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Dossier : Allergy

Emerging pollen allergens

Rosalía Rodríguez*, Mayte Villalba, Eva Batanero, Oscar Palomares, Guillermo Salamanca

*Depto. Bioquímica y Biología Molecular, Facultad de Ciencias Químicas, Universidad Complutense de Madrid,
Ciudad Universitaria s/n, 28040 Madrid, Spain*

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Abstract

Numerous pollen allergens have been reported over the last few years. Most of them belong to well-known families of proteins but some others constitute the first member of new allergenic families. Some of the factors that can contribute to the detection and identification of new pollen allergens are: a) advances in the technology tools for molecular analysis; and b) the deep knowledge of many allergenic sources. The combination of these factors has provided vast information on the olive pollen allergogram and the identification of minor allergens that become major ones for a significant population. The close taxonomical relationship between olive tree and ash -both Oleaceae- has permitted to identify Fra e 1 (the Ole e 1-like allergen) in ash pollen and to detect the presence of protein homologues of Ole e 3 and Ole e 6. In the other hand, extensive areas of south Europe are suffering an increasing desertification. As a consequence of this, new botanical species are spontaneously growing in these areas or being used in greening ground programs: *Chenopodium album* and *Salsola kali* are some examples recently recognized as allergenic woods. The identification of the complete panel of allergens from the hypersensitizing sources might help to develop more accurate diagnosis, and efficient and safer therapy tools for Type-I allergic diseases.

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1. Introduction

Over the last decade, lots of allergens have been characterized, and the number of available amino acid sequences and three-dimensional structures of allergenic proteins has been spectacularly increased. Recompilation of new allergens has been possible thanks to several database banks -many of them with access through web sites (e.g., allergen.com; allergy.net; allergom.com; Informall database, etc.)- that have been designed and created to facilitate the knowledge and understanding of this type of health-related molecules. However, new allergens are waiting to be detected and analysed, and the next few years will be crucial for the identification and study of the role of novel proteins related to IgE-mediate allergy disorders. The accurate diagnosis of hypersensitive patients and

the designing of the mixtures for a safer and effective immunotherapy definitely depend on the knowledge of the pattern of allergenic components from a biological source.

Two main factors can be related to the increasing discovery of new molecules involved in allergy:

- a) The emergence of new technologies that improve the analysis and detection of minor components in complex mixtures of allergenic extracts. Sensitivity, resolution and reproducibility are crucial factors that the new technologies frequently guarantee, and they provide a high degree of purification which is needed to assure the unequivocal identification of a new allergen. In this concern, the proteomic tools give very satisfactory results as deduced from the number of works including this technology over the last few years. In addition, the possibility to analyse the biological role of proteins, the manufacturing of helpful enzymes as tool for molecular biology methodology, the improvement of NMR and crystallization devices and protocols,

* Corresponding author. Tel.: +34 91 394 4260; fax: +34 91 394 4159.

E-mail address: rrg@bbm1.ucm.es (R. Rodríguez).

and the implementation of faster computer systems, have necessarily facilitated the research on molecular allergy.

- b) The advance in the knowledge of the components from related allergen sources -or even non-related sources- may provide the rules for the utilization of specific techniques and protocols that lead to an easier and faster detection or identification of the homologous or related allergens. Information on the properties of a protein allergen – solubility, stability, molecular weight, pI, etc.,- may in fact facilitate the discovery of the corresponding counterparts in different allergenic sources.

Finally, the changes in the environment or the diet habits of the hypersensitive patients can lead to new responses involving novel allergens. This factor is especially important when considering pollinosis as changes in the climatic environment of specific geographical areas may come up allergens that in the previous conditions were unnoticeable. One of the most evident climate changes is the increasing desertification which promotes the growth and propagation of adapted vegetables, both in a natural way or through greening programs.

We are going to analyse how these factors are being -and will be in the near future- responsible of the detection and identification of new allergens involved in several pollinosis.

2. Emergence of new technologies

Proteomic methodology has been successfully employed for many goals in the field of allergy research. It is especially valuable for the detection and identification of allergens coming from allergenic sources that display a complex allergenic mixture and whose monodimensional electrophoresis analysis gives a pattern of multiple bands. The difficulty of the study increases when the amount of the allergens in the extract is very different. This is the case of the allergenic olive pollen. Olive (*Olea europaea*) tree is an important source of allergy in Mediterranean countries, and it has been also documented in areas from America, South Africa, Japan and Australia. More than 20 IgE-reactive bands can be seen in sodium dodecyl sulphate-polyacrylamide gel electrophoresis of this extract by immunostaining with a pool of sera from patients reactive to olive pollen [1–3]. To date, ten allergens-Ole e 1 to Ole e 10- have been characterized in the saline extract, and the levels of these IgE-reactive components goes from 20% to less than 0.01% of the total protein. The main allergen of olive pollen is Ole e 1, a glycoprotein of 145 amino acids length that exhibits a major band at 20.0 kDa of apparent molecular mass and a minor non-glycosylated variant at 18.5 kDa [4–8]. More than 75% of patients allergic to olive pollen contain IgE reactive to this major allergen [4,9–11]. In spite of the large number of IgE-reactive bands in the olive pollen extract, the finding of the second allergen took eight years. This was probably for the reason that Ole e 1 constitutes up to 20% of total protein content of the saline extract, and other allergens appear at much lower concentration in the pollen (Ole e 2 concentration is lesser than 0.2%, and Ole e 9 less than 0.1%). Detection and identification of minor allergens is much more difficult and slow. Over the last ten years,

however, nine allergens (Ole e 2 to Ole e 10) have been identified and characterized from olive pollen. Profilin (Ole e 2) [12,13], two calcium-binding proteins (Ole e 3 -so called polcalcine- with two sites for binding calcium and Ole e 8 with four sites to bind the ion) [14–16], superoxide dismutase (Ole e 4) [17], LTP (Ole e 7) [18], 1,3- β -glucanase (Ole e 9) [19], as well as carbohydrate binding module (Ole e 10) [20] are biochemical roles attributed for olive pollen allergens. For three allergens -Ole e 1, Ole e 5 [17] and Ole e 6 [21]- no biochemical activities have been defined, at date. Several of the olive allergens belong to families of panallergens and show strong cross-reactivity with other members of the same family [14,15]; and Ole e 6, Ole e 7, Ole e 9 and Ole e 10 display a prevalence higher than 50% in allergic populations exposed to high levels of olive pollen in the air during the pollination season [11,18–22]. Most of the olive pollen allergens have been isolated and characterized by conventional methodologies: ion-exchange, size exclusion, reverse-phase high pressure liquid chromatographies, amino acid analysis, spectroscopic studies, molecular biology techniques, etc. However, and in spite of the number of olive pollen allergens characterized, several proteins remain to be analysed. The allergenic pattern shows the existence of several IgE-reactive bands around 40–50 kDa [2,3], but only Ole e 9 (46 kDa) has been described at this range of molecular masses. This indicates that several allergens remain to be identified. Neither ion-exchange nor size exclusion chromatographies allowed to isolate allergens of molecular mass higher than 40 kDa other than Ole e 9. Here and now, proteomic plays a major role.

The two-dimensional electrophoretic analysis -by combining isoelectrofocusing and sodium dodecyl sulphate-polyacrylamide gel electrophoresis- of the saline extract of the olive pollen has rendered a complex map of the protein components (Fig. 1A). The staining with AgNO₃ is able to detect less than 1 ng of protein and it almost guarantees the visualization of most of the spots corresponding to putative allergenic proteins. As above mentioned, the region of the map of the olive pollen extract comprising proteins of molecular mass around 40–50 kDa should contain new and unknown allergens. When this portion of the map is immunostained with a pool of sera from patients allergic to this pollen, the reactivity to several proteins is clearly detected (Fig. 1B). One of this allergens was identified as Ole e 9 (a polymorphic olive 1,3- β -glucanase with 45 kDa and pI around 5–6.5). However, the remaining spots might correspond to unidentified and significant allergens. The analysis of several IgE-reactive spots by matrix-assisted laser desorption ionization mass spectrometry led us to identify the pectin-methylesterase from olive pollen as a new family of allergenic components.

The possibility to analyze protein-ligand interactions by electrophoresis of the proteins under native conditions gave recently the opportunity to elucidate the biological activity of the olive allergen Ole e 10 [23]. Ole e 10 was demonstrated to bind laminarin, a 1,3- β -glucose polysaccharide. The allergen constituted the first member of a new family of carbohydrate binding modules which was named as CBM43 [23]. Now, we have used this methodology to analyse the activity of the C-terminal domain of Ole e 9. Ole e 9 is composed of two structurally defined

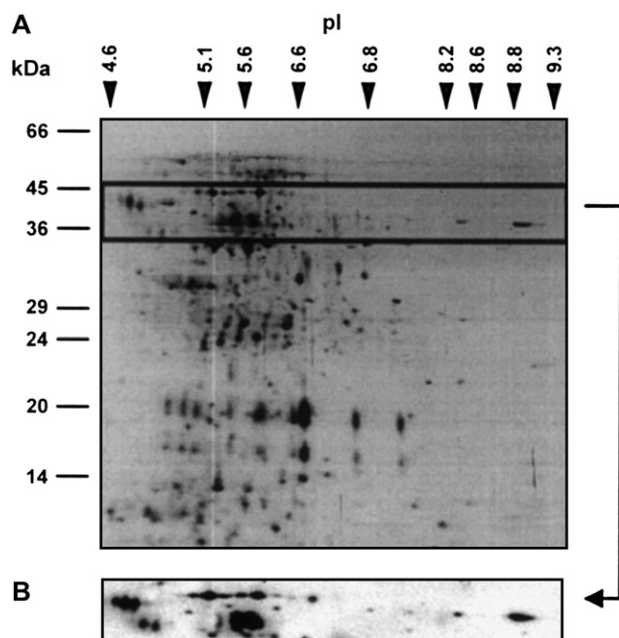


Fig. 1. Two-dimensional analysis of the olive pollen extract. A) NO_3Ag staining; and B) Immunostaining of the marked section with a pool of sera from patients allergic to olive pollen. pI and molecular mass (in kDa) of standard proteins are indicated.

domains: the N-terminal domain (36 kDa) which possesses the active site and, therefore, the catalytic activity to hydrolyze 1,3- β -glucans; and the C-terminal domain (10 kDa) which is homologous to Ole e 10 (53% identity, 69% similarity). Both the C-terminal and the N-terminal domains of Ole e 9 have been expressed by recombinant DNA technology in the yeast *Pichia pastoris*, and the molecules obtained were tested for the IgG and IgE reactivities [24,25]: the recombinant products displayed reactivity against specific polyclonal and monoclonal antibodies as well as against sera from patients allergic to Ole e 9. This recombinant C-terminal domain has been now assayed for its capacity to bind laminarin.

Protein-ligand interaction between the C-terminal domain of Ole e 9 and laminarin has been demonstrated by using affinity gel electrophoresis. This technology makes use of polyacrylamide gel electrophoresis in native conditions, e. g. in the absence of denaturing reagents such as sodium dodecyl sulphate, urea or β -mercaptoethanol [23]. The comparison between the electrophoretic mobility in the presence and absence of the ligand molecule -in this case the polysaccharide laminarin- should show the capacity of interaction of the protein (or polypeptide domain) with the putative ligand. Fig. 2 illustrates the result of the study for the C-terminal domain of Ole e 9 in comparison with the data obtained to Ole e 10 as positive control and bovine serum albumin as negative control. The mobility of the C-terminal domain was slower in the presence than in the absence of laminarin. This demonstrates that the C-terminal module of Ole e 9 is able to interact with 1,3- β -glucans. The homology of the amino acid sequence of this domain with that of the allergen Ole e 10 and the similar biochemical capacity to bind 1,3- β -glucans suggest that it

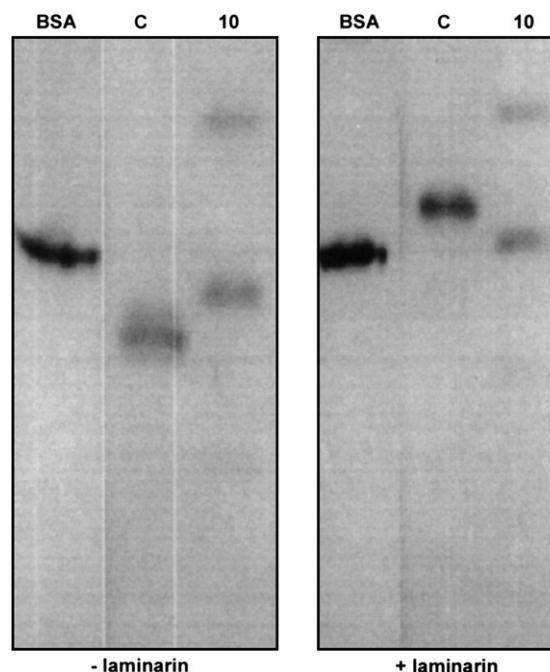


Fig. 2. Electrophoresis in native conditions of the C-terminal domain of Ole e 9 (lane C) in comparison with Ole e 10 (lane 10) and bovine serum albumin (lane BSA) in the absence of laminarin (– laminarin) and in the presence of the polysaccharide (+ laminarin).

also belongs to the CBM43 family. We can hypothesize that the role of the C-terminal domain of the whole 1,3- β -glucanase protein would be the capture of the substrate in order to be hydrolysed in the active site of the N-terminal domain.

3. Benefits of the advance on the knowledge of related allergens

Over the last decade the research on protein allergens has dramatically increased the number of well-known allergens. Many of them have been incorporated to the database banks of amino acid sequences and have been grouped in families. Families of proteins are composed by members with identical biochemical role but belonging to different sources. Sometimes, the families contain isoforms of the same protein expressed by multigen families. The members of a specific family of proteins share most of the structural and functional properties, although particular characteristics can be also displayed. The amino acid sequences of the members of a family can show different degrees of similarity, but conformational structure use to be highly conserved. The knowledge of a member of a family of protein allergens can give the clue to the identification of new members of the family. A good example can be given by the Ole e 1-like family: the major olive pollen allergen, Ole e 1, led to the research on the corresponding counterparts from lilac (*Syringa vulgaris*), privet (*Ligustrum vulgare*), and more important from ash (*Fraxinus excelsior*) [26–29]. In fact, these species also belong to the Oleaceae family and are close taxonomically related. Although the importance of olive pollen allergy is much more high than that of

the remaining Oleaceae pollens, the existence of cross-reactivity at the level of IgE, early described in the literature [30–33], made interesting the analysis of the putative allergens from these pollens. In addition, and although for a long time the importance of ash pollen as allergy elicitor has been overlooked, several recent reports have recognized its clinical importance in areas of Central Europe. Ole e 1 is a well-known allergen, which has been proposed as a marker of the sensitization to Oleaceae pollens [34]. It is a small protein with a limited degree of glycosylation and a putative role in fertility of the pollen. The olive pollen contains very high amount of the allergen, which is very soluble in saline solutions (it is easily extracted from the pollen grain). These properties can explain the high incidence of this allergen among the olive allergic patients. Interestingly, lilac, privet and ash tree contain homologous counterparts of Ole e 1 with highly conserved amino acid sequences. Although privet and lilac have not been reported as significant allergy sources, patients allergic to olive tree are at a risk of suffer symptoms when they are exposed to these pollens. It has been explained because they contain high amounts of the corresponding Ole e 1-like counterpart. The high similarity of the chemicophysical properties of these proteins from lilac and privet with those of Ole e 1 facilitated the isolation and study of Lig v 1 and Syr v 1, to which IgE-binding capacity was demonstrated [26,27]. These allergens have probably a low clinical significance, although allergy to privet is not uncommon in some regions where this Oleaceae is being used as ornamental plant. Fra e 1, the Ole e 1-like member from ash tree, has been recently reported as a significant allergen among the population from Central Europe; IgE antibodies from ash-sensitized patients bound to Fra e 1 with a prevalence of 75% [28,29]. From all these studies, it can be deduced that identity of amino acid sequence between the Ole e 1-like Oleaceae members is higher than 80% and they are the main components responsible for the strong cross-reactivity that these pollens show. This feature enhances the interest in the knowledge of their specific antigenicity as the utilization of one member of the family could facilitate the diagnosis and/or the immunotherapy of patients sensitized to any Oleaceae member.

Taking into account the similarity of the sodium dodecyl sulphate-polyacrylamide gel electrophoresis profiles of protein components of olive tree, ash, lilac and privet pollens [3,32] and the results on the isolation and characterization of olive pollen allergens, it can be deduced that their saline extracts contain some other allergens in addition to the members of the Ole e 1-like family. In order to detect the presence of proteins homologous to other olive allergens, we have performed enzyme linked immunosorbent assays of the extract obtained from ash pollen by using the specific antisera raised in rabbits against several olive pollen allergens as tools for immunostaining. The study was performed in comparison with the experiments for the corresponding olive pollen allergen as a positive control of the reactivity. The IgG binding results are shown as absorbance values (Fig. 3), and they indicate that all the allergens tested are present in ash pollen extract. Ole e 1 and Fra e 1 display a significant similarity in their responses to the Ole e 1-specific polyclonal

antibody (Fig. 3A), which is in agreement with the data of amino acid sequences of both proteins and their widely documented cross-reactivity. The polcalcins from olive and ash pollen show the highest degree of similarity in their responses to the specific antibodies (Fig. 3B), which can be explained by the high similarity expected for their amino acid sequences. The higher values of IgG titles obtained for ash polcalcin versus that of the olive pollen could be explained by the presence of

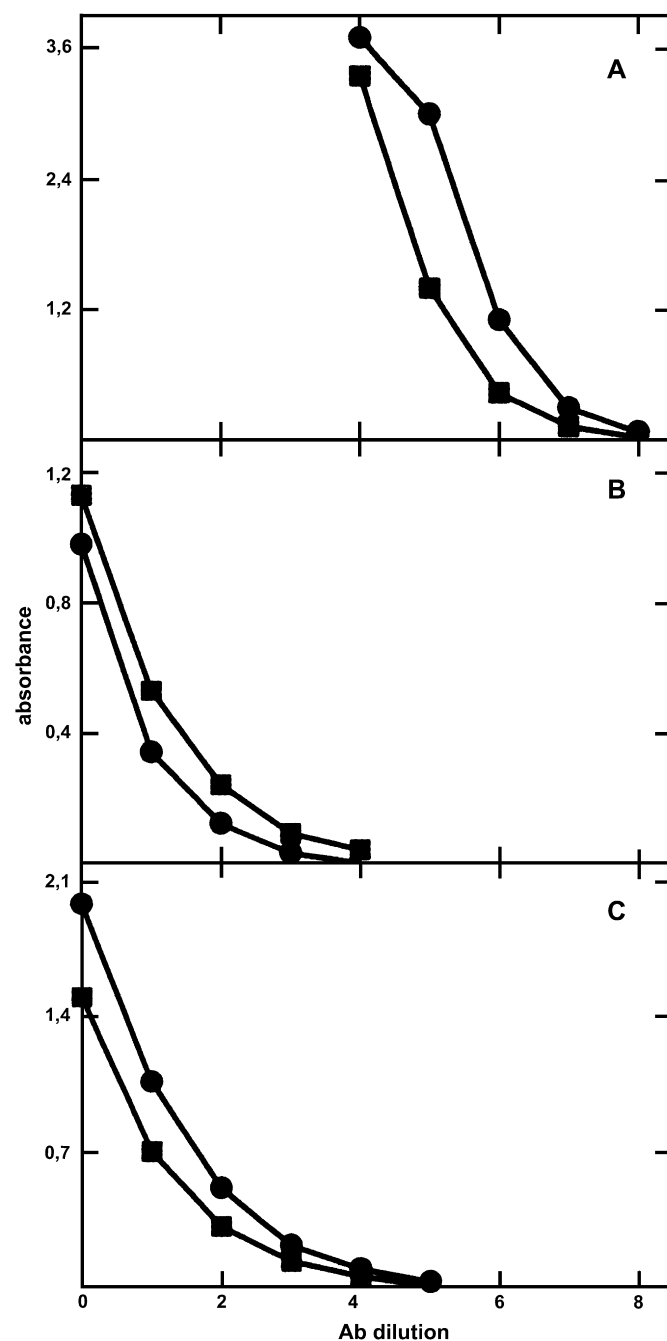


Fig. 3. Enzyme linked immunosorbent assays of ash pollen extract coated wells (■) incubated with specific antibodies obtained against olive pollen allergens: A) Ole e 1-specific antibody; B) Ole e 3-specific antibody; and C) Ole e 6-specific antibody. The corresponding olive pollen allergen (●) was also used for comparison. Serial dilutions ($1/100 \times 4^x$) of antibody were used. Absorbance at 492 nm was measured.

higher amounts of the panallergen in the former pollen extract. Concerning Ole e 6 counterparts, they also show great similarity in their IgG reactivities (Fig. 3C); it is probably due to the high identity of the sequences of their components. The three-dimensional structure of Ole e 6 has been recently reported and it shows two α -helices join together in a near parallel form linked by three disulphide bonds [35]. This should determine a strong restriction to the changes of amino acids along these segments of the polypeptide chain as they maintain a number of contacts between the side chains of the amino acid residues that constitute the helices. Therefore, it is expected the amino acid sequences of the homologous Ole e 6-like allergens to be highly conserved. For this reason, they could be detected and immunologically analyzed by means of Ole e 6-specific monoclonal and polyclonal antibodies. Based on the same reasoning, it is also expected a degree of IgE cross-reactivity of Ole e 6 with other Oleaceae counterparts what should be taken into account because of its putative clinical impact.

4. Arising of novel allergen sources

It is well-known that climatic conditions determine the developing of different plants, depending on the temperature and humidity of the environment, the extent of seasons, dryness and salinity of soils, etc. It is also clear that the desert areas are increasing all around the world. South Europe is in the scope of the environment analysts because of great regions of the Mediterranean countries are suffering enlarged summer seasons and shortened winters and autumns. Dry summers and mild winters promote the growth of specific vegetation that is able to survive in such aggressive climatic conditions. Some of these plants are members of the Chenopodiaceae and Amarantaceae families. Weeds such Lamb's quarter (*Chenopodium album*) or Russian thistle (*Salsola kali-pestifer*) easily develop in very dry and saline soils. These vegetable species are also cultivated in desert countries such as Saudi Arabia, Kuwait, and United Arab Emirates, as a part of the greening ground programs or to avoid erosion of drained zones. These weeds can shed large amounts of pollen and are also spreading big areas of United States and temperate regions of southern Europe. Chenopod has been reported to cause allergy in desert countries where it is well adapted [36–39]. A significant feature of chenopod sensitivity is its concomitant appearance with other pollinoses and probably explains the little attention that this allergy has received [40,41].

Three allergens have been recently isolated and characterized from chenopod: Che a 1, Che a 2 (profilin) and Che a 3 (polcalcin). Che a 1 belongs to the Ole e 1-like family (30% identity with Ole e 1), and shows very low reactivity against Ole e 1-specific antibodies [42,43]. Che a 2 and Che a 3 display high cross-reactivity with the corresponding profilin and polcalcin from olive pollen, as expected because of their conserved amino acid sequences [44–46]. These allergens have been isolated from pollen, and their IgE reactivity to sera from patients allergic to chenopod pollen was demonstrated [45]. Che a 2 and Che a 3 were also produced by recombinant technology in *Escherichia coli*, and Che a 1 in *Pichia pastoris*. Profilin and polcalcin from chenopod showed high prevalence values around 50%,

and the patients sensitive to these allergens possessed high IgE titres [45]. In a recent work, we showed the high cross-reactivity degree at the level of both polyclonal and monoclonal antibodies that exist among polcalcins from taxonomically related and non-related pollens such as those from olive, Timothy grass, lilac and chenopod, and indicated that diagnosis of polcalcin-sensitized patients could be performed whatever polcalcin used [47]. However, in the same work, some specific behaviour was also detected for any polcalcin, suggesting that for immunotherapy, the identification of the allergenic source responsible of the primary sensitization should be considered.

Allergy to *Salsola kali* pollen has been less explored, so far. *S. kali* also belong to the Chenopodiaceae family. It comes from Eurasia, but today is widely distributed all around the planet. *S. kali* is well adapted to saline-enriched and dry soils, and is able to support great variations in pH and aggressive climates. Its presence in southern Europe as allergenic inducer is in high rising probably due to the progressive desertification of wide areas. Extensive cross-reactivity among members of the Chenopodiaceae family has been demonstrated by different methodologies [41,48]. Allergens of 14 kDa molecular mass have been reported in chenopod and *Salsola* [48]. Isolation and characterization of Che a 2 [45,46] would be in agreement with the data obtained by Lombardero et al. (1985), but the allergenic component of *Salsola* remained to be identified. Immunoblotting of *S. kali* pollen extract with Ole e 2-specific polyclonal antiserum is shown in Fig. 4 in comparison with

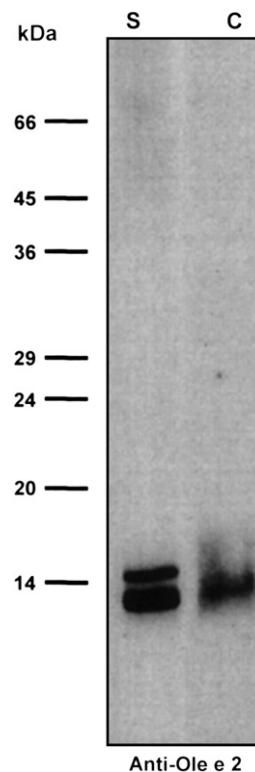


Fig. 4. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transference to membranes of *Salsola kali* (lane S) and *Chenopodium album* (lane C) pollen extracts. The immunostained was obtained with anti-Ole e 2 (olive profilin) specific antibody. Molecular mass markers are indicated in kDa.

the result obtained for chenopod extract. Two protein bands of around 14 kDa are detected in *S. kali*, whereas chenopod extract shows only a reactive protein band. This result indicates that *S. kali* pollen contains a cross-reactive profilin that could be involved in allergenicity. Clarification of the role of profilin in sensitization to *S. kali* pollen could help to allergy diagnosis of patients sensitive to Chenopodiaceae.

5. Conclusions

The improvement of the technology tools -mainly at the level of sensitivity and resolution- that can be currently applied to the study of new allergy inducers as well as the recent advances obtained on the knowledge of many allergen sources may help to complete, in a near future, the panels of allergens needed for an accurate diagnosis and a more efficient and safer immunotherapy of allergy diseases.

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References

- [1] Blanca M, Boulton P, Brostoff J, González-Reguera I. Studies of the allergens of *Olea europea* pollen. Clin Allergy 1983;13:473–8.
- [2] Rodríguez R, Villalba M, Monsalve RI, Batanero E. The spectrum of olive pollen allergens. Int Arch Allergy Immunol 2001;125:185–95.
- [3] Rodríguez R, Villalba M, Batanero E, González EM, Monsalve RI, Huecas S, et al. Allergenic diversity of the olive pollen. Allergy 2002; 57:6–16.
- [4] Villalba M, López-Otín C, Martín-Orozco E, Monsalve RI, Palomino P, Lahoz C, et al. Isolation of three allergenic fractions of the major allergen from *Olea europea* pollen and N-terminal amino acid sequence. Biochem Biophys Res Commun 1990;172:523–8.
- [5] Villalba M, Batanero E, López-Otín C, Sánchez LM, Monsalve RI, González de la Peña MA, et al. The amino acid sequence of Ole e I, the major allergen from olive tree (*Olea europaea*) pollen. Eur J Biochem 1993;216:863–9.
- [6] Villalba M, Batanero E, Monsalve RI, González de la Peña MA, Lahoz C, Rodríguez R. Cloning and expression of Ole e I, the major allergen from olive tree pollen. Polymorphism analysis and tissue specificity. J Biol Chem 1994;269:15217–22.
- [7] 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;31:31–7.
- [8] Lombardero M, Barbas JA, Moscoso del Prado J, Carreira J. cDNA sequence analysis of the main olive allergen, Ole e I. Clin Exp Allergy 1994;24:765–70.
- [9] Lauzurica P, Maruri N, Galocha B, González J, Díaz R, Palomino P, et al. Olive (*Olea europea*) pollen allergens—II. Isolation and characterization of two major antigens. Mol Immunol 1988;25:337–44.
- [10] Wheeler AW. Hypersensitivity to the allergens of the pollen from the olive tree (*Olea europaea*). Clin Exp Allergy 1992;22:1052–7.
- [11] Quirarte J, Florido F, Arias de Saavedra JM, Gómez E, Sáenz de San Pedro B, González E, et al. Olive allergen-specific IgE responses in patients with *Olea europaea* pollinosis. Allergy 2002;57:47–52.
- [12] Ledesma A, Rodríguez R, Villalba M. Olive-pollen profilin. Molecular and immunologic properties. Allergy 1998;53:520–6.
- [13] 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;100:365–72.
- [14] 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;241:772–8.
- [15] 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;258:454–9.
- [16] 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;466:192–6.
- [17] Boluda L, Alonso C, Fernández-Caldas E. Purification, characterization, and partial sequencing of two new allergens of *Olea europaea*. J Allergy Clin Immunol 1998;101:210–6.
- [18] 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.
- [19] Huecas S, Villalba M, Rodríguez R. Ole e 9, a major olive pollen allergen, is a 1, 3- β -glucanase. Isolation, characterization, amino acid sequence, and tissue specificity. J Biol Chem 2001;276:27959–66.
- [20] Barral P, Batanero E, Palomares O, Quirarte J, Villalba M, Rodríguez R. A major allergen from pollen defines a novel family of plant proteins and shows intra- and inter-species cross-reactivity. J Immunol 2004;172: 3644–51.
- [21] 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;410:293–6.
- [22] Palomares O, Villalba M, Quirarte J, Rodríguez R. Allergenic contribution of the IgE reactive domains of the 1, 3- β -glucanase Ole e 9: diagnostic value in olive pollen allergy. Ann Allergy Asth Immunol 2006;97: 61–5.
- [23] Barral P, Suárez C, Batanero E, Alfonso C, Alché JD, Rodríguez-García MI, et al. An olive pollen protein with allergenic activity, Ole e 10, defines a novel family of carbohydrate-binding modules and is potentially implicated in pollen germination. Biochem J 2005;15: 77–84.
- [24] Palomares O, Villalba M, Rodríguez R. The C-terminal segment of the 1, 3- β -glucanase Ole e 9 from olive pollen is an independent domain with allergenic activity. Expression in *Pichia pastoris* and characterization. Biochem J 2003;369:593–601.
- [25] Palomares O, Villalba M, Quirarte J, Polo F, Rodríguez R. 1, 3- β -glucanases as candidates in latex-pollen-vegetables food cross-reactivity. Clin Exp Allergy 2005;35:345–51.
- [26] 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; 221:187–93.
- [27] 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: 1401–10.
- [28] Barderas R, Purohit A, Papanikolaou I, Rodríguez R, Pauli G, Villalba M. Cloning, expression, and clinical significance of the major allergen from ash pollen Fra e 1. J Allergy Clin Immunol 2005;115: 351–7.
- [29] Barderas R, Purohit A, Rodríguez R, Pauli G, Villalba M. Isolation of the main allergen Fra e 1 from ash (*Fraxinus excelsior*) pollen. Equivalence between the natural and the recombinant forms. Ann Allergy Asth Immunol 2006;96:557–63.
- [30] 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;69:493–6.
- [31] Obispo TM, Melero JA, Carpizo JA, Carreira J, Lombardero M. The main allergen of *Olea europaea* (Ole e I) is also present in other species of the Oleaceae family. Clin Exp Allergy 1993;23:311–6.
- [32] Martín-Orozco E, Cárdbaba B, del Pozo V, de Andrés B, Villalba M, Gallardo S, et al. Ole e I: epitope mapping, cross-reactivity with other

- oleaceae pollens and ultrastructural localization. *Int Arch Allergy Immunol* 1994;104:160–70.
- [33] 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* 1995;23:41–6.
- [34] Palomares O, Swoboda I, Villalba M, Belic N, Spitzauer S, Rodríguez R, et al. The major allergen of olive pollen Ole e 1 is a diagnostic marker for sensitization to Oleaceae. *Int Arch Allergy Immunol* 2006;141:110–8.
- [35] Treviño MA, García-Mayoral MF, Barral P, Villalba M, Santoro J, Rico M, et al. NMR solution structure of Ole e 6, a major allergen from olive tree pollen. *J Biol Chem* 2004;279:39035–41.
- [36] Ezeamuzie CI, al-Mousawi M, Dashti H, al-Bashir A, al-Hage M, al-Ali S. Prevalence of allergic sensitization to inhalant allergens among blood donors in Kuwait- a desert country. *Allergy* 1997;52:1194–200.
- [37] Suliaman FA, Holmes WF, Kwick S, Khouri F, Ratard R. Pattern of immediate type hypersensitivity reactions in the Eastern Province, Saudi Arabia. *Ann Allergy Asthma Immunol* 1997;78:415–8.
- [38] Lestringant GG, Bener A, Frossard PM, Abdulkhalik S, Bouix G. A clinical study of airborne allergens in the United Arab Emirates. *Allerg Immunol* 1999;31:263–7.
- [39] Dowaisan A, al-Ali S, Khan M, Hijazi Z, Thomson MS, Ezeamuzie CI. Sensitization to aeroallergens among patients with allergic rhinitis in a desert environment. *Ann Allergy Asthma Immunol* 2000;84:433–8.
- [40] Lombardero M, Duffort O, Carreira J. Allergenic significance of chenopod pollen. In: D'Amato G, Spieksma FTh M, Bonini S, editors. Allergenic pollen and pollinosis in Europe. Oxford: Blackwell Scientific Publications; 1991. p. 128–31.
- [41] Wurtzen PA, Nelson HS, Lowenstein H, Ipsen H. Characterization of Chenopodiales (*Amaranthus retroflexus*, *Chenopodium album*, *Kochia scoparia*, *Salsola pestifer*) pollen allergens. *Allergy* 1995;50:489–97.
- [42] Barderas R, Villalba M, Lombardero M, Rodríguez R. Identification and characterization of Che a 1 allergen from *Chenopodium album* pollen. *Int Arch Allergy Immunol* 2002;127:47–54.
- [43] Barderas R, Villalba M, Rodríguez R. Che a 1: recombinant expression, purification and correspondence to the natural form. *Int Arch Allergy Immunol* 2004;135:284–92.
- [44] Barderas R, Villalba M, Batanero E, Pascual CY, Rodríguez R. Role of profilin and polcalcin in chenopod pollen allergy. *J Allergy Clin Immunol* 2003;111:1132–3.
- [45] Barderas R, Villalba M, Pascual CY, Batanero E, Rodríguez R. Profilin (Che a 2) and polcalcin (Che a 3) are relevant allergens of *Chenopodium album* pollen. Isolation, amino acid sequences and immunological properties. *J Allergy Clin Immunol* 2004;113:1192–8.
- [46] Barderas R, Villalba M, Rodríguez R. Recombinant expression, purification and cross-reactivity of chenopod profilin: rChe a 2 as a good marker for profilin sensitization. *Biol Chem* 2004;385:731–7.
- [47] Ledesma A, Barderas R, Westrischnig K, Quiralte J, Pascual C, Valenta R, et al. A comparative analysis of the cross-reactivity in the polcalcin family including Syr v 3, a new member from lilac pollen. *Allergy* 2006;61:477–84.
- [48] Lombardero M, Duffort O, Sellés JG, Hernández J, Carreira J. Cross-reactivity among Chenopodiaceae and Amaranthaceae. *Ann Allergy* 1985;54:430–6.