a 1 and to Ole e 1 were performed with sera from patients exhibiting pollinosis to both *O. europaea* and *C. album* pollens, and no correlation was observed between the level of response of each serum to both allergens. Additional experiments should be carried out to assess the extension of IgE cross-reactivity between Ole e 1 and Che a 1.

Role of N-linked glycan on cross-reactivity

Several pollen allergens are glycoproteins. Plant glycoproteins (and also insect glycoproteins) have structural elements not found on mammalian glycoproteins, which have been claimed to be responsible for cross-reactivity between glycosylated allergens from different sources. In this sense, it has been demonstrated that the α 1,3-fucose of the innermost N-acetylglucosamine residue of bee venom phospholipase A2 forms an IgE-reactive determinant, which is also present in plant glycoproteins as, for instance, bromelain (27).

Most of the Ole e 1 molecules contain an N-linked glycan which can be released with a high yield with PNGase F (28), and similar results were found with the glycans of Ole e 1 homologues in other Oleaceae (9). The Ole e 1 N-glycan binds specific IgE antibodies from human sera and induces histamine release from sensitized human basophils (28). The primary structure of the glycans of Ole e 1 and Lol p 11 has recently been determined (29) and several differences have been found. In agreement with this, no significant correlation was found with the IgE responses to the N-glycans, thus indicating the absence of IgE-binding equivalence between them. There are no data yet on the primary structure of the Pla l 1 glycan, but preliminary results (treatment with glycosidases) suggest differences with respect to the Ole e 1 glycan (23).

IgE responses to proteinase K-digested extracts of grass (*D. glomerata*), birch (*B. verrucosa*) and mugwort (*A. vulgaris*) pollens demonstrated a low correlation (29). All these results indicate that the IgE reactivity to the carbohydrate moiety of glycosylated allergens, in general, and Ole e 1-related proteins, in particular, is not simply accounted for the presence of specific structural features (i.e. β 1,2-xylose and/or α 1,3-fucose residues), but other factors must be involved. Consequently, carbohydrate-mediated IgE cross-reactivity may be more limited than initially thought.

Conclusions

Ole e 1 homologues of different Oleaceae species have a very similar amino acid sequence ($\approx 90\%$ identity) and *in vivo* and *in vitro* reactivities indicating a relevant role in the IgE cross-reactivity detected in Oleaceae pollens at least in mono- and oligo-sensitized patients.

In the cross-reactivity between olive and other taxonomic groups (i.e. grasses) profilins, polcalcins and allergen glycans are surely involved. However, the presence of Ole e 1-related proteins in grasses, plantain and chenopods discloses sources of potential cross-allergenicity that should be further investigated.

The level of exposure of patients to the pollen has an effect on the allergen prevalence (i.e. minor pollen components may be more allergenic in areas of high pollen exposure, and

mechanisms of tolerance against major pollen components may appear). This should have an impact on the pattern of cross-reactivity detected in patients.

The recent detection of an LTP-like allergen in olive pollen (R. Rodríguez; personal communication) could be important in cases of cross-reactivity between Oleaceae pollen and vegetable foods. Plant LTPs form a family of 9 kDa polypeptides that is widely distributed throughout the plant kingdom. They are very stable, resistant to protease digestion and probably involved in severe symptoms of food allergy.

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