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The spectrum of olive pollen allergens. From structures to diagnosis and treatment

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ABSTRACT

Olive tree is one of the main allergy sources in Mediterranean countries. The identification of the allergenic repertoire from olive pollen has been essential for the development of rational strategies of standardization, diagnosis, and immunotherapy, all of them focused to increase the life quality of the patients. From its complex allergogram, twelve allergens – Ole e 1 to Ole e 12 – have been identified and characterized to date. Most of them have been cloned and produced as recombinant forms, whose availability have allowed analyzing their three-dimensional structures, mapping their T-cell and B-cell epitopes, and determining the precise allergenic profile of patients for a subsequent patient-tailored immunotherapy. Protein mutant, hypoallergenic derivatives, or recombinant fragments have been also useful experimental tools to analyze the immune recognition of allergens. To test these molecules before using them for clinic purposes, a mouse model of allergic sensitizations has been used. This model has been helpful for assaying different prophylactic approaches based on tolerance induction by intranasal administration of allergens or hypoallergens, used as free or integrated in different delivery systems, and their findings suggest a promising utilization as nasal vaccines. Exosomes – nanovesicles isolated from bronchoalveolar lavage fluid of tolerogenic mice – have shown immunomodulatory properties, being able to protect mice against sensitization to Ole e 1.

1. Introduction

Olive (*Olea europaea*) pollen is one of the most important causes of pollinosis in the Mediterranean basin, and reaches the first position in several Spanish and Italian provinces where the cultivation of the olive tree is very intensive [1–3]. The origin of this long-life tree seems to be Asia Minor, where the birthplace of the cultivated olive tree took place six millennia ago. From there it spread to Europe and North Africa, and in the sixteenth century to the American Continent, Mexico, California, Peru, Chile and Argentina. More recently the olive tree has continued to expand and today is farmed in South Africa, Australia, Japan and China. The olive tree, which presents around 3000 varieties, is source of wood, fruit and oil, the latter being much appreciated because of its healthy properties [4]. The olive pollen displays the suitable conditions to be allergenic: it is small (around 18 μm), spread by the wind (anemophilous), and their daily pollen counts reach in many areas values higher than 5000 grains/m^3, maintaining these levels for weeks [3–7]. Allergy to olive pollen is well documented in countries such as Spain or Italy where the pollination season - from middle of April to the end of June-lead to a high number of affected individuals and frequently severe symptoms. An interesting feature of the olive pollinosis is the high dependence on the gradient of pollen counts, a fact that strongly influences the sensitization of patients to different allergens: thus, the prevalence of several allergens, minor in certain populations living in low pollen-count regions, turn into major allergens in areas of raised pollen charge [8–13]. To make matters worse, these minor allergens are related to severity and/or persistence of symptoms. The olive tree belongs to the Oleaceae family which also includes ash (*Fraxinus* L.), privet (*Ligustrum* L.), lilac (*Syringa* L.), forsythia (*Forsythia* L.), and jasmine (*Jasminum* L.) genus, some of which were also reported as cause of allergy processes. Interestingly, ash pollen has been recently recognised as a significant allergy inducer in central Europe and clinicians are paying attention to the study of its allergens [14].

The protein extract of olive pollen presents a complex and varied allergogram: at least twenty IgE-reacting protein bands appear in Western blots immunostained with sera from olive allergic individuals [9,10,15–21], and patients can be sensitized to very different combinations of them. In Fig. 1A, a diversity of allergenic profiles obtained after SDS–PAGE and immunoblotting with sera from allergic patients are shown. Immunoblotting results agree

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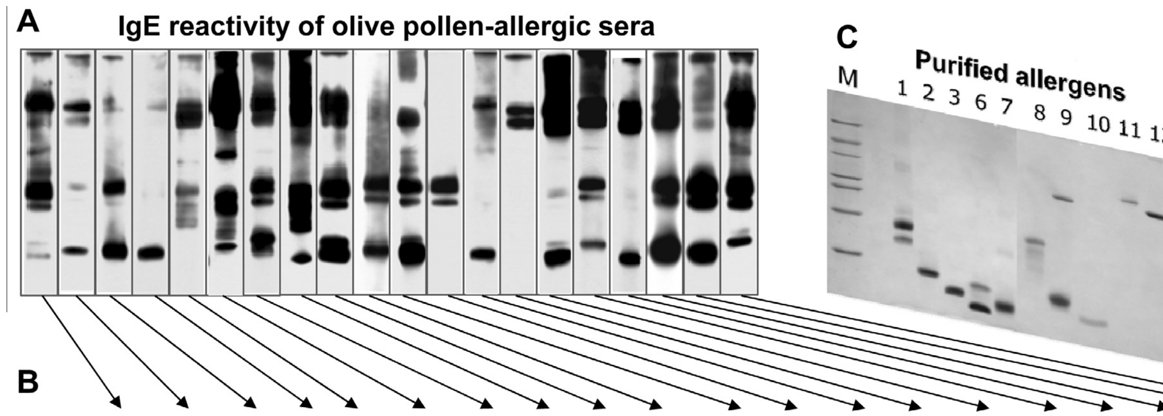

B

Ole e 1	0.75	1.76	0.39	0.11	0.15	1.23	1.40	0.89	2.50	1.42	2.50	0.83	0.00	0.29	1.68	0.61	0.32	2.50	2.50	2.50
Ole e 2	0.48	0.31	0.11	0.07	1.52	1.54	0.04	0.98	2.10	0.20	0.08	0.08	0.10	0.09	0.20	0.09	0.10	0.42	0.17	1.50
Ole e 3	0.00	0.00	0.00	0.04	0.08	0.11	0.00	2.50	2.50	0.00	0.02	0.02	0.02	0.00	0.00	0.00	0.00	0.03	0.05	0.00
Ole e 6	0.71	1.40	1.95	1.12	0.03	0.00	1.55	0.00	1.23	1.05	2.50	0.00	0.09	0.03	0.05	1.35	0.41	2.50	2.22	0.00
Ole e 7	0.93	2.22	2.50	1.88	0.00	0.01	2.50	0.01	1.85	1.88	1.76	0.05	1.16	0.00	0.11	2.18	1.54	2.50	2.50	0.00
Ole e 8	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ole e 9	1.51	0.54	0.18	0.05	0.24	2.29	2.32	0.11	0.66	0.11	1.62	0.11	0.03	0.06	1.95	0.11	0.12	0.11	0.38	2.50
Ole e 10	0.81	0.70	0.82	0.71	0.04	1.33	1.11	0.00	1.56	0.47	1.01	0.04	0.53	0.00	0.50	0.51	0.38	1.28	0.71	2.10
Ole e 11	0.11	0.02	0.02	0.00	0.28	0.00	0.03	0.04	0.30	0.03	0.00	0.02	0.22	0.03	0.32	0.00	0.04	0.02	0.16	0.21
Ole e 12	0.31	0.01	0.01	0.0	0.00	0.00	0.00	0.01	0.23	0.00	0.01	0.01	0.02	0.00	0.00	0.00	0.01	0.02	0.00	0.00

Fig. 1. Allergenic profile of olive pollen-allergic patients and purified allergens. Immunoblotting of sera from twenty olive pollen allergic patients shows very different IgE-binding profiles (A), which correlate well with ELISA values obtained with purified allergens (B) SDS–PAGE stained with Coomassie Blue of purified allergens from olive pollen (C). Ole e 1 shows the glycosylated (20 kDa) and non-glycosylated (18.5 kDa) forms. Ole e 6 shows the bands that correspond to different conformations. Ole e 9 corresponds to a mixture of N-terminal and C-terminal domains. ELISA values are given in optical density units; dark, light, and no shadow correspond to >0.5, 0.1–0.5, and <0.1 (negative), respectively.

with the data obtained from ELISA experiments (Fig. 1B) by using purified allergens [13,22,23]. At present, twelve allergenic proteins (Ole e 1 to Ole e 12) with diverse clinical incidence have been identified in olive pollen [11,24] (Fig. 1C), and the use of the powerful proteomic tools promises new future findings.

2. Identification and characterization of allergens

The knowledge of the allergenic profiles of a biological source is a prerequisite for any improvement in the design of protocols for a correct diagnosis and an adequate immunotherapy. The use of purified allergens, rather than the extracts currently available, increases the possibility of identifying the sensitizing antigen. The big efforts carried out in the past two decades to identify the most significant allergens of olive pollen have been essential to know which is the allergen or allergens responsible for the sensitization of each particular patient (Table 1).

2.1. Sensitivity to Ole e 1 indicates genuine sensitization to Oleaceae pollens

From pioneering studies on olive pollen allergy, a major IgE-binding protein was detected [15,16,18,25,26]. It was named Ole e 1 [27] and characterized as a glycosylated protein [28,29]. To date no biological function has been assigned to this allergen, but it seems to be involved, by homology to the cysteine-rich extracellular protein LAT52, to pollen hydration and/or germination processes [30]. Ole e 1 is an abundant protein of the pollen and probably this is the reason because it displays very high values of allergenic incidence: most of the population allergic to olive pollen presents around 80% of prevalence for Ole e 1. It is the best characterized allergen from olive, specifically expressed in pollen and therefore not present in olive fruit. The protein has a length of 145 amino acids (16,331 kDa) with a partially N-glycosylated Asn at position 111. Both protein and the oligosaccharide show a high degree of polymorphism and contribute to the allergenicity of the molecule [31–34]. The ultrastructural location of Ole e 1 has been determined [35,36] and suggests the involvement of the allergen in secretion routes that would be in agreement with a suggested biological role in fertilization processes. The association between the IgE response to Ole e 1 and the DR7-DQ2 haplotype [37], and the analysis of the T-cell and B-cell epitopes have been also reported [38–40].

Allergenic counterparts of Ole e 1 have been reported in other pollens, and they constitute the ubiquitous Ole e 1-like family. The proteins exhibit a broad degree of amino acid sequence identity, being high (>80%) among Oleaceae members (Fra e 1 from ash, Lig v 1 from privet, and Syr v 1 from lilac) [21,35,41–45] and low (25–60%) within not botanically related species such as ryegrass (*Lolium perenne*), Lambs' quarter (*Chenopodium album*), English plantain (*Plantago lanceolata*), rice (*Oriza sativa*), maize (*Zea mays*), tomato (*Lycopersicum esculentum*), birch (*Betula verrucosa*), rapeseed (*Brassica napus*), *Canary grass* (*Phalaris coerulescens*) or mouse-ear cress (*Arabidopsis thaliana*) [21]. The high similarity among Oleaceae members of the Ole e 1-like family explains their high cross-reactivity reported, although there are patients specifically reactive to a single member of the family [11]. The character of Ole e 1 as a marker of primary sensitization to Oleaceae pollens has been demonstrated [46]. Ole e 1 [29,47], Oleaceae-homologous proteins [39], as well as well as several mutants [48] have been recombinantly produced and used to study diverse immunological properties of the allergen (Fig. 2).

2.2. Panallergens: profilin and polcalcin

Several allergens are involved in vital functions and therefore are ubiquitously distributed in many biological sources. These panallergens are responsible for the polysensitization observed in many patients. Their conserved structures allow the allergen-specific IgE cross-react with homologues belonging to the family. Profilin has been isolated from olive pollen and identified as an allergen. It has named Ole e 2 [49]. Molecular and immunologic properties of Ole e 2 do not significantly differ from those of other profilins, exhibiting a prevalence of around 15% in patients allergic