Modelling Diseases: The Allergens of *Olea europaea* Pollen

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Abstract

This study analyzes the influence of the IgE response to certain olive pollen allergens in the modulation of the different clinical phenotypes of allergic disease and their relationship with the level of exposure to pollen and genetic factors.
Patients from high-exposure areas had a complex IgE antibody response to allergens of *Olea euroapea*, which included 3 or more allergens in 75% of cases. The majority allergens were Ole e 1, Ole e 2 (profilin), Ole e 7 (lipid transporting protein), Ole e 9 (glucanase), and Ole e 10.
The existence of the antigen HLA-DR2(15) led to a higher risk of sensitization to Ole e 10 and a greater trend towards the development of severe asthma, which increased in the presence of an anti-profilin IgE.
Thirty percent of patients suffering from pollinosis simultaneously presented allergy to vegetable foods. Anti-Ole e 7 IgE was significantly associated with fruit anaphylaxis and anti-profilin IgE was detected in 90% of patients with oral syndrome.
Finally, we analyzed the role of glucanase and Ole e 10 as causes of the pollen-latex-fruit syndrome.

Key words: Olive pollen allergens. Bronchial asthma. Food allergy.

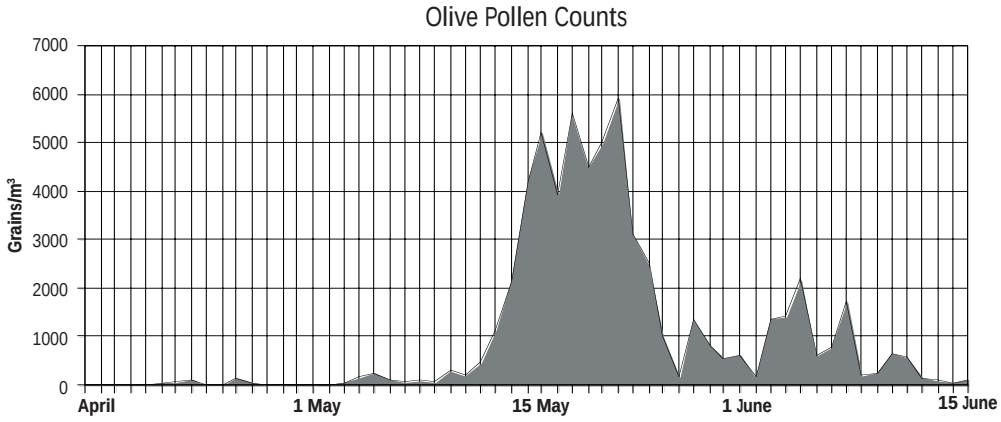
Resumen

En este trabajo, se analiza la influencia de la respuesta IgE frente a algunos alérgenos de polen de olivo en la modulación de los distintos fenotipos clínicos de la enfermedad alérgica, y su relación con el nivel de exposición a polen y los factores genéticos.
Los pacientes de zonas de alta exposición presentaron una respuesta compleja de anticuerpos IgE frente a alérgenos de *Olea euroapea*, que incluía a 3 ó mas de ellos en el 75% de los casos. Los alérgenos mayoritarios fueron Ole e 1, Ole e 2 (profilina), Ole e 7 (proteína transportadora de lípidos), Ole e 9 (glucanasa) y Ole e 10.
La existencia del antígeno HLA-DR2(15) determinaba un mayor riesgo de sensibilización a Ole e 10, así como una mayor tendencia al desarrollo de asma grave, que aumentaba si coexistía, además, con una IgE anti-profilina.
Un 30 % de pacientes polínicos, presentaron simultáneamente alergia a alimentos vegetales. La IgE anti-Ole e 7 se asoció significativamente con anafilaxia por frutas, y la IgE anti-profilina se detectó en un 90% de pacientes con síndrome oral.
Por último, hemos analizado el papel de la glucanasa y Ole e 10 como candidatos responsables del síndrome polen-latex-frutas.

Key words: Polen de olivo. Alérgenos. Alergia a alimentos.

Introduction

Olive pollen is one of the most important causes of respiratory allergy in the Mediterranean area [1]. Although several reports have described the high prevalence of *Olea europaea*-induced nasal and conjunctival symptoms [2], this pollen may also induce exacerbation of asthma between late April and early June [3]. However, the threshold of *O europaea* pollen required to elicit symptoms of seasonal allergic rhinitis is extremely high, around 400 grains/m^3 [4]. In Jaén (Southern Spain), the atmospheric pollen concentration varies between 500 and 1000 grains/m^3 during at least half of the pollination season, with peaks of more than 5000 grains/m^{3}by mid-May (figure). At these levels, rhinitis and/or bronchial asthma are more severe [3,4].



Olive pollen counts for the period May to June 2000 in Jaén (southern Spain)

Table 1. The Olive Pollen Allergens.

	Molecular Mass	IgE Prevalence	Family
Ole e 1	18.5-20 kDa	55%-90%	Unknown
Ole e 2	14-16 kDa	24%	Profilin
Ole e 3	9.2 kDa	20%-30%	Polcalcin
Ole e 4	32 kDa	80%	Unknown
Ole e 5	16 kDa	35%	Cu/Zn superoxide dismutase
Ole e 6	5.8 kDa	10%-55%	Unknown
Ole e 7	10 kDa	47%	Lipid transfer protein
Ole e 8	18.8 kDa	5%	Ca^{2+}-binding protein
Ole e 9	46 kDa	65%	1,3-β-glucanase
Ole e 10	10 kDa	55%-69.2%	CBM43*

*Carbohydrate-binding module

Standard laboratory methods have shown that olive pollen extract contains at least 20 protein bands with allergenic activity [6]. Many allergens show sequence homology to proteins from different vegetable tissues.

In this article, we examine the recent advances in our understanding of these extensively reviewed allergens [7-17] in an attempt to answer 3 questions: i) How many kinds of allergens have been described? ii) How do environmental conditions and genetic restrictions regulate allergen-specific IgE responses? and iii) How do these specific responses affect clinical disease?

Olive Pollen Allergens

Over the last few years, intense efforts have been made to define the molecular content of *O europaea* pollen [6-17]. Knowing the precise allergenic composition would enable us to understand the mechanisms involved in the development of pollinosis.

The description of the *Olea europaea* allergen, Ole e 1, is extremely important because it has been found to contribute significantly to the total allergenicity of olive pollen extract and its concentration is closely related to the allergenic reactivity of the whole pollen extract [7,8,18]. This allergen is one of the major allergens of *O europaea* extracts with an IgE-binding frequency of almost 80% among patients with olive pollinosis. Ole e 1 has been expressed in the yeast *Pichia pastoris*, a heterologous expression system that is efficient for the production of well-folded cys-containing proteins. It displays a conformational structure and an IgE-binding capability in vitro [19] and using the skin prick test [20] that is identical to that of the natural counterpart.

Besides Ole e 1, a further 9 additional olive pollen allergens have been purified and characterized from *O europaea* pollen extract (Table 1). Several of these allergenic proteins, eg, Ole e 6 [13], fail to show any homology to known protein sequences and, therefore, the biochemical function of these gene products remains unknown. Many other allergens belong to well-known families of proteins, such as profilin (Ole e 2) [9], superoxide dismutase (Ole e 5) [11,12], calcium-binding proteins (Ole e 3 and Ole e 8) [10,15], lipid transfer proteins (Ole e 7) [14], and 1,3-β-glucanases (Ole e 9) [16]. Ole e 10 is a new major allergen (54% of IgE recognition) with a molecular mass of 10 789 Da and pI of 5.8 that shows homology with the C-terminal domain of Ole e 9, thus explaining the remarkable cross-reactivity detected between them [17].

Genetics, Environment, and IgE Prevalence

This array of allergenic proteins could substantially improve the diagnosis of sensitization to olive pollen [21] and would allow the identification of specific factors that influence clinical phenotypes in *O europaea*-related diseases [3] and their natural history.

However, olive pollen allergy is a complex disease involving environmental and genetic factors. The failure to show any model of inheritance in atopic disorders means that the most likely possibility is that several genes are interacting with a strong environmental component. A number of genes influencing atopic diseases have now been identified [22,23], and one of the most widely studied areas has been the correlation between specific IgE antibody responses to well-defined allergens and HLA class II antigens [23]. In this sense, we reported the relationship between HLA class II antigens and the IgE antibody response to Ole e 1 [24], Ole e 3 [25], and Ole e 2 [3] in 2 nonrelated populations and we found a strong association between HLA-DRB1*0701/02 and HLA-DQB1*0201 and their responses [24,25].

We also recently performed genomic DNA typing (HLA-DRB and DQB loci) in 156 unrelated patients with olive pollinosis who had specific IgE antibodies against 8 different *O europaea* pollen allergens [3,21] detected by ELISA and skin prick test (Table 2). Interesting results were found in Ole e 10-sensitized patients. The DR2 (15) antigen was associated with the IgE response against this allergen and appeared to be a risk factor for this specific reactivity [3]. These data are especially relevant if we consider that one of the asthma-associated phenotypes studied, anti-Ole e 10 IgE antibodies, was significantly associated with very severe bronchial asthma. Furthermore, HLA-DR2 antigen could have affected the specific Ole e 10 response [3]. These data suggest that the genetic control of allergen-specific IgE responses in olive allergy may play a role in clinical disease.

Moreover, there is evidence to suggest that certain environmental factors could have an effect on allergen-specific IgE prevalence. Preliminary studies have shown that the allergen profiles of olive-allergic patients from areas with a high pollen count (such as Jaén in Andalusia, southern Spain) are notably different from those in areas with a low count (eg, Madrid, central Spain) [26]. Therefore, we have observed that the highest allergenicity of Ole e 6 [13], Ole e 7 [14], Ole e 9 [16], and Ole e 10 [17] is reached only in areas of intense olive tree cultivation. Although exposure to high or low levels of pollen has been suggested as a possible risk factor in the IgE-response to certain olive pollen allergens [3], high olive pollen counts have been shown to be one of the main predictors of intensity of rhinitis and asthma symptoms [4] in susceptible individuals during the pollen season.

The olive pollen allergen IgE phenotype could result from the interaction between these genetic factors and environmental stimuli. Therefore, and despite this complexity, olive pollen allergy may be considered an excellent model for the analysis of the IgE response.

It is no surprise that some researchers have tried to study specific IgE responses in several well-defined populations

Table 2. Allergen-Specific IgE Reactivity Profile in 156 Patients With Olive Pollen Allergy

IgE Reactivity Pattern Profile	Allergens of *Olea europaea* Pollen	Number of Patients
One allergen	1	12
9.6%	2	3
Two allergens	1 +2	14
17.3%	1+9	5
	1+10	2
	6+7	2
	1+7	2
	2+3	1
	2+9	1
Three allergens	1+2+9	11
17.3%	1+2+3	5
	1+6+7	4
	1+2+7	2
	6+7+9	1
	1+9+10	1
	1+2+8	1
	1+2+10	1
	1+7+10	1
Four allergens	1+2+9+10	11
13.5%	1+6+7+10	3
	3+6+7+10	2
	1+2+3+9	1
	1+2+6+9	1
	2+6+7+10	1
	1+3+6+7	1
	1+2+6+7	1
Five allergens	1+2+3+9+10	3
9.6%	1+6+7+9+10	3
	1+3+6+7+9	2
	1+3+6+7+10	2
	1+2+3+6+7	1
	1+2+6+9+10	1
	1+2+6+7+9	1
	1+2+6+7+10	1
	1+2+7+9+10	1
Six or more allergens	1+2+3+6+7+9+10	20
32.6%	1+2+6+7+9+10	10
	1+2+3+6+7+10	9
	1+2+3+6+7+8+9+10	5
	1+2+3+6+7+8+10	2
	1+2+3+6+8+10	1
	1+2+3+6+9+10	1
	1+2+3+7+9+10	1
	1+2+3+6+7+9	1
	2+3+6+7+8+9+10	1

in order to better understand pollen disease and find new treatments and prevention strategies. However, the sera used for allergen purification and characterization were only selected on the basis of a radioallergosorbent test value in patients from different geographic areas [7-17] and therefore exposed to different levels of pollen. Furthermore, the patients were poorly defined in terms of their clinical characteristics, such as the type and severity of the allergy syndrome and/or association with other atopic conditions.

We studied the allergen-specific IgE reactivity profile of a population with *O europaea* pollinosis [3, 21] using molecular techniques (Table 2). The recruitment criteria in this population give cause for concern. First, the clinical phenotype was the primary inclusion criterion. By using a combination of regular symptom recording and peak expiratory flow measurements during the spring season, the course of asthma and/or rhinitis was monitored in all patients. Second, our patient-group lived in the same heavy-exposure area (Jaén), where more than 5000 grains/m^3 are recorded from late April to June [4].

Patients showed complex IgE responses with different frequencies among allergens (major vs minor allergens) and distinctive allergen-specific IgE reactivity patterns with 1 to 8 of the allergens tested. According to our results, the major allergens identified (with more than 50% frequency) were Ole e 1, Ole e 7, Ole e 9, and Ole e 10. Moreover, the IgE reactivity pattern involving more than 3 allergens was observed in almost 75% of patients, but 45 different IgE patterns were observed when 8 allergens were tested in a group of 156 patients. The IgE binding frequency and IgE reactivity patterns of the olive allergens are summarized in Table 2.

The IgE prevalence of most allergens tested in our patient group agrees with that of other studies, except for Ole e 2, the olive profilin. The prevalence of specific IgE for profilin in different populations ranged from 15% to 24% as detected after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the purified protein and immunoblotting [5]. In our study, we demonstrated an Ole e 2-specific IgE response in more than 50% of the patients. Therefore, it may be considered a major allergen. The reason why we found a higher value is not clear, but it could be related to the population studied, which was made up mainly of asthmatic patients heavily exposed to olive pollen. Once again, genetic and environmental factors could be involved in the different sensitivity individuals can show to a specific allergen. However, the impact of successive exposure to high or low pollen counts each year on susceptible patients in terms of their specific allergen IgE prevalence remains largely unknown. For this purpose, studies with an adequate clinical follow-up in well-defined patient populations, such as those with seasonal rhinitis and bronchial asthma, will be indispensable in understanding the impact of allergen biology on the clinical outcome of this pollinosis.

Disease and Olive Pollen Allergens

An important challenge for the near future will be the correlation between the increasing quantities of molecular data on allergens and clinical information. It is clear that olive pollen allergens can induce a number of specific IgE reactivity profiles, including a differential expression of allergen-specific IgE when it is evaluated in terms of major *vs* minor allergens or their specific olive allergen IgE reactivity patterns. There is evidence to indicate that these IgE reactivity profiles could play an important role in the clinical events of pollen-related diseases [3,27].

The physical-chemical and biochemical characteristics of the *O europaea* allergens are listed in Table 1. Several of these molecules occur as homologous allergens in *Oleaceae* and non-*Oleaceae* species and could account for cross-reactivity between different pollens. For example, it has been shown that anti-Ole e 1 IgE can act as an immunological marker for sensitivity against the *Oleaceae* family (*Olea*, *Fraxinus*) [28] and, by contrast, Ole e 2, Ole e 3, Ole e 7, and Ole e 9 belong to highly conserved protein families both in phylogenetically and nonphylogenetically related species [6]. These observations support the low incidence of patients monosensitized to *O europaea* extract when large-scale surveys have been performed. Moreover, inhibitor assays of IgE binding of olive allergic sera to different crude pollen extracts suggest that olive pollen should be a primary sensitization in patients who are exposed to large quantities to *O europaea* pollen. Therefore, the susceptible patient with *O europaea* pollinosis has seasonal rhinitis and/or bronchial asthma (both diseases are present in the same individual in more than 75% of cases) and above 90% of these patients had a positive skin prick test to non-*Oleaceae* pollens.

Ole e 10 and Olive Profilin: the Asthma Markers

We analyzed the involvement of each allergen in the pathogenesis of the different *O europaea*-related respiratory diseases [3]. This report analyzed the IgE-antibody response to 2 olive pollen allergens, Ole e 2 and Ole e 10, and showed a strong association with bronchial asthma.

The relationship between allergen-specific IgE responses and these clinical phenotypes showed a statistically significant association between Ole e 2 reactivity and asthma (P = .04, odds ratio [OR]: 2.2, confidence interval [CI]: 0.9-5.1) and between Ole e 10 reactivity and asthma (P = .007, OR: 2.8, CI: 1.3-6.1). This association was not detected with the remaining 4 olive pollen allergens tested (Ole e 1, Ole e 3, Ole e 6, Ole e 7) in the same population [3].

The analysis of IgE reactivity to Ole e 2 and Ole e 10 has also revealed how both allergens were associated with the asthma phenotype [3]. There was a statistically significant correlation between the existence of anti-Ole e 2 IgE and the asthma phenotype and between the highest levels of IgE antibodies and crude olive pollen extract. This correlation was also observed between Ole e 10 sensitization and asthma, and with high levels of total IgE, olive pollen extract, and Ole e 10 IgE antibodies. However, the combined analysis showed how the risk of developing asthma in patients who were sensitized to both allergens was higher (OR 4.3) than in patients only sensitized to 1 of them (OR 1.9 and 3.2 for Ole e 2 and Ole e 10 patients, respectively) [3]. In addition, patients who showed Ole e 10-specific IgE antibodies also

Table 3. Specific Allergen IgE Prevalence in 39 Patients With Olive Pollen–Fruit Syndrome.

	Fruit Allergy	
Olive Pollen Allergen	Oral Allergy Syndrome (%)	Anaphylaxis (%)
Ole e 1	89.7	100
Ole e 2	89.7	61.1
Ole e 3	53.8	44.4
Ole e 6	43.6	72.2
Ole e 7	35.5	77.8
Ole e 8	7.7	4.2
Ole e 10	51.2	55.6

had more severe bronchial asthma, because the number of asthma days was significantly higher than that observed in non-Ole e 10-sensitized patients (13.4 ± 8.7 days vs 9.2 ± 9.1 days, Mann Whitney U Test, $P < .05$). These data suggest that the existence of anti-Ole e 10 IgE in patients with *O europaea* bronchial asthma would identify patients at risk of more severe disease.

Ole e 7 and Plant-Derived Food Anaphylaxis

Sensitization to food allergens may occur as a consequence of an allergic sensitization to inhalant allergens (class 2 food allergens), most of which are highly cross-reactive plant-derived proteins. Interestingly, several differences have been reported concerning the frequent association of pollen allergy with allergic reactions to fruits. The clinical spectrum may range from patients with symptoms limited to the oropharyngeal mucosa, as in the oral allergy syndrome (OAS), to those suffering from generalized urticaria, angioedema, or systemic anaphylaxis [29].

We recently evaluated allergenic patterns in patients with olive pollinosis and fruit allergy [27]. In this study, we evaluated 134 *O europaea* pollen-allergic patients, 40 of whom were sensitive to plant-derived food on the basis of clinically relevant allergy demonstrated by an unequivocal history of anaphylaxis due to any fresh fruit or nut, or diagnosed with OAS by positive double blind placebo-controlled oral challenges (DBPCOC) with fruits [27].

The main fruits involved in DBPCOC were peach, pear, melon, kiwi, and nuts. The IgE prevalence of olive pollen allergens was different when OAS or anaphylaxis patients were evaluated (Table 3). Firstly, Ole e 7 was clearly associated with more severe clinical symptoms in patients who had had anaphylactic reactions. Secondly, anti-Ole e 2 IgE antibodies were detected in 90% of OAS patients, whereas patients with fruit anaphylaxis showed a lower frequency than that observed in the control group of pollinic patients without food allergy [27].

The identification of the patient's individual IgE reactivity profile in the *Olea* pollen–fruit syndrome in a large population of olive allergic patients can improve the diagnosis and treatment of food allergy. A vaccine based on the specific IgE response to *O europaea* allergens would probably improve the clinical efficacy of treatment and influence the natural history of the fruit allergy. However, this effect remains to be determined in future clinical trials.

Ole e 9 as a Candidate Involved in the Pollen–Latex–Fruit Syndrome

Many researchers have noted clinical associations between allergy to latex and to several fruits and vegetables, most commonly kiwi, banana, chestnut, and avocado (also known as the latex–fruit syndrome) [30]. Most patients with latex allergy are highly atopic, with personal histories of seasonal rhinitis and asthma caused by pollen and other aeroallergens.

The major allergen of olive pollen Ole e 9 is a 1,3-β-glucanase belonging to the PR-2 protein family [16]. The conformational structure of Ole e 9 is composed of two domains: the N-terminal domain (36-kDa molecular mass, approximately 330 amino acid residues) and the C-terminal domain (10.6-kDa molecular mass, approximately 100 amino acid residues) connected by a segment of 10 to 15 amino acids in length. The N-terminal domain is common to other 1,3-β-glucanases [31].

Purified Ole e 9 is not available for specific immunotherapy and diagnosis protocols because it is obtained at very low yields from olive pollen. Moreover, the recombinant production of Ole e 9 has not been successful to date. An alternative approach to make the allergen available involves the production of the 2 N- and C-terminal domains by recombinant technology in the yeast *Pichia pastoris*, which has been reported to yield high levels of these molecules with the proper conformation while retaining the ability to bind IgE from olive pollen-allergic patients [32].

The ubiquity of 1,3-β-glucanases in plant derived tissues and the existence of an allergenic counterpart, Hev b 2, in latex extract indicates that this protein family could be involved in IgE cross-reactivity between different vegetable sources, including latex, fruits, and pollen [30]. This is supported by our report that serological cross-reactivity found by inhibition assays of IgE-binding to the N-terminal domain made it possible to detect IgG- and IgE-reactive peptide epitopes common to Ole e 9 in extract from tomato, potato, banana, and latex. The clinical relevance of these cross-reactivity allergens remains to be determined in the

pollen–latex–fruit syndrome, although the N-terminal domain could be used in diagnostic protocols as a means of identifying pollen-allergic patients at risk of developing allergic symptoms to other vegetable sources.

We also recently evaluated the in vitro diagnostic use of recombinant N-terminal (rNtD) and C-terminal domains (rCtD) to identify 33 Ole e 9 allergic patients and classify them according to their sensitization profiles [31]. Ninety-four percent (31/33) of the patients were reactive to rNtD or rCtD. Seventy-nine percent (26/33) gave a positive response to rNtD, 67% (22/33) to rCtD, and 52% (17/33) to both. Interestingly, 27% (9/33) were reactive to rNtD alone and 15% (5/33) to rCtD alone, indicating that many Ole e 9 allergic patients (42%) can be specifically sensitized to the N- or the C-terminal domains of the allergen.

This molecular (or component-resolved) diagnostic approach has shown different IgE responses to several epitopes of a single olive allergen. There is evidence [31] that these IgE responses to each domain may be useful markers of disease in at least 2 subsets of patients with olive pollen allergy: i) individuals who are at risk of developing allergic symptoms to fruit, vegetables, and latex due to sensitization against rNtD, as this part of the molecule has been shown to be involved in latex–pollen–vegetable food cross-reactivity processes [10], and ii) patients who could develop asthma due to sensitization to rCtD because this domain shows homology (53% identity) and high cross-reactivity with Ole e 10, which has been associated with more severe bronchial asthma in olive-allergic patients [3].

The discovery of Ole e 10 has allowed us to define a novel family of plant proteins with high interspecies IgE cross-reactivity and which was also a candidate for the pollen–latex–fruit syndrome [17]. Thus, homology with the CtD of Ole e 9 can explain the level of cross-reactivity detected with this allergen. However, Ole e 10 also shared IgE B-cell epitopes with proteins present in saline pollen extracts from other *Oleaceae*, *Gramineae*, *Betulaceae*, *Chenopodiaceae*, *Cupressaceae*, and *Parietaria* species, and it also exhibited cross-reactivity with latex and several plant-derived foods, such as tomato, kiwi, potato, and peach.

Furthermore, natural rubber latex also contained allergens that show structural homology with those found in olive pollen: Hev b 8 (a profilin, like Ole e 2) and Hev b 10 (a manganese superoxide dismutase, like Ole e 5) [32], which could be involved as putative cross-reactive allergens in the pollen–latex–fruit syndrome

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