

The main allergen of *Olea europaea* (*Ole e* I) is also present in other species of the oleaceae family

T. M. OBISPO, J. A. MELERO*, J. A. CARPIZO, J. CARREIRA and M. LOMBARDEO

Research and Analytical Departments, Alergia e Inmunología Abelló S.A. and *Instituto de Salud Carlos III, Centro Nacional de Microbiología, Virología e Inmunología Clínica, Madrid, Spain

Summary

Three major pollen allergens from *Fraxinus excelsior*, *Ligustrum vulgare* and *Syringa vulgaris* belonging to the Oleaceae family were purified. Monoclonal antibodies previously raised against the main allergen of *Olea europaea* (*Ole e* I) were used for their purification by affinity chromatography. The three new purified allergens were able to bind human IgE from serum of olive-allergic patients in a way analogous to *Ole e* I. Crossed radioimmuno-electrophoresis of the four allergens, using anti-olive extract rabbit serum, showed a unique immunoprecipitation arc with the same characteristics. The four purified proteins had similar molecular weights on SDS-PAGE and the N-terminal sequences for the first 20 amino acids were identical. Furthermore, the concentration of the allergens could be determined using a two-site solid phase assay previously developed for the allergen *Ole e* I. Our results indicate that the four purified proteins share, to a great extent, antigenic and allergenic epitopes leading to cross-reactivities which could cause common clinical manifestations. We propose for the newly purified allergens the nomenclature of *Fra e* I, *Lig v* I and *Syr v* I.

Clinical and Experimental Allergy, Vol. 23, pp. 311–316. Submitted 12 August 1992; revised 30 October 1992; accepted 16 November 1992.

Introduction

The pollen of *Olea europaea* is an important cause of hay fever and asthma in the Mediterranean area. In contrast, in Northern and Central Europe, where olive trees are uncommon, the incidence of hypersensitivity to olive pollen seems to be very low. However, in these areas other members of the Oleaceae family, for instance *Fraxinus excelsior* (ash), *Ligustrum vulgare* (privet) and *Syringa vulgaris* (lilac) do exist, which can produce allergic symptoms [1]. Bousquet *et al.* [2] have described a high degree of allergenic crossreactivity among four species of Oleaceae pollens, but the molecular basis for this cross-reactivity is not yet known.

Studies by Blanca *et al.* [3] and Lauzurica *et al.* [4] have described the existence of a major olive allergen which, on SDS-PAGE, shows a main band at a molecular weight of 19 kD and a minor band at 17 kD. The N-terminal sequence of this allergen, which was called *Ole e* I, has been determined by Villalba *et al.* [5].

More recently, Lombardero *et al.* [6] have studied the clinical relevance of the allergen *Ole e* I determining the effect of allergen depletion, by affinity chromatography, on skin reactivity and histamine release tests, and the results indicated that *Ole e* I is, by far, the most important olive allergen.

In the work presented here we have investigated the presence of '*Ole e* I-like' allergens in the other Oleaceae species. We have purified three proteins from the Oleaceae species *F. excelsior*, *L. vulgare* and *S. vulgaris* with monoclonal antibodies (MoAb) produced previously against *Ole e* I. A high degree of structural homology and antigenic and allergenic crossreactivity was observed among these proteins which explains, from the molecular point of view, the high crossreactivity observed between Oleaceae species.

Materials and methods

Allergenic extracts

Fraxinus excelsior and *O. europaea* (kindly provided by J. A. Jiménez, Madrid, Spain), *L. vulgare* and *S. vulgaris*

Correspondence: Dr T. M. Obispo, Research Department, Alergia e Inmunología Abelló S.A., Miguel Fleita 19, 28037 Madrid, Spain.

bodies we were able to purify, in each of the Oleaceae pollen extracts investigated, a protein which closely resembles, from the physicochemical point of view *Ole e I* allergen, as indicated by the following experiments: (i) they have the same molecular weight on SDS-PAGE (about 20 kD) and (ii) the N-terminal sequence for the first 20 amino acids, which corresponds to about 10% of the protein, was identical. This sequence was in agreement with that reported by Villalba *et al.* [5] for *Ole e I* except for the presence of tyrosine in position 12, instead of histidine.

The high structural homology between the purified Oleaceae proteins is reflected in the immunochemical resemblance of these proteins. Thus, two different epitopes defined by MoAbs OL-2 and OL-7 on *Ole e I* [6] are also present in *Fra e I*, *Lig v I* and *Syr v I*, as demonstrated by the purification procedure used and the behaviour of these proteins on the two-site MoAb-based assay. Furthermore, the four proteins gave a very similar precipitation arc on CIE against an antiserum obtained from rabbits immunized with *O. europaea* pollen extract.

A high degree of allergenic crossreactivity between these proteins has also been demonstrated. (i) The CIE precipitation arc obtained with *Fra e I*, *Lig v I* and *Syr v I* can be revealed with a human serum pool from olive-allergic patients. (ii) All patients in a sample of 15 with specific IgE to *Ole e I* also had specific IgE to *Fra e I*, *Lig v I* and *Syr v I* and there was a good correlation between the IgE titres obtained. All these patients lived in the Mediterranean area, where ash tree, privet and lilac are uncommon pollens. (iii) The RAST inhibition curves obtained with the four Oleaceae allergens were very similar, indicating a high homology between the IgE binding epitopes of all the allergens.

Taking all these results together, the presence in *Olea*, *Fraxinus*, *Ligustrum* and *Syringa*, and probably in all the Oleaceae family, of a common major allergen with very similar characteristics is demonstrated. Bousquet *et al.* [2] have described the existence of a high degree of cross-reactivity between *O. europaea*, *F. excelsior*, *L. vulgare* and *Phillyrea angustifolia* using techniques such as RAST inhibition, iso-electrofocusing and tandem CIE. However, at that time the allergenic composition of the Oleaceae pollens had not yet been characterized so that the molecular basis for that crossreactivity could not be identified. The results obtained here indicate that the presence in all these pollens of an '*Ole e I*-like' allergen is mainly responsible for the crossreactivity, although the existence of other crossreactivities at the level of minor allergens cannot be ruled out. A parallel situation has been found in the Betulaceae family in which the major allergens of alder (*Ahn g I*), birch (*Bet v I*), hazel (*Cor a I*) and hornbeam (*Car b I*) resemble one another from the

physicochemical point of a view to a high degree although they are not so alike immunochemically [16].

The existence of a common major allergen in the Oleaceae family can have clinical implications due to the fact that these plants have different geographical distributions. *Olea* is mainly present in the Mediterranean area but *Fraxinus* and *Ligustrum* are very common in Northern Europe and North America. In this sense, a patient allergic to olive pollen living in the Mediterranean area could, when travelling to North Europe, present allergic symptoms due to inhalation of pollen from other Oleaceae. On the other hand, although the olive pollen seems to be the most important from the allergenic point of view, other Oleaceae pollens can be responsible for allergic sensitizations. Thus, Kernerman *et al.* [17] have reported that 19 out of 103 Michigan residents had positive skin tests with Oleaceae pollens and Lelong *et al.* [18] have pointed out that in the north of France the pollen of *Fraxinus* can reach important atmospheric concentrations during the pollination season and that ash sensitization can be much more important than previously suspected.

To conclude, we have demonstrated the presence of a shared major allergen in the Oleaceae family which explains the previously detected crossreactivity among the pollens of this family and which can cause common clinical manifestations.

Acknowledgments

This work has been supported by Alergia Inmunología Abelló, S.A. We would like to thank J. A. Jiménez for his botanical assistance, Dr Carmen Audera for critical reading of the manuscript and I. Courinha for secretarial work.

References

- 1 D'Amato G, Mullins J, Nolard N, Spieksma FThM, Wachter R. City spore concentrations in the European Economic Community (EEC). VII. Oleaceae (*Fraxinus*, *Ligustrum*, *Olea*). Clin Allergy 1988; 18:541-7.
- 2 Bousquet J, Guérin B, Hewitt B, Lim S, Michel FB. Allergy in the Mediterranean area III: cross reactivity among Oleaceae pollens. Clin Allergy 1985; 15:439-48.
- 3 Blanca M, Boulton P, Brostoff J, González-Reguera I. Studies of allergens of *Olea europaea* pollen. Clin Allergy 1983; 13:473-8.
- 4 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; 25:337-44.
- 5 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. *Biophys Biochem Res Commun* 1990; 172:523-8.
- 6 Lombardero M, Quirce S, Duffort O, Barber D, Carpizo J, Chamarro MJ, Lezaun A, Carreira J. Monoclonal antibodies against *O. europaea* major allergen. Allergen activity of affinity-purified allergen and depleted extract and development of a radioimmunoassay for the quantitation of the allergen. *J Allergy Clin Immunol* 1992; 89:884-94.
 - 7 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-phenol reagent. *J Biol Chem* 1951; 193:265-75.
 - 8 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T₄. *Nature* 1972; 227:680-5.
 - 9 Weeke B, Lowenstein H. Allergen identified in crossed-radioimmuno-electrophoresis (CRIE). *Scand J Immunol* 1973; 2 (suppl 1):149-53.
 - 10 Sánchez-Madrid F, Morago G, Corbi AL, Carreira J. Monoclonal antibodies to three distinct epitopes on human IgE: their use for determination of allergen-specific IgE. *J Immunol Methods* 1984; 73:367-78.
 - 11 Vanderkerckhove J, Bauw G, Puype M, Van Damme J, Van Montagu M. Protein-blotting on polybrene-coated fiber sheets. A basis of acid hydrolysis and gas-phase sequencing of picomole quantities of protein previously separated on SDS-polyacrylamide gel. *Eur J Biochem* 1985; 152:9-19.
 - 12 Yman L, Ponterius G, Brandt T. RAST-based allergen assay methods. *Dev Biol Stand* 1987; 29:151-65.
 - 13 Ceska M, Eriksson R, Varga JM. Radio-immunosorbent assay for allergens. *J Allergy* 1972; 49:1-12.
 - 14 Jimeno L, Carreira J, Lombardero M. Obtention of monoclonal antibodies against human IgG₄ using two different immunization strategies: Development of a biotin-based ELISA for IgG₄ quantitation. *Int Arch Allergy Appl Immunol* 1990; 92:175-82.
 - 15 Marsh DG, Goodfriend L, King TP, Lowenstein H, Platts-Mills TAE. Allergen nomenclature. *Clin Allergy* 1988; 18:201-9.
 - 16 Ipsen H, Hansen OC. The NH₂-terminal amino acid sequence of the immunochemically partial identical major allergens of alder (*Alnus glutinosa*) *Aln g I*, birch (*Betula verrucosa*) *Bet v I*, hornbeam (*Carpinus alba*) *Car b I* and oak (*Quercus alba*) *Que a I* pollens. *Mol Immunol* 1991; 28:1279-88.
 - 17 Kernerman SM, McCullough J, Ownby DR. Evidence of cross-reactivity between olive, privet, and Russian olive tree pollens. *J Allergy Clin Immunol* 1991; 87:187.
 - 18 Lelong M, Catelain MC, Sulmont G. Existe-t-il, en France, une pollinose au frêne? *Rev Fr Allergol* 1992; 32:41-2.