

The Spectrum of Olive Pollen Allergens

Rosalía Rodríguez Mayte Villalba Rafael I. Monsalve Eva Batanero

Departamento Bioquímica y Biología Molecular, Facultad de Química, Universidad Complutense, Madrid, España

Key Words

Olea europaea · Olive pollen allergens

Abstract

Olive pollen is one of the most important causes of seasonal respiratory allergy in Mediterranean countries, where this tree is intensely cultivated. Among the high number of protein allergens detected in this pollen, 8 – Ole e 1 to Ole e 8 – have been isolated and characterized. Ole e 1 is the most frequent sensitizing agent, affecting more than 70% of the patients suffering of olive pollinosis, although others, such as Ole e 4 and Ole e 7, have also been shown to be major allergens. In this context, the prevalence of many olive pollen allergens seems to be dependent on the geographical area where the sensitized patients live. Some of the olive allergens have been revealed as members of known protein families: profilin (Ole e 2), Ca²⁺-binding proteins (Ole e 3 and Ole e 8), superoxide dismutase (Ole e 5) and lipid transfer protein (Ole e 7). No biological function has been demonstrated for Ole e 1, whereas Ole e 4 and Ole e 6 are new proteins without homology to known sequences from databases. cDNAs encoding for Ole e 1, Ole e 3 and Ole e 8 have been overproduced in heterologous systems. The recombinant products were correctly folded and exhibited the functional activities of the natural allergens. In addi-

tion to the Oleaceae family, other species, such as Gramineae or Betulaceae, contain pollen allergens structurally or immunologically related to those of the olive tree. This fact allows to detect and evaluate antigenic cross-reactivities involving olive allergens. The aim of this research is the development of new diagnostic tools for olive pollinosis and new approaches to improve the classical immunotherapy.

Copyright © 2001 S. Karger AG, Basel

Introduction

The olive (*Olea europaea*) is a long-lived tree from which humans have obtained oil, fruit and wood for more than 5,000 years [1, 2]. It is widely cultivated in countries of the Mediterranean basin [3], but was also introduced into America, Australia, Japan and South Africa. The olive tree displays a great number of botanical varieties – up to 165 are found in Spain [4] –, including a wild form called *Olea oleaster*. Olive pollen grains are small (mean diameter between 17 and 21 µm), slightly elliptic, and tricolporate, with a dense reticulation. Its spreading is eminently entomophilous but also anemophilous when pollen production is abundant [5]. The pollination period of olive trees lasts from the middle of April to the end of June [6]. The average atmospheric concentration of pol-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2001 S. Karger AG, Basel
1018–2438/01/1253–0185\$17.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Dr. Rosalía Rodríguez
Departamento Bioquímica y Biología Molecular
Facultad de Química
Universidad Complutense
E-28040 Madrid (Spain)

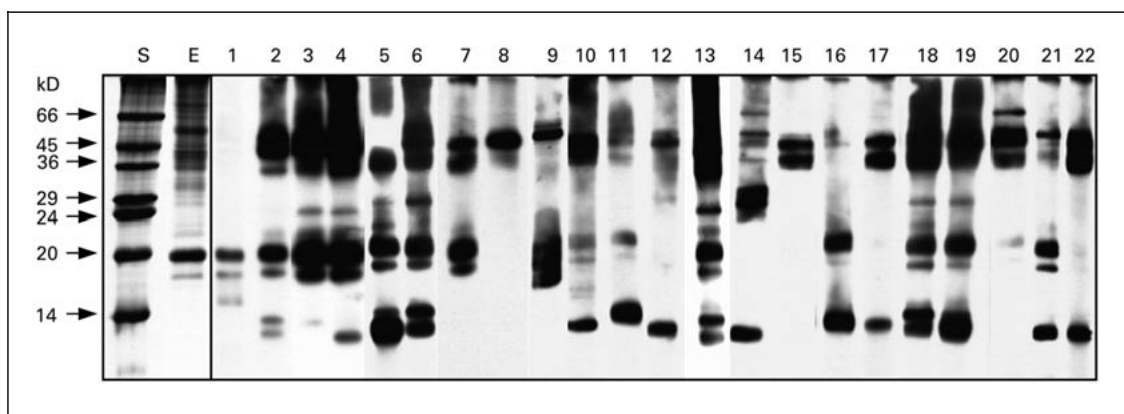


Fig. 1. Allergenic patterns of sera from patients hypersensitive to olive tree pollen. The IgE binding patterns to olive pollen extract of 22 sera were selected as representative for the variability and complexity of the responses in individuals suffering of olive pollinosis. S = Molecular mass standard proteins; E = Coomassie blue staining of olive pollen extract.

len in areas of olive cultivation is between 100 and 200 grains/m³, although up to 3,000–4,000 grains/m³ have been collected in areas where this tree is intensively cultivated, such as Jaén and Córdoba (Andalucía) in Spain and Bari (Puglia) in Italy [7, 8]. Topics on aerobiology and clinical aspects of olive pollinosis have been reviewed by Liccardi et al. [9].

Former analyses on olive pollen allergens pointed at proteins of about 65 and 32 kD apparent molecular mass, as determined by SDS-PAGE [10, 11], and preliminary attempts at their purification were carried out [11, 12]. Afterwards, proteins of 17, 19, 41–42 and 50–60 kD were identified as major allergens of olive pollen [13–15], although other bands of 8 and 60–70 kD were also detected as IgE-reactive molecules in pollen extracts [8, 14]. Some of these IgE-reactive proteins of high molecular mass have recently been identified as aggregates (multimeric forms) of a major allergen of 20 kD. The first data on the amino acid sequence of an olive allergen were reported in 1990 by Villalba et al. [16]. In this paper, the 1986 allergen nomenclature [17] was adopted, and thus the identified protein was named *Ole e 1*. Effective isolation procedures allowed its molecular and immunological characterization. Prevalence data over a large population of sensitive individuals were also obtained. As a result, *Ole e 1* was recognized as the main allergen from olive pollen. The isolation of another allergen from this biological source took 6 years. So far, 8 olive pollen allergens are known. This article provides a brief review of the structural and immunological properties of these allergens.

Complexity of the IgE-Binding Pattern of Olive Pollen Extracts

The SDS-PAGE pattern of the allergenic components of olive pollen extract – in other words, the *allergogram* of the olive pollen – is very complex, since it contains a large number of protein bands recognized by the IgE antibodies of the sera from patients hypersensitive to this pollen (fig. 1). Early studies showed the presence of 20 fractions with allergenic activity [11]. Furthermore, several authors have reported allergenic patterns of olive pollen extracts by using individual sera, all of them showing the existence of a large number of IgE-reactive protein bands [15, 18, 19–23]. After the identification of *Ole e 1*, and over the past 5 years, an increasing number of olive pollen allergens have been purified and characterized, thus confirming former prospects. To date, 8 allergens have been identified, purified and characterized, i.e. *Ole e 1*–*Ole e 8* according to the last recommendations of the Allergy Nomenclature Subcommittee of the International Union of Immunology Societies [24]. But many others of high clinical significance have been detected and their study is under way [unpubl. results]. From our experience in the study of allergens from olive pollen, we have observed that the number of allergens to which an olive allergic-patient is sensitized is often greater than 2 and reaches 8–10 in individuals with a RAST value of 5–6 for the whole olive pollen extract.

Table 1. Relevant properties of olive pollen allergens

Allergen	MW, kD ¹	pI ²	Cross-reactivity ³	Prevalence ⁴ %	Recombinant ⁵ expression	References ⁶
Ole e 1	16.3/18.5*	5.5–6.5*	yes*	>70	<i>E. coli</i> (0.1 mg/l) <i>P. pastoris</i> (60 mg/l)	16, 19, 27, 37, 38
Ole e 2	14–16**	5.1**	yes	>40	<i>E. coli</i> (20–30 mg/l)	49, 50
Ole e 3	9.2*	4.3*	yes	20	<i>E. coli</i> (25 mg/l)	51, 52
Ole e 4	32**	4.6–5.1*	ND	80*	–	57
Ole e 5	16**	5.1–6.5*	ND	35*	–	57
Ole e 6	5.8*	4.2*	no	5–20**	–	58
Ole e 7	9.9–10.3*	>9*	ND	>60	–	23
Ole e 8	18.8*	4.5**	ND	3–4	<i>E. coli</i> (20 mg/l)	53

¹ Molecular weights were obtained by mass spectrometry (*) or SDS-PAGE (**). For Ole e 1, MW of glycosylated and nonglycosylated forms are indicated.

² Isoelectric point (pI) was determined experimentally (*), or calculated from the amino acid sequence (**).

³ Ole e 1 exhibits cross-reactivity only with homologous counterparts from taxonomically closely related species. ND = Not determined.

⁴ Prevalence data for many allergens strongly depend on the population used in the study. The data for (*) are preliminary. The data for (**) have been recently revised by the authors.

⁵ The yield of the expression is indicated.

⁶ For Ole e 1, only references on isolation and sequencing are indicated.

Structural Properties and Functional Roles of Olive Pollen Allergens

Ole e 1: The Most Prevalent Allergen of Olive Pollen

Although the prevalence of Ole e 1 differs in different geographical areas, ranging from 55 to 90%, it has always been shown to be the most prevalent allergen of olive pollen extract [13, 15, 16, 25]. Moreover, removal of Ole e 1 from the whole extract led to the nearly total disappearance of the allergenic activity of the extract [26]. These data, along with the fact that Ole e 1 is the most abundant protein component of the pollen extract, make it the best-characterized olive allergen so far (table 1).

Isolation procedures of Ole e 1 have been reported by several authors, differing in techniques and yields [13, 16, 19]. It was characterized as a glycoprotein, and its primary structure was determined by Edman degradation of peptides obtained by proteolysis [27]. The protein consists of a single polypeptide chain of 145 amino acid residues with a calculated molecular mass of 16,331 D [27], and contains one N-linked glycan moiety located at asparagine 111, which increases the molecular mass of the allergen to about 1.5 kD [28]. Because of the presence of the sugar, the SDS-PAGE pattern of purified Ole e 1 exhibits a multiple-band profile with two main components, a nonglycosylated form of 18.5 kD (10% of the

whole allergen) and a glycosylated variant of 20.0 kD (85%) [13, 19, 27, 28]. The allergen frequently exhibits two faint bands at 22 and 40 kD as a result of the presence of a hyperglycosylated component and the 20-kD dimer, respectively [27]. The polypeptide chain possesses six cysteine residues arranged in three disulfide bridges whose topology has recently been elucidated [29].

The glycan moiety of Ole e 1 has a heterogeneous structure and seems to be involved in the antigenic and allergenic activities of the allergen; it is also responsible for cross-reactivity with glycoproteins from plants taxonomically unrelated to the olive tree [28, 30, 31]. The Ole e 1 glycan is composed of two main oligosaccharide species, whose primary structures have been determined: I) a high mannose component (Man 7), and II) a complex type N-glycan containing β (1,2)-xylose and β (1,2)-GlcNAc-Man(1,3) linked to the ManNAcGlc-NacGlc core [32]. The complex type component can explain the antigenicity of the carbohydrate, since xylose has been largely shown to be involved in the immunogenicity of plant glycoproteins [33]. Minor components (5–10% of the whole oligosaccharide), containing α (1,3)-fucose, were also detected in the glycan of Ole e 1 [31, 32], and their structures were recently elucidated [34]. The presence of fucose increases the antigenicity of such oligosaccharides. As mentioned above, the glycan moiety of Ole e 1 is involved in

the allergenicity of the protein: the isolated glycan contains an IgE epitope, which is recognized by more than 60% of the sera from patients allergic to Ole e 1 [31]. However, the contribution of the glycan to the IgE reactivity of the whole allergen should not be highly relevant, as deduced from the analysis of the allergenic equivalence between the natural and recombinant forms of Ole e 1, which exhibited very similar IgE-binding activities [35] although they possess different N-glycans. This observation is also supported by studies performed with a nonglycosylated mutant of Ole e 1 (N111Q), expressed in the yeast *Pichia pastoris*, which exhibited an immunologic behavior very similar to that of the glycosylated natural allergen [36].

The nucleotide sequence of several cDNA clones encoding Ole e 1 was determined by molecular biology techniques showing a high degree of polymorphism since more than 40 positions of the polypeptide chain were found to be heterogeneous [37, 38]. The allergen belongs to a family of proteins whose members have been found to be expressed almost exclusively in pollen tissue. Ole e 1 exhibits the highest amino acid sequence identity (>85%) with its close relatives from other Oleaceae, such as privet and lilac. It also displays a low but significant identity (ranging from 24 to 31%) to Lol p 11 – the allergen from ryegrass –, and to the homologous proteins from rice, maize, birch, *Arabidopsis thaliana*, *Sambucus nigra*, *Brassica napus*, and *Phalaris coerulescens* [39–41 and sequence databases].

Ole e 1 is a protein specifically expressed in pollen tissue, as was demonstrated by Northern blot [37]. This is in agreement with the results obtained from studies on the ultrastructural localization of the allergen with Ole e 1-specific monoclonal antibodies and its corresponding mRNA by in situ hybridization. The allergen was localized in the endoplasmic reticulum, the pollen wall and the tapetum during pollen development [42, 43]. Both synthesis and storage take place in the reticulum, suggesting a biological role as a secretion protein [43]. This would be in agreement with the presence of signal peptides in the amino acid sequences deduced from the cDNAs encoding this family of proteins. These findings have suggested a potential role for Ole e 1 in establishing the osmotic gradient needed for hydration of the pollen during germination [43], but functional analyses should be performed to assess this biochemical activity.

Mapping of immunodominant B and T cell epitopes of Ole e 1 has been carried out. Using several Ole e 1-specific monoclonal antibodies, the existence of at least four IgG antigenic determinants was demonstrated [42]. Analyzing

the proliferating response that synthetic peptides of the allergen induce in the peripheral blood mononuclear cells from Ole e 1-sensitized patients versus nonsensitized controls, Cárdena et al. [44] have found immunodominant T cell epitopes located at positions 91–102 and 109–130 of the amino acid sequence of Ole e 1, which are not recognized by IgE antibodies. However, the segments of the polypeptide chain of Ole e 1 involved in B cell determinants remain to be defined.

Genetic studies of an Ole e 1-sensitive population have shown that the specific response to the allergen is associated with the DR7 and DQ2 HLA class II antigens [45]. Ole e 1 IgG titers are positively correlated with HLA-DRB1*0701 and HLA-DQB1*0201 alleles, and DQB1*0201 was also found to be correlated with Ole e 1 IgE titers [45, 46]. In addition, it was demonstrated that IgE antibodies against Ole e 1 were associated with FcεRI-β [47].

Ole e 2: An Olive Profilin

Profilin is a ubiquitous protein found in a large number of vegetable and animal biological sources. It binds actin polymers and is involved in signal transduction events through phosphoinositide pathways. It has been established as a pan-allergen [48]. Profilin has also been purified from olive pollen extracts and, as other members of this family of proteins, has been found to be allergenic [49]. Molecular and immunochemical properties of Ole e 2 do not differ from those of other profilins, exhibiting a prevalence of 24 and >40% in patients allergic to olive pollen, as shown by immunoblotting and ELISA, respectively [49]. This indicates that the allergen loses IgE-binding capacity against the sera of a large number of hypersensitive individuals when it is assayed under denaturing conditions. Olive profilin has been sequenced and expressed in *Escherichia coli* [50], showing 86 and 73% identity to birch and grass profilins, respectively, which is in agreement with the high antigenic and allergenic cross-reactivities found between them [49].

Ole e 3 (Olive Polcalcin) and Ole e 8: Two Ca²⁺-Binding Proteins

The biochemical activity of a protein is one of the main features that allows to know its behavior. Two olive allergens, Ole e 3 and Ole e 8, have been identified as Ca²⁺-binding proteins [51–53]. Both allergens belong to the EF-hand Ca²⁺-binding protein family, since they possess functional segments consisting of 12 consecutive amino acid residues flanked by two α-helices called EF-hand motifs, which form a conformational loop that is able to

bind calcium ion. However, and according to their behavior in affinity columns, they should display different biological roles in the pollen cell: whereas Ole e 3 belongs to the buffering type Ca^{2+} -binding protein subfamily [52], Ole e 8 should develop a regulatory function involved in signal transduction pathways [53]. In addition, Ole e 8 contains four EF-hand motifs along its polypeptide sequence, whereas Ole e 3 contains only two.

Ole e 3 is a small (9.2 kD) and acidic (pI 4.2–4.3) protein isolated from the pollen [51]. The protein consists of a single chain of 84 residues which exhibits high homology to allergenic pollen proteins from rapeseed, Bermuda grass, timothy grass, birch and alder (60–85% of amino acid sequence identity and 85–95% of similarity), and strong cross-reactivity with them [51, 52]. Moreover, the presence of Ole e 3-like proteins has also been shown in other unrelated species, since an Ole e 3-specific polyclonal antiserum recognized proteins in the pollen from wheat, mugwort, pine, cypress and rose [51, 52]. Because of its specific expression in pollen tissue and its Ca^{2+} -binding capacity, the name of polcalcin has been proposed for this subfamily of Ca^{2+} -binding proteins, which has also been established as a new pan-allergen.

Ole e 8 (18,795 D) consists of a single polypeptide chain of 171 amino acid residues that displays an apparent molecular mass of 20 kD in SDS-PAGE [53]. The olive pollen extract contains very low amounts of this allergen. Therefore, the strategy to obtain manageable levels of the protein was the recombinant expression in *E. coli* [53]. The molecular properties of Ole e 8 were those typical of Ca^{2+} -binding proteins, exhibiting a high level of α -helix secondary structure and undergoing conformational changes, which depend on the presence of Ca^{2+} ions [53].

Interestingly, the IgE-binding capability of Ca^{2+} -binding allergens is related to the presence of calcium bound to their polypeptide chains [54, 55]. This fact would allow the designing of recombinant derivatives with a decreased capability of binding Ca^{2+} ions, although maintaining the antigenic properties; thus, their allergenic activity would be diminished and they could be used in immunotherapy [56].

Ole e 4, Ole e 5, and Ole e 6: Three Allergens to be Explored

Allergens Ole e 4 and Ole e 5 were purified and partially characterized by Boluda et al. [57]. Both proteins have an acidic pI value, and display apparent molecular masses of 32 and 16 kD, respectively. Both are composed of a single polypeptide chain and exhibited polymorphism

since their pIs are heterogeneous. The N-terminal end of Ole e 4 was blocked, but internal amino acid sequences were obtained from two tryptic peptides of the protein. Comparison of such sequences with those of proteins contained in databases showed no homology with any known protein. By contrast, homology with superoxide dismutases of several plant species, such as spinach, sweet potato, grasses or tomato, was deduced from the N-terminal amino acid sequence of Ole e 5. Prevalence analyses of these allergens are still preliminary and should be revised, since a small series of 20 Spanish allergic individuals was selected for the study. Moreover, the authors suggested the existence of variability in the allergen content of the pollen collected in California (USA) [57].

Ole e 6 is the smallest allergen found in olive pollen so far. This highly soluble protein was purified, its cDNA was cloned and sequenced, and no microheterogeneity was detected [58]. Although the protein exhibits an apparent molecular mass in SDS-PAGE of 10 kD, it consists of a single very acidic polypeptide chain of 50 amino acids (5,830 D by mass spectrometry), with a high content of Cys residues arranged in two sets of a particular sequential motif, i.e. Cys- X_3 -Cys- X_3 -Cys [58]. The allergen shows no homology with any protein contained in the nucleotide sequence databases, but the Cys-enriched motif appears in the amino acid sequence deduced from *Tap1*, a stamen-specific gene from snapdragon (*Antirrhinum majus*) and tobacco (*Nicotiana tabacum*). Data on the Ole e 6 prevalence are preliminary, but seem to be dependent on the geographical area of sensitization of the patients.

These three olive allergens remain to be more deeply analyzed, since complete sequences of Ole e 4 and Ole e 5, the presence of homologous counterparts for three proteins, cross-reactivities with other pollen allergens, and even their allergenic properties are yet unknown.

Ole e 7: A Lipid Transfer Protein

The protein fraction of low molecular weight (around 10 kD) of olive pollen extract is rich in allergens. Besides Ole e 3 and Ole e 6, it also contains Ole e 7, and we have now evidence for the existence of more allergenic components with a high clinical significance in this fraction [unpubl. results]. The SDS-PAGE analysis of these allergens shows a very similar pattern, and their specific responses cannot be distinguished when immunoblotting the whole pollen extract against sera from individual patients (see fig. 1, in which 9- to 10-kD bands include all these allergens).

Ole e 7 has been purified and characterized [23]. Only 21 amino acid residues from the N-terminal end of the polypeptide chain were determined by Edman degradation, exhibiting a high degree of microheterogeneity. The polymorphism of the protein was corroborated by mass spectrometry since it showed several peaks in the range of 9,876–10,091 kD. As a result of sequence homology searches using the short portion of the obtained primary structure, similarities with nonspecific lipid transfer proteins (nsLTP) from plant tissue were detected [unpubl. results]. Lipid transfer proteins (LTPs) are very stable molecules, widely distributed in different species and tissues, whose function is related to the ability of binding fatty acids and which possess antibacterial and antifungal activities [59]. This family of proteins was originally associated with intracellular trafficking of lipids, but now a role in the cuticle formation of aerial parts of the plant and in defensive mechanisms against pathogens is accepted [60]. Moreover, in some plant species there are isoforms of LTP which may have different functions and are differentially expressed in a tissue-specific manner. The N-terminal amino acid sequence of Ole e 7 was found to be most similar (around 50%) to LTP from tobacco, rapeseed, sunflower and barley.

LTP have been identified as relevant allergens in the Rosaceae family (peach, apricot, apple, plum) and other vegetable foods [61, 62], and they emerge slowly as pollen allergens [63]. Because of the ubiquitous distribution of LTP in different plant species, they have been proposed as a novel pan-allergen with a potential spectrum of cross-reactivity comparable to that reported for profilins [63, 64]. Therefore, Ole e 7 could be involved in allergenic cross-reactivity between fruits and olive pollen.

IgE-Binding Proteins Not Yet Reported

In light of the individual allergenic patterns (fig. 1), it is easy to deduce the existence in the olive pollen of allergens with a high molecular mass and a high clinical prevalence that have not been examined yet. Thus, proteins of 36–50 kD seem likely to be recognized by the IgE antibodies of a wide population of hypersensitive patients. Recent studies have shown the existence of two allergens of 36 and 45–46 kD, but neither has been characterized either structurally or immunologically [65, 66]. Our group is now investigating the 46-kD allergen [67], and its properties will soon be reported. Moreover, the analysis of the allergenic pattern of the olive pollen proteins extracted using a short time of hydration (15 min) were different from those of those extracted for a longer time (3 h). Thus, Ole e 6 and Ole e 7 are highly soluble, and they are almost completely

released during the former period, whereas Ole e 3 require longer extraction time [23, 51, 58]. From these studies, we have evidence of the presence of an important allergen in the fraction of 10 kD in addition to the reported proteins Ole e 3, Ole e 6 and Ole e 7 [unpubl. results].

Geographic Dependence on the Prevalence of Olive Allergens

From the analysis by immunoblotting of individual IgE responses of olive-allergic patients belonging to different Spanish communities, a different degree of complexity was observed between the allergograms of patients exposed to high or low levels of olive pollen [22]. Hypersensitive individuals living in geographic areas such as Andalucía, where the olive tree is abundant, exhibited sensitization to a significantly larger number of allergens than those of regions with low pollen counts (for instance from Madrid). In addition, the later patients showed a preferential reactivity to Ole e 1, and a high percentage of them were only reactive to this allergen [22]. This is supported by the existence of a correlation between the abundance of olive trees in the patients' neighborhood and the incidence of skin-tested sensitivity among individuals with suspected atopic respiratory allergy from a population of Israelis [68]. These facts could help to explain the differences found by several authors among allergenic patterns as well as for the prevalence of olive allergens [15, 18, 19, 21], since they would depend on the particular sera used in each study, besides the specific technique employed for the analysis. As an example, the prevalence of Ole e 6 was found to range from 5 to 20% depending on the geographical area of sensitization of the patients suffering of olive pollinosis. In summary, the prevalence of individual allergens depends, to some extent, on the level of pollen in the atmosphere, and therefore is closely related to the geographical area in which the patients were sensitized. Interestingly, it has been shown that patients monosensitized to *Olea* seem to need exceptionally high levels of olive pollen (around 400 grains/m³) to suffer from symptoms of allergic rhinitis [69].

The fact that most early studies on olive allergens were done on sera from patients living in areas with lower pollen counts [13, 15, 16, 26, 27] may explain the focussing on Ole e 1 during the early nineties. More recently, the use of sera from patients living in an area with a high density of olive trees has led to the discovery of several allergens, many of which have a clinically significant potential [22, 52, 53, 57, 58].

On the other hand, differences have been shown in the allergenic components of various cultivars of *O. europaea* [14, 21], even different levels of particular allergens – such as Ole e 1 – were also measured in olive pollen extracts depending on the natural tribes and the commercial suppliers [70, 71]. Although preliminary results have shown a correlation between the incidence of the allergic response in a population of Israel and different olive cultivars [68], the potential involvement of such differences – both qualitative and quantitative – in the antigenicity of this pollen is to be analyzed. In addition, exposure of the hypersensitive individuals to other biological sources, mainly to pollens, may build up allergenic cross-reactivity in olive-allergic patients, and the prevalence measured for a given allergen could be the result of primary and secondary sensitizations.

Cross-Reactivity: Related and Unrelated Pollens

Cross-reactivity is a process by which the IgE induced against an allergen can be reactive to another molecule from a different biological source. The structural similarity between two allergens is a crucial factor in the degree of allergenic cross-reactivity. Therefore, homologous proteins are frequently candidates for cross-reactivity. In addition, when there are close phylogenetic relationships between the biological sources of the allergens, the possibility of cross-reactivity increases. Although the olive tree produces the most allergenic pollen among the Oleaceae, other species, such as the ash (*Fraxinus excelsior*), privet (*Ligustrum vulgare*), lilac (*Syringa vulgaris*) and forsythia (*Forsythia suspensa*) also contain potential allergens. In fact, cross-reactivity among Oleaceae pollens was observed in early studies [3] and confirmed in the early nineties [72–74]. Afterwards, three other olive allergens have been demonstrated to be involved in cross-reactivity processes: Ole e 1, profilin and Ole e 3.

The presence of Ole e 1-like proteins in several Oleaceae pollens, such as privet, ash, lilac, and forsythia, has been demonstrated [42, 75], and several members of this family of proteins were characterized and produced as recombinant forms [39, 41]. Those produced by lilac and privet were called Syr v 1 and Lig v 1, respectively, because of the detection of specific IgE to these proteins in the sera of olive-sensitive patients [39, 41, 75]. The amino acid sequences of Syr v 1 and Lig v 1 are very similar to that of the olive allergen (>85% identity) [39, 41], and thus they should share antigenic determinants with Ole e 1. This could explain the strong allergenic cross-reactivity

largely observed between Oleaceae pollens, since these proteins are, as Ole e 1, major components of these pollen extracts [16, 39, 41, 75]. However, significant differences were detected in the antigenic behavior of these allergens against both Ole e 1-specific monoclonal antibodies and sera from olive-sensitive patients [39, 41, 75, 76]. Therefore, Ole e 1-like proteins from Oleaceae pollens, although possessing conserved polypeptide sequences, show structural differences detectable by both IgE and monoclonal antibodies and result in protein-specific antigenic determinants. On the basis of the reduced IgE-binding ability of many Ole e 1-like proteins, their use as hypoallergenic vaccines can be suggested.

On the other hand, Ole e 1-like proteins from pollen sources taxonomically unrelated to olive do not cross-react with Ole e 1 at a significant level [unpubl. results].

The amino acid sequences of Ole e 1 homologous counterparts from putative allergenic pollens from plants, such as ryegrass, birch, rice, corn, rapeseed, and others are different enough (24–31% identity) to explain the absence of cross-reactivity among them. However, it should also be considered that members of the Ole e 1-like protein family exhibit intrinsic allergenic capacity when the hypersensitive individuals were in contact with their biological sources. In fact, the homologous Ole e 1 counterpart from ryegrass (Lol p 11) has been reported to induce type I allergy [40], and very recently it has been found that the main allergen from the pollen of *Plantago lanceolata*, Pla l 1, is the Ole e 1-like protein from this species, based on amino acid sequence identity of the N-terminal end of the isolated allergen to Ole e 1 [77]. Could the biochemical activity of this family of proteins, which exhibit low conserved amino acid sequences, be involved in the allergenicity of their members? This is a question that remains to be answered.

The existence of an IgE epitope located in the xylose- and fucose-containing glycan of Ole e 1 [28, 30–32, 34] and the close similarity of the carbohydrate structures of plant glycoproteins [33] could enlarge the group of molecules responsible of the cross-reactivity between olive pollen and other unrelated vegetable sources.

Besides Ole e 1, olive pollen contains a number of pan-allergens which have been extensively explored in other vegetable sources: profilin and polcalcin. The involvement of olive profilin in cross-reactivity was analyzed by inhibition assays using both anti-olive profilin rabbit serum and human sera from olive-allergic patients [49]. The specific IgG polyclonal antiserum recognized profilins from all the assayed pollen extracts: ash, birch, Bermuda grass and maize. The degree of inhibition of the IgE-

binding to olive profilin achieved by the extracts of these pollens was very different. Gramineae profilins displayed low inhibitory capability, whereas the *Oleaceae* profilins showed high inhibitory capacity. However, when allergic sera were used in the inhibition assays, the inhibitory capacities of the different extracts were very similar: total inhibition was always obtained, indicating a high similarity in the allergenic behavior of different profilins against the IgE from olive-allergic sera [49].

As mentioned above, Ole e 3 belongs to a widespread family of Ca^{2+} -binding proteins, whose polypeptide sequences are highly conserved, with a strong degree of allergenic cross-reactivity among their members. Immunochemical similarities between Ole e 3 and other members of the polcalcin family were analyzed by ELISA inhibition assays, and by Western and Northern blots at protein and mRNA levels, using respectively Ole e 3-specific rabbit antiserum, and Ole e 3-specific cDNA probes [51, 52]. The allergenic pollen sources employed in these studies were olive, lilac, ash, birch, mugwort, ryegrass, maize, Bermuda grass, cypress, and pine, as well as the nonallergenic rose pollen. ELISA inhibition data indicated that the polcalcins from pollen extracts share antigenic epitopes with Ole e 3, as they are capable of inhibiting the binding of the specific IgG to the olive allergen by at least 50% [51]. Immunoblotting analyses of the pollen extracts showed differences in the recognition of polcalcins from different sources. A similar result was obtained in hybridization experiments [52], indicating that the members of this family of allergens share antigenic determinants but are immunochemically and structurally distinct.

The fact that a structural motif such as the EF-hand loop, common to a huge number of Ca^{2+} -binding proteins, could be a recognition site for IgE of allergic sera, extends the range of proteins which can be involved in cross-reactivity, even considering scarce similarity in the rest of the molecule. Other allergenic Ca^{2+} -binding proteins have been described in animal and vegetable sources: parvalbumin [78], Bet v 3 [79] and Jun o 2 [80], whose amino acid sequences only share the EF-hand motifs. The putative antigenic relationships among different types of EF-hand Ca^{2+} -binding proteins have not been explored. Ole e 3 and Ole e 8 may be a good model for analyzing allergenic cross-reactivity between EF-hand Ca^{2+} -binding proteins with unrelated polypeptide sequences.

Scarce data have been published on cross-reactivity between whole olive pollen extracts and those from unrelated pollens. Baldo et al. [72] demonstrated the existence of cross-reactivity of olive and privet allergens with grass pollen proteins, and, more recently, it has been found that

pollen proteins from birch, mugwort, pine, and cypress share IgE epitopes with olive allergens [66]. These data could explain the frequent polysensitization of olive-allergic patients, and should be analyzed in depth since it may help in the diagnostic of olive allergy and other pollinoses.

Recombinant Allergens

Recombinant DNA engineering has been a very useful tool for cloning, sequencing and production of olive allergens. This technology provides homogeneous molecules at high yield, overcoming the troubles associated with the polymorphism of the natural proteins and the low yield and difficulties in their purification. Two heterologous hosts have been used for the expression of four olive proteins: *E. coli* for Ole e 1, profilin, Ole e 3 and Ole e 8 [37, 50, 52, 53], and the yeast *P. pastoris* to produce Ole e 1 and its relatives from lilac and privet [76, 81].

The recombinant expression in *E. coli* of one cDNA clone encoding Ole e 1 as a fusion protein linked to the enzyme glutathione S-transferase produced an antigenically active allergen [37]. However, the low yield and limited solubility of the bacterial product led us to use an alternative host for the sizeable expression of this protein. *P. pastoris* provided an overproductive eukaryotic system for the synthesis of recombinant Ole e 1, allowing the isolation of a properly folded, soluble and secreted form of the protein [81]. The glycosylation ability and the adequate folding mechanisms of the yeast for arranging disulfide bridges probably explain the production of a recombinant glycosylated form of the allergen, whose immunochemical properties were indistinguishable from those of the natural protein purified from pollen extracts [81]. In fact, both in vitro (ELISA and immunoblotting) and in vivo (prick tests) analyses performed to validate recombinant Ole e 1 demonstrated its immunological equivalence to the natural form, although the glycan added to the recombinant allergen was not identical to that of the natural Ole e 1 [35, 81]. Therefore, these analyses supported once again the limited significance of the oligosaccharide-specific IgE antibodies in the whole response to Ole e 1.

Bacterial systems provided satisfactory hosts for the recombinant production of olive profilin, Ole e 3 and Ole e 8 [52, 53]. These proteins do not contain disulfide bridges or glycan moieties and, thus, the absence of posttranslational modifications in the produced proteins makes *E. coli* a successful expression system. Both natural and recombinant forms of Ole e 3 were able to bind/

release calcium, these processes inducing conformational changes in the protein, as demonstrated by SDS-PAGE analysis and circular dichroism studies, which were responsible for the differences observed in the IgE-binding properties of the apo- and Ca²⁺-bound forms of the allergen [52].

All the studies on the production of olive pollen proteins by DNA technology confirm the recombinant allergens as excellent products for research and clinical applications. Cocktails of recombinant allergens can be prepared with selected levels to replace natural allergens in allergy diagnosis and specific immunotherapy [82]. In

addition, the availability of high levels of homogeneous molecules provides essential tools to investigate aspects associated with molecular and immunological features of these proteins including their three-dimensional structure, biochemical activity, biological role, epitope mapping.

Acknowledgments

This work has been supported by grant PM98/0094 from the DGESIC (MEC, Spain).

References

- Chevalier A: L'origine de l'olivier cultivé et ses variations. *Rev Int Bot Appl Agric Trop* 1948; 28:1–25.
- Manousis T, Moore NF: Exploited plants. The olive tree. *Biologist* 1988;35:7–12.
- Bousquet J, Cour P, Guerin B, Michel FB: Allergy in the Mediterranean area. I. Pollen counts and pollinosis of Montpellier. *Clin Allergy* 1984;14:249–258.
- Barranco D, Rallo L: Las variedades del olivo cultivadas en Andalucía: Consejería de Agricultura y Pesca de la Junta de Andalucía, Madrid, MAPA, 1984.
- D'Amato G, Mullins J, Nolard N, Spiekma FT, Wachter R: City spore concentrations in the European Economic Community (EEC). VII. Oleaceae (*Fraxinus*, *Ligustrum*, *Olea*). *Clin Allergy* 1988;18:541–547.
- D'Amato G, Lobefalo G: Allergenic pollens in the southern Mediterranean area. *J Allergy Clin Immunol* 1989;83:116–122.
- Galán C, Ruiz de Clavijo E, Infante F, Gallego G: Annual and daily variation of pollen from *Olea europaea* L. in the atmosphere of Córdoba (Spain) along two years of sampling. *Experientia* 1987;51:109–112.
- Frenguelli G, Bricchi E, Romano B, Mincigruci G, Spiekma FTM: A predictive study of the beginning of the pollen season for Graminaeae and *Olea europaea* L. *Aerobiologia* 1989; 5:64–70.
- Liccardi G, D'Amato M, D'Amato G: Oleaceae pollinosis: A review. *Int Arch Allergy Immunol* 1996;111:210–217.
- Vela C, Platas C, Gurbindo C, Tricas L, Subiza E, García R, Lahoz C: Fractionation and biological characterization of *Olea europaea* pollen extract. *Int Arch Allergy Appl Immunol* 1982; 68:289–294.
- Blanca M, Boulton P, Brostoff J, González-Reguera I: Studies of the allergens of *Olea europaea* pollen. *Clin Allergy* 1983;13:473–478.
- Rubio N, Brieve A, Alcázar B: Purification of allergens by high-performance liquid chromatography. IV. Purification of the allergen of olive pollen (*Olea europaea*). *J Chromatogr* 1987;403:312–318.
- Lauzurica P, Maruri N, Galocha B, González J, Díaz R, Palomino P, Hernández D, García R, Lahoz C: Olive (*Olea europaea*) pollen allergens. II. Isolation and characterization of two major antigens. *Mol Immunol* 1988;25:337–344.
- Barber D, Carpizo J, García-Rumbao MC, Polo F, Juan F: Allergenic variability in olea pollen. *Ann Allergy* 1990;64:43–46.
- Wheeler AW, Hickman BE, Fox B: Heterogeneity of a major allergen from olive (*Olea europaea*) pollen. *Mol Immunol* 1990;27:631–636.
- Villalba M, López-Otín C, Martín-Orozco E, Monsalve RI, Palomino P, Lahoz C, Rodríguez R: Isolation of three allergenic fractions of the major allergen from *Olea europaea* pollen and N-terminal amino acid sequence. *Biochem Biophys Res Commun* 1990;172:523–528.
- Marsh DG, Goodfriend L, King TP, Lowenstein H, Platts-Mills TA: Allergen nomenclature. *Bull World Health Organ* 1986;64:767–774.
- Lauzurica P, Gurbindo C, Maruri N, Galocha B, Díaz R, González J, García R, Lahoz C: Olive (*Olea europaea*) pollen allergens. I. Immunochemical characterization by immunoblotting, CRIE and immunodetection by a monoclonal antibody. *Mol Immunol* 1988;25:329–335.
- De Cesare F, Pini C, Di Felice G, Caiaffa MF, Macchia L, Tursi A, Tinghino R, Palumbo S, Sallusto F, Federico R: Purification and fine characterization of a major allergen from *Olea europaea* pollen extract. *Allergy* 1993;48:248–254.
- Rodríguez R, Villalba M, Batanero E, Monsalve RI, López-Otín C, Lahoz C, González de la Peña MA: Allergens from olive tree and related species. Bologna, Monduzzi, 1995.
- Waisel Y, Geller-Bernstein C, Keynan N, Arad G: Antigenicity of the pollen proteins of various cultivars of *Olea europaea*. *Allergy* 1996; 51:819–825.
- Rodríguez R, Villalba M, Monsalve RI, Batanero E, Ledesma A, González E, Tejera ML, Huecas S, García M, García I: Perfiles de reconocimiento alérgico del polen de olivo. Implicación en la diagnosis e inmunoterapia. *Rev R Acad Cienc Exact Fis Nat (Esp)* 1998; 92:253–258.
- Tejera ML, Villalba M, Batanero E, Rodríguez R: Identification, isolation, and characterization of Ole e 7, a new allergen of olive tree pollen. *J Allergy Clin Immunol* 1999;104:797–802.
- King TP, Hoffman D, Lowenstein H, Marsh DG, Platts-Mills TA, Thomas W: Allergen nomenclature. *Allergy* 1995;50:765–774.
- Wheeler AW: Hypersensitivity to the allergens of the pollen from the olive tree (*Olea europaea*). *Clin Exp Allergy* 1992;22:1052–1057.
- Lombardero M, Quirce S, Duffort O, Barber D, Carpizo J, Chamorro MJ, Lezaun A, Carreira J: Monoclonal antibodies against *Olea europaea* major allergen: Allergenic activity of affinity-purified allergen and depleted extract and development of a radioimmunoassay for the quantitation of the allergen. *J Allergy Clin Immunol* 1992;89:884–894.
- Villalba M, Batanero E, López-Otín C, Sánchez LM, Monsalve RI, González de la Peña MA, Lahoz C, Rodríguez R: The amino acid sequence of Ole e I, the major allergen from olive tree (*Olea europaea*) pollen. *Eur J Biochem* 1993;216:863–869.
- Batanero E, Villalba M, Rodríguez R: Glycosylation site of the major allergen from olive tree pollen. Allergenic implications of the carbohydrate moiety. *Mol Immunol* 1994;31:31–37.
- González E, Monsalve RI, Puente XS, Villalba M, Rodríguez R: Assignment of the disulfide bonds of Ole e I, a major allergen of olive tree pollen involved in fertilization. *J Pept Res* 2000;55:18–23.

- 30 Batanero E, Villalba M, Monsalve RI, Rodríguez R: Cross-reactivity between the major allergen from olive pollen and unrelated glycoproteins: Evidence of an epitope in the glycan moiety of the allergen. *J Allergy Clin Immunol* 1996;97:1264–1271.
- 31 Batanero E, Crespo JF, Monsalve RI, Martín-Esteban M, Villalba M, Rodríguez R: IgE-binding and histamine-release capabilities of the main carbohydrate component isolated from the major allergen of olive tree pollen, Ole e 1. *J Allergy Clin Immunol* 1999;103:147–153.
- 32 van Ree R, Cabanes-Macheteau M, Akkerdaas J, Milazzo JP, Loutelier-Bourhis C, Rayon C, Villalba M, Koppelman S, Aalberse R, Rodríguez R, Faye L, Lerouge P: Beta(1,2)-xylose and alpha(1,3)-fucose residues have a strong contribution in IgE binding to plant glycoallergens. *J Biol Chem* 2000;275:11451–11458.
- 33 Faye L, Chrispeels MJ: Common antigenic determinants in the glycoproteins of plants, molluscs and insects. *Glycoconj J* 1988;5:245–256.
- 34 Kolarich D, Altmann F: N-Glycan analysis by matrix-assisted laser desorption/ionization mass spectrometry of electrophoretically separated nonmammalian proteins: Application to peanut allergen Ara h 1 and olive pollen allergen Ole e 1. *Anal Biochem* 2000;285:64–75.
- 35 Quiralte J, González E, Arias de Saavedra JM, Villalba M, Florido JF, Sáenz de San Pedro B, Rodríguez R: Biological activity of recombinant Ole e 1 in patients with *Olea europaea* pollinosis. *Int Arch Allergy Immunol* 1999;122:101–107.
- 36 Villalba M, Huecas S, González E, Batanero E, Tejera ML, García-Cao I, Rodríguez R: Expression in *Pichia pastoris* and characterization of a non glycosylated mutant of Ole e 1 (abstract 333). *J Allergy Clin Immunol* 1999;103: S88.
- 37 Villalba M, Batanero E, Monsalve RI, González de la Peña MA, Lahoz C, Rodríguez R: Cloning and expression of Ole e 1, the major allergen from olive tree pollen. Polymorphism analysis and tissue specificity. *J Biol Chem* 1994;269:15217–15222.
- 38 Lombardero M, Barbas JA, Moscoso del Prado J, Carreira J: cDNA sequence analysis of the main olive allergen, Ole e 1. *Clin Exp Allergy* 1994;24:765–770.
- 39 Batanero E, Villalba M, López-Otín C, Rodríguez R: Isolation and characterization of an olive allergen-like protein from lilac pollen. Sequence analysis of three cDNA encoding protein isoforms. *Eur J Biochem* 1994;221:187–193.
- 40 van Ree R, Hoffman DR, van Dijk W, Brodard V, Mahieu K, Koeleman CA, Grande M, van Leeuwen WA, Aalberse RC: Lol p XI, a new major grass pollen allergen, is a member of a family of soybean trypsin inhibitor-related proteins. *J Allergy Clin Immunol* 1995;95:970–978.
- 41 Batanero E, González de la Peña MA, Villalba M, Monsalve RI, Martín-Esteban M, Rodríguez R: Isolation, cDNA cloning and expression of Lig v 1, the major allergen from privet pollen. *Clin Exp Allergy* 1996;26:1401–1410.
- 42 Martín-Orozco E, Cárdaña B, del Pozo V, de Andrés B, Villalba M, Gallardo S, Rodríguez-García MI, Fernández MC, Alché JD, Rodríguez R, Palomino P, Lahoz C: Ole e 1: Epitope mapping, cross-reactivity with other *Oleaceae* pollens and ultrastructural localization. *Int Arch Allergy Immunol* 1994;104:160–170.
- 43 Alché JD, Castro AJ, Olmedilla A, Fernández MC, Rodríguez R, Villalba M, Rodríguez-García MI: The major olive pollen allergen (Ole e 1) shows both gametophytic and sporophytic expression during anther development, and its synthesis and storage takes place in the RER. *J Cell Sci* 1999;112:2501–2509.
- 44 Cárdaña B, Del Pozo V, Jurado A, Gallardo S, Cortegano I, Arrieta I, Del Amo A, Tramón P, Florido F, Sastre J, Palomino P, Lahoz C: Olive pollen allergy: Searching for immunodominant T-cell epitopes on the Ole e 1 molecule. *Clin Exp Allergy* 1998;28:413–422.
- 45 Cárdaña B, Vilches C, Martín E, de Andrés B, del Pozo V, Hernández D, Gallardo S, Fernández JC, Villalba M, Rodríguez R, et al.: DR7 and DQ2 are positively associated with immunoglobulin-E response to the main antigen of olive pollen (Ole e 1) in allergic patients. *Hum Immunol* 1993;38:293–299.
- 46 Cárdaña B, Moffat M, Quiralte J, Wiltshire S, Del Pozo V, Florido F, Cortegano I, Arrieta I, Gallardo S, Rojo M, Palomino P, Rodríguez R, Cookson B, Lahoz C: Genetic study of olive pollen allergy (abstract). *Int Arch Allergy Immunol* 1999;119:1–2.
- 47 Cárdaña B, Cortegano I, Florido F, Arrieta I, Aceituno E, del Pozo V, Gallardo S, Rojo M, Palomino P, Lahoz C: Genetic restrictions in olive pollen allergy. *J Allergy Clin Immunol* 2000;105:292–298.
- 48 Valenta R, Duchene M, Ebner C, Valent P, Sillaber C, Deviller P, Ferreira F, Tejkl M, Edelmann H, Kraft D, Scheiner D: Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;175:377–385.
- 49 Ledesma A, Rodríguez R, Villalba M: Olive-pollen profilin. Molecular and immunologic properties. *Allergy* 1998;53:520–526.
- 50 Asturias JA, Arilla MC, Gómez-Bayón N, Martínez J, Martínez A, Palacios R: Cloning and expression of the panallergen profilin and the major allergen (Ole e 1) from olive tree pollen. *J Allergy Clin Immunol* 1997;100:365–372.
- 51 Batanero E, Villalba M, Ledesma A, Puente XS, Rodríguez R: Ole e 3, an olive-tree allergen, belongs to a widespread family of pollen proteins. *Eur J Biochem* 1996;241:772–778.
- 52 Ledesma A, Villalba M, Batanero E, Rodríguez R: Molecular cloning and expression of active Ole e 3, a major allergen from olive-tree pollen and member of a novel family of Ca²⁺-binding proteins (polcalcins) involved in allergy. *Eur J Biochem* 1998;258:454–459.
- 53 Ledesma A, Villalba M, Rodríguez R: Cloning, expression and characterization of a novel four EF-hand Ca²⁺-binding protein from olive pollen with allergenic activity. *FEBS Lett* 2000;466:192–196.
- 54 Hayek B, Vangelista L, Pastore A, Sperr WR, Valent P, Vrtala S, Niederberger V, Twardosz A, Kraft D, Valenta R: Molecular and immunologic characterization of a highly cross-reactive two EF-hand calcium-binding alder pollen allergen, Aln g 4: Structural basis for calcium-modulated IgE recognition. *J Immunol* 1998;161:7031–7039.
- 55 Niederberger V, Hayek B, Vrtala S, Laffer S, Twardosz A, Vangelista L, Sperr WR, Valent P, Rumpold H, Kraft D, Ehrenberger K, Valenta R, Spitzauer S: Calcium-dependent immunoglobulin E recognition of the apo- and calcium-bound form of a cross-reactive two EF-hand timothy grass pollen allergen, Phl p 7. *Faseb J* 1999;13:843–856.
- 56 Valenta R, Hayek B, Seiberler S, Bugajska-Schretter A, Niederberger V, Twardosz A, Natter S, Vangelista L, Pastore A, Spitzauer S, Kraft D: Calcium-binding allergens: From plants to man. *Int Arch Allergy Immunol* 1998;117:160–166.
- 57 Boluda L, Alonso C, Fernández-Caldas E: Purification, characterization, and partial sequencing of two new allergens of *Olea europaea*. *J Allergy Clin Immunol* 1998;101:210–216.
- 58 Batanero E, Ledesma A, Villalba M, Rodríguez R: Purification, amino acid sequence and immunological characterization of Ole e 6, a cysteine-enriched allergen from olive tree pollen. *FEBS Lett* 1997;410:293–296.
- 59 García-Olmedo F, Molina A, Segura A, Moreno M: The defensive role of nonspecific lipid-transfer proteins in plants. *Trends Microbiol* 1995;3:72–74.
- 60 Wirtz KW: Phospholipid transfer proteins revisited. *Biochem J* 1997;324:353–360.
- 61 Pastorello EA, Farioli L, Pravettoni V, Ortolani C, Ispano M, Monza M, Baroglio C, Scibola E, Ansaloni R, Incorvaia C, Conti A: The major allergen of peach (*Prunus persica*) is a lipid transfer protein. *J Allergy Clin Immunol* 1999;103:520–526.
- 62 Sánchez-Monge R, Lombardero M, García Selles FJ, Barber D, Salcedo G: Lipid-transfer proteins are relevant allergens in fruit allergy. *J Allergy Clin Immunol* 1999;103:514–519.
- 63 Díaz-Perales A, Lombardero M, Sánchez-Monge R, García-Selles FJ, Pernas M, Fernández-Rivas M, Barber D, Salcedo G: Lipid-transfer proteins as potential plant panallergens: Cross-reactivity among proteins of Artemisia pollen, Castanea nut and Rosaceae fruits, with different IgE-binding capacities. *Clin Exp Allergy* 2000;30:1403–1410.
- 64 Asero R, Mistrello G, Roncarolo D, de Vries SC, Gautier MF, Ciurana CL, Verbeek E, Mohammadi T, Knul-Brettlova V, Akkerdaas JH, Bulder I, Aalberse RC, van Ree R: Lipid transfer protein: A pan-allergen in plant-derived foods that is highly resistant to pepsin digestion. *Int Arch Allergy Immunol* 2000;122:20–32.
- 65 Martínez A, Asturias JA, Palacios R, Sanz ML, Sánchez G, Oehling A, Martínez J: Identification of a 36-kDa olive-pollen allergen by in vitro and in vivo studies. *Allergy* 1999;54:584–592.

- 66 González EM, Villalba M, Rodríguez R: Allergic cross-reactivity of olive pollen. *Allergy* 2000;55:658–663.
- 67 Huecas S: Molecular biology and immunologic characterization of two major allergens from olive pollen: Ole e 1 and Ole e 9; thesis, Universidad Complutense, Madrid, 2001.
- 68 Geller-Bernstein C, Arad G, Keynan N, Lahoz C, Cárdbaba B, Waisel Y: Hypersensitivity to pollen of *Olea europaea* in Israel. *Allergy* 1996; 51:356–359.
- 69 Florido JF, Delgado PG, de San Pedro BS, Quiralte J, de Saavedra JM, Peralta V, Valenzuela LR: High levels of *Olea europaea* pollen and relation with clinical findings. *Int Arch Allergy Immunol* 1999;119:133–137.
- 70 Boluda L, Sastre J, Casanovas M, Fernández-Caldas E: Determination of Ole e 1 by enzyme immunoassay and scanning densitometry. Validation by skin-prick testing. *J Immunol Methods* 1999;223:17–26.
- 71 van Ree R, Aalbers M, Kea O, Marco De La Calle FM, Sempere Ortells JM, Villalba M, Rodríguez R, Aalberse RC: A sensitive monoclonal antibody sandwich ELISA for the measurement of the major olive pollen allergen Ole e 1. *Int Arch Allergy Immunol* 2000;122:224–228.
- 72 Baldo BA, Panzani RC, Bass D, Zerboni R: Olive (*Olea europea*) and privet (*Ligustrum vulgare*) pollen allergens. Identification and cross-reactivity with grass pollen proteins. *Mol Immunol* 1992;29:1209–1218.
- 73 Kernerman SM, McCullough J, Green J, Ownby DR: Evidence of cross-reactivity between olive, ash, privet, and Russian olive tree pollen allergens. *Ann Allergy* 1992;69:493–496.
- 74 Liccardi G, Russo M, Saggese M, D'Amato M, D'Amato G: Evaluation of serum specific IgE and skin responsiveness to allergenic extracts of Oleaceae pollens (*Olea europaea*, *Fraxinus excelsior* and *Ligustrum vulgare*) in patients with respiratory allergy. *Allergol Immunopathol (Madr)* 1995;23:41–46.
- 75 Obispo TM, Melero JA, Carpizo JA, Carreira J, Lombardero M: The main allergen of *Olea europaea* (Ole e 1) is also present in other species of the oleaceae family. *Clin Exp Allergy* 1993;23:311–316.
- 76 González E, Villalba M, Rodríguez R: Immunological and molecular characterization of the major allergens from lilac and privet pollens overproduced in *Pichia pastoris*. *Clin Exp Allergy* 2001;31:313–321.
- 77 Calabozo B, Barber D, Polo F: Purification and characterization of the main allergen of *Plantago lanceolata* pollen, Pla l 1. *Clin Exp Allergy* 2001;31:322–330.
- 78 Elsayed S, Titlestad K, Apold J, Aas K: A synthetic hexadecapeptide derived from allergen M imposing allergenic and antigenic reactivity. *Scand J Immunol* 1980;12:171–175.
- 79 Seiberler S, Scheiner O, Kraft D, Lonsdale D, Valenta R: Characterization of a birch pollen allergen, *Bet v III*, representing a novel class of Ca²⁺ binding proteins: Specific expression in mature pollen and dependence of patients' IgE binding on protein-bound Ca²⁺. *EMBO J* 1994; 13:3481–3486.
- 80 Tinghino R, Barletta B, Palumbo S, Afferni C, Iacovacci P, Mari A, Di Felice G, Pini C: Molecular characterization of a cross-reactive *Juniperus oxycedrus* pollen allergen, Jun o 2: A novel calcium-binding allergen. *J Allergy Clin Immunol* 1998;101:772–777.
- 81 Huecas S, Villalba M, González E, Martínez-Ruiz A, Rodríguez R: Production and detailed characterization of biologically active olive pollen allergen Ole e 1 secreted by the yeast *Pichia pastoris*. *Eur J Biochem* 1999;261:539–546.
- 82 Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Gröndlund H: The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CDR and CRIT). *Clin Exp Allergy* 1999;29:896–904.

Copyright: S. Karger AG, Basel 2001. Reproduced with the permission of S. Karger AG, Basel. Further reproduction or distribution (electronic or otherwise) is prohibited without permission from the copyright holder.