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***Mercurialis annua*: Characterization of Main Allergens and Cross-Reactivity with Other Species**

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Abstract

A multicentric study was conducted to evaluate the frequency of *Mercurialis annua* pollen sensitization in several areas of Spain and to select a population sample to characterize the main allergenic components in *M. annua* pollen. Patients were recruited from six hospitals in Spain. Out of 420 patients sensitized to pollens, 195 (46.4%) showed positive skin tests to *M. annua*, thus evidencing the high level of sensitization to the pollen of this plant in Spain. Thirty-seven sera with RAST class values to *M. annua* ≥ 3 were selected for SDS-PAGE immunoblotting analysis. Two main allergenic components with molecular weights of 15.8 and 14.1 kD were detected in 59 and 51% of the sera, respectively, and they were identified as profilins. Isolation of the relevant allergens was made by affinity chromatography on a poly-L-proline-Sepharose column, followed by gel filtration and anion exchange chromatography in the micropreparative SMART® System. A significant but low antigenic cross-reactivity between *M. annua* and *Olea europaea*, *Fraxinus elatior*, *Ricinus communis*, *Salsola kali*, *Parietaria judaica* and *Artemisia vulgaris* was demonstrated by several in vitro techniques.

Key Words

Pollen allergy

Mercurialis annua

Pollen antigenic cross-reactivity

Relevant allergen

Profilin

Introduction

Mercurialis annua is a 40- to 60-cm-high weed of the Euphorbiaceae family, widespread through Europe, North America and northern Africa [1]. Its pollen is small (25 by 22 μm), tricolporated and reticulated [2]. It is wind pollinated, and its flowering period is long lasting and varies widely; in our area it extends through winter and spring [3].

Sensitization to *M. annua* pollen (positive skin tests, specific IgE and provocation tests) has recently been reported in patients from the Mediterranean areas of Spain and Italy [4, 5]. Some of these patients had clinical rhinoconjunctivitis and/or asthma related to the *M. annua* pollinating season and some were monosensitized [4, 5]. On the other hand,

cross-reactivity between *M. annua* pollen and pollen from *Olea europaea* and grasses has also been reported [5]. Two main groups of allergenic proteins, with isoelectric point of 10.2 and 5.0–5.5, were previously identified by immunoblotting, using sera from 13 patients sensitized to *M. annua* [4].

Taking into account the high incidence of sensitization to *M. annua* pollen found in our area, as already reported in a previous study [4], we attempted to conduct a multicentric trial to assess: (1) the prevalence of sensitization to *M. annua* pollen in other areas of Spain; (2) the characterization of major allergens of *M. annua*, based on a large population of patients, and (3) the degree of antigenic cross-reactivity with other pollens prevalent in our area.

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Patients and Methods

Patients

Patients were recruited from the outpatient allergy units of five general hospitals of Spain, located on the northern Mediterranean coast (Hospitalet de Llobregat and Tarragona), on the southern Mediterranean coast (Elche), on the Atlantic coast in southern Spain (Huelva) and inland (Segovia). Over 6 months, all new patients accepted for respiratory allergy study had a prick test performed with one single batch of *M. annua* pollen extract. *M.-annua*-specific IgE was determined in patients with positive skin tests. Only sera with radioallergosorbent test (RAST) class values ≥ 3 were selected for studies of cross-reactivity and characterization of major allergens.

Methods

Preparation of *M. annua* Pollen Extract. Pollen was collected from *M. annua* specimens in the Barcelona area during February 1993. Collected pollen was processed as previously described [4]. Pollen collection revealed 97% purity, and pollen extract yielded 2.1 g of allergenic material/100 g pollen. Protein content measured by Lowry's method [6] was 74% (w/w). Carbohydrate content measured by the anthrone [7] and orcinol methods [8] was 12 (w/w) and 14% (w/w), using glucose and xylose as standards, respectively. Aliquots of lyophilized extract of the same batch were used for all in vitro and in vivo tests.

Skin Tests. *M. annua* pollen extract was diluted in phenolated saline solution at concentrations of 0.1, 1.0 and 10.0 mg/ml according to García-Ortega et al. [4]. Standardized lancets (Phazet uncoated lancet, Pharmacia Diagnostics, Uppsala, Sweden) were used for prick test. Histamine dihydrochloride (10 mg/ml) and phenolated saline were used as positive and negative controls. Results were recorded after 15 min. The mean wheal area produced by each allergen concentration was plotted as a function of the allergen concentration in a log-system. The allergen concentration eliciting a wheal equal to the one produced by histamine (10 mg/ml) was defined as 1 histamine equivalent prick unit [9]. 1 unit was found to be equivalent to 0.788 mg/ml of allergen extract.

RAST. Solid-phase antigen was obtained by coupling the extract solution to the 6-mm-diameter CNBr-activated paper disks according to Ceska and Lundqvist [10]. RAST was performed according to Wide et al. [11] with Phadezym RAST reagents (Pharmacia Diagnostics) following the manufacturer's instructions. RAST was performed using the following solid-phase allergenic extracts: *M. annua*, *Lolium perenne*, *Artemisia vulgaris*, *Parietaria judaica*, *Salsola kali*, *Ricinus communis* and *Olea europaea*.

SDS-PAGE Immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out as described by Laemmli [12]. Polyacrylamide concentrations of 12.5 and 3% were used for separating and stacking gels, respectively. Proteins dissolved in 0.125 M HCl-Tris buffer (pH 6.8) were dissociated with 0.1% SDS and 5% (v/v) β -mercaptoethanol by treatment at 100°C for 5 min. Twenty micrograms of protein estimated by Bradford's method [13] were applied per well.

After electrophoresis, gels were stained by diffusion in 0.1% Coomassie brilliant blue R-250 dissolved in methanol/acetic acid/distilled water (4:1:5). Destaining was also performed by diffusion in the same mixture without dye. Separated protein bands were electrophoretically transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Milford, Mass., USA), essentially by the method described by Towbin et al. [14]. Immunochemical staining was performed by first blocking the Immobilon membrane with 9% (w/v) defatted milk

in 50 mM Tris-buffered saline (TBS) (1 h at 37°C). After three washes with TBS, the blots were incubated with 0.5 ml of undiluted human serum (16 h at 4°C). The blots were washed three times with TBS and incubated with 2.5 ml of 1:25 diluted rabbit immunoglobulins to human ϵ -chains (DAKO, Glostrup, Denmark) conjugated to peroxidase. Bound peroxidase was detected by the addition of 3 ml of freshly prepared 0.06% w/v of 4-chloro-1-naphthol solution (Bio-Rad Lab, Richmond, Calif., USA) and 0.01% (v/v) H_2O_2 in TBS. After 30 min, the blots were rinsed in distilled water and air-dried. Antigen-antibody binding was identified by purple-stain deposition [15]. Analysis of electrophoretic components was performed by image analysis in the Bio-Image System (Millipore).

SDS-PAGE immunoblotting using rabbit serum anti-*Helianthus annuus* profilin was performed according to the same procedure but using antiserum diluted at 1:3,000, 1:30,000 and 1:300,000 and an 1:1,000 diluted anti-rabbit IgG peroxidase conjugate.

Agarose Isoelectric Focusing. Isoelectric focusing was performed on Isogel agarose plates (FMC Bio Products, Rockland, Me., USA), over the pH range of 3–10, and 20 μ g of protein were applied per lane. The isoelectric point (pI) was estimated from the migration of a set of proteins with known pI. The resulting electrophoretic patterns were evaluated by image analysis in the Bio-Image System (Millipore).

Production of Experimental Antisera. Immunization was performed according to Gallart et al. [16] in white male adult New Zealand rabbits. Weekly injections of 10 mg of *M. annua* extract (freeze-dried material), with Freund's complete adjuvant, were given subcutaneously. After eight injections, the animals were bled, and the serum collected. Antiserum against purified profilin from sunflower (*Helianthus annuus*) pollen was obtained according to the same schedule but injecting 1 mg of protein per dose. This profilin used as reference had been previously isolated and identified by amino acid microsequencing of internal peptides (to be published elsewhere).

ELISA Inhibition. Enzyme-linked immunosorbent assay (ELISA) inhibition was done according to Martínez et al. [17]. The *M. annua* pollen extract was dissolved to an optimal solid phase concentration [18] (0.125 mg/ml) in 0.1 M carbonate-bicarbonate buffer, pH 9.6 and dispensed (100 μ l/well) in polystyrene microdilution plates (Maxisorp, NUNC, Roskilde, Denmark). After solid phase coupling, inhibitory extracts of *M. annua*, *L. perenne*, *P. judaica*, *S. kali*, *A. vulgaris*, *R. communis*, *O. europaea* and *Fraxinus excelsior* were dissolved in PBS-Tween 20-bovine serum albumin at different concentrations (0.6–20,000 μ g/ml) and dispensed (50 μ l/well); rabbit serum anti-*M. annua* pollen extract diluted 1/2,660 (50 μ l/well) was added. Plates were incubated for 3 h (room temperature, moist chamber) and supernatants removed and washed as before. Swine γ -globulin fraction anti-rabbit immunoglobulins, affinity purified and conjugated to horseradish peroxidase (DAKO), was then used, and peroxidase activity was measured with a substrate solution of 3-dimethyl aminobenzoic acid, 3-methyl-2-benzothiazolinone hydrazone [19]. Optical densities at 600 nm and concentrations of inhibitor antigen were correlated by linear regression. Inhibition percentages were calculated on and plotted against this concentration basis and the antigen concentrations needed for 50% inhibition (Ag_{50}) calculated.

Double Diffusion. Double diffusion was performed according to Ouchterlony and Nilsson [20] using a support of 1% (w/v) agarose in 0.05 M pH 8.6 barbital buffer. 50 mg/ml (freeze-dried extract) of allergen from each of the following species were used: *M. annua*, *L. perenne*, *P. pratense*, *Dactylis glomerata*, *A. vulgaris*, *P. judaica*, *S. kali*, *Plantago lanceolata*, *R. communis*, *H. annuus*, *O. europaea*, *F. excelsior*, *Ligustrum vulgaris* and undiluted *M. annua* rabbit immunosera.

Passive immunodiffusion was carried out for 48 h and washed once with 5% (w/v) trisodium citrate (1 h, room temperature) and 3 times with saline. After drying, agarose-protein staining with amido black was performed.

SDS-PAGE Immunoblotting Inhibition. Aliquots of the diluted 1:10,000 anti-*M. annua* rabbit antiserum (IgG immunoblotting inhibition) were incubated for 10 min with equal volumes of the different pollen extracts, at a final concentration of 1 mg protein/ml. Mixtures were then centrifuged and supernatants incubated with *M. annua*-blotted polyvinylidene difluoride strips, followed by immunodetection as described in the SDS-PAGE immunoblotting section, but using an anti-rabbit IgG peroxidase conjugate (DAKO) diluted 1:1,000. The amount of specific IgG bound to each strip was estimated in terms of optical density detected in the Bio-Image analyzer.

Chromatographic Purification of Profilin. The first step in profilin purification was accomplished by affinity purification in a poly-L-proline (PLP) CNBr-activated Sepharose column, as described by Lindberg et al. [21]. Briefly, 2 g of CNBr-activated Sepharose 4B (Pharmacia Biotech, Uppsala, Sweden) were swollen in 100 ml of 1 M HCl for 15 min. The resin was then washed in a filtering funnel (approximately 7 ml) and suspended in two volumes of the coupling buffer (0.1 M KHCO₃, pH 8.3, 0.5 M KCl); 30 mg of PLP (M_r 5,000, ICN Biochemicals, Aurora, Ohio, USA) were dissolved in 2 ml of distilled water for 12 h. Nonsoluble material was discarded and the supernatant was added to the resin suspended in coupling buffer. The mixture was left at room temperature for 2 h. After blocking of active groups and washing of ligand excess, the resulting PLP-Sepharose conjugated was mounted onto a 1×24 cm column and equilibrated with PBS. Chromatography was carried out at a flow of 10 ml/h; 500 mg of *M. annua* pollen extract were dissolved in 2.5 ml of PBS and applied to the column. 150 ml of the unretained fraction, containing most of the proteins, were collected. The adsorbed fraction (mainly profilin) was later eluted in 70 ml of 6 M urea in PBS.

Proteins co-eluted with profilin were removed by high-resolution chromatography in the SMART™ system (Pharmacia). A gel filtration column, Superdex 75 PC 3.2/30, was used. Samples were applied at a concentration of 20 mg/ml in 0.05 M phosphate buffer, 0.15 M NaCl pH 7.0 (PBS), and injected with a 20-μl loop. A flow rate of 20-μl/min was maintained during the whole chromatographic process. The column was calibrated with a set of proteins of known molecular weight. Chromatography was done at 8°C. An eluted peak from gel filtration corresponding to profilin was analyzed by chromatography in the SMART system using an anion exchange MonoQ PC 1.6/5, column. A linear gradient elution from 0 to 1.0 M NaCl was applied.

Results

Prevalence

During the first stage of the study (6 months), 420 patients sensitized to pollen were recruited from five different areas of Spain. Of these, 195 (46.4%) showed a positive prick test to *M. annua* pollen extract. The prevalence observed in the different areas studied varied widely; the lowest prevalence was detected in Hospital de Llobregat, an urban area on the northern Mediterranean coast, where 28% of patients sensitized to any pollen were also sensitized to

M. annua, and the highest in Elche, an urban-rural area on the southern Mediterranean coast, where the prevalence reached 54% in patients sensitized to any pollen. Only 3 patients in all were monosensitized to *M. annua* pollen. The 195 patients sensitized to *M. annua* included 98 males and 97 females, with ages ranging from 5 to 51 years, who suffered from rhinoconjunctivitis (139 patients), rhinoconjunctivitis and asthma (51 patients), asthma (3 patients), allergic cough (1 patient) and urticaria (1 patient). RAST class ≥3 was found in just 37 sera. Only these sera were used for the experimental stage of the study. RAST scores to the different pollens studied for the 37 sera are shown in figure 1.

Allergen Characterization and Purification

SDS-PAGE immunoblotting of 37 sera with RAST values ≥3 and its allergograms evaluated by image analysis (Bio-Image analyzer, Millipore) are outlined in figures 2a and b. Forty-two different IgE-binding components were detected. Two bands of 14.1 and 15.8 kD were revealed in 51 and 59% of the sera studied, respectively. Both bands were presumably profilins, since they were able to bind IgG from a rabbit polyclonal antiserum anti-*H. annuus* pollen profilin by SDS-PAGE immunoblotting. These profilins were firstly fractionated by affinity chromatography on PLP-CNBr-Sepharose 4B column. An eluted fraction from the PLP-CNBr-Sepharose column corresponding to proteins bound to PLP was then applied to the gel filtration column (SMART system), and the eluted fraction corresponding to a 17.5- to 19.7-kD interval with maximum at 15.8 kD, and containing relevant allergens, was further chromatographed by anion exchange chromatography.

The elution profile showed one sharp peak which eluted at 200 mM NaCl (peak No. 1 in fig. 3) containing the two relevant allergens. Analysis of the SDS-PAGE pattern corresponding to this peak (Bio-Image analyzer, Millipore) demonstrated two components with molecular weights of 14.1 and 15.8 kD. Both protein bands bound rabbit IgG anti-profilin from *H. annuus* as proved by SDS-PAGE immunoblotting (fig. 4). Isoelectric focusing of anion-exchange purified components revealed (figure not shown) three different bands with pIs of 4.5, 4.7 and 4.9, respectively.

Anion exchange purified proteins responsible for peaks 1 and 2 (fig. 3) were again chromatographed by gel filtration, in order to estimate the native molecular weights of this fraction; both gave rise to a single peak which corresponded to 15.5 kD (profile not shown).

SDS-PAGE immunoblotting of purified profilin proteins incubated with human sera from selected sensitized patients demonstrated two IgE-binding components with 14.6 and 15.8 kD (fig. 5), together with other bands at around 30.0, 43.0 and 60.0 kD.

PRU/ml

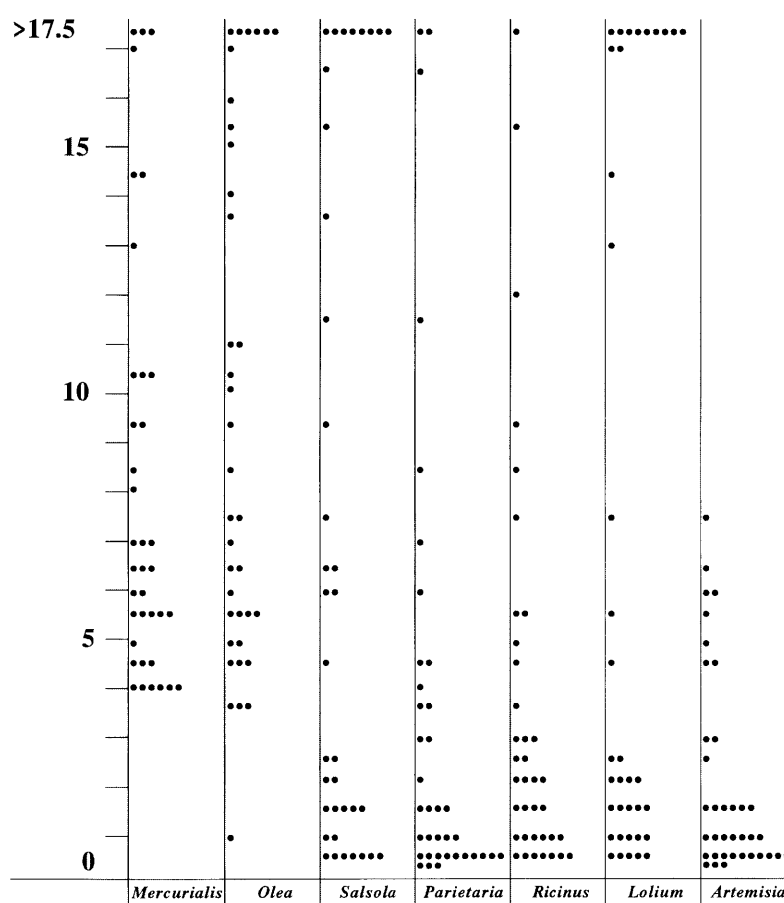


Fig. 1. RAST scores to different pollen allergens obtained with the selected 37 sera showing *M. annua* RAST values ≥ 3 . PRU = Phadezym RAST units.

Antigenic Cross-Reactivity

Antigenic cross-reactivity between *M. annua* and some other common regional pollen allergens was assayed by means of ELISA inhibition (fig. 6), using a rabbit antiserum anti-*M. annua* pollen extract. All the heterologous extracts layed within the same range of inhibitory potency, far from the degree of inhibition caused by the *M. annua* extract. The homologous extract (*M. annua*) gave rise to an Ag_{50} value of 0.006 mg/ml, while the heterologous extracts originated much higher Ag_{50} values: *F. excelsior* 20.4 mg/ml, *L. perenne* 31.7 mg/ml, *P. judaica* 37.1 mg/ml, *A. vulgaris* 44.6 mg/ml, *O. europaea* 100 mg/ml, *R. communis* 148 mg/ml and *S. kali* 416 mg/ml (the last three values were indeed extrapolated ones).

Double diffusion using anti-*M. annua* rabbit serum revealed five strong precipitating systems when homologous antigen was used. One strong precipitating system was ob-

served with *Ricinus*, *Fraxinus* and *Olea*, while a weak precipitating system was observed with *Salsola*, *Parietaria*, *Artemisia* and *Phleum*. No precipitating systems were observed with the remaining extracts.

In order to contrast the ELISA inhibition experiments, and also to detect the protein fractions responsible for the antigenic cross-reactivity, SDS-PAGE immunoblotting-inhibition experiments were carried out using the rabbit antiserum anti-*M. annua* (fig. 7).

In the obtained IgG immunoblotting pattern, the homologous (*M. annua*) extract completely abolished the IgG binding, while the other pollen inhibitors reached lower inhibition levels, ranging from the 80% caused by *P. judaica* extract to the 26% inhibition of *L. perenne*. Overall, the IgG binding inhibition affected mainly the 94.0- to 30.0-kD interval.

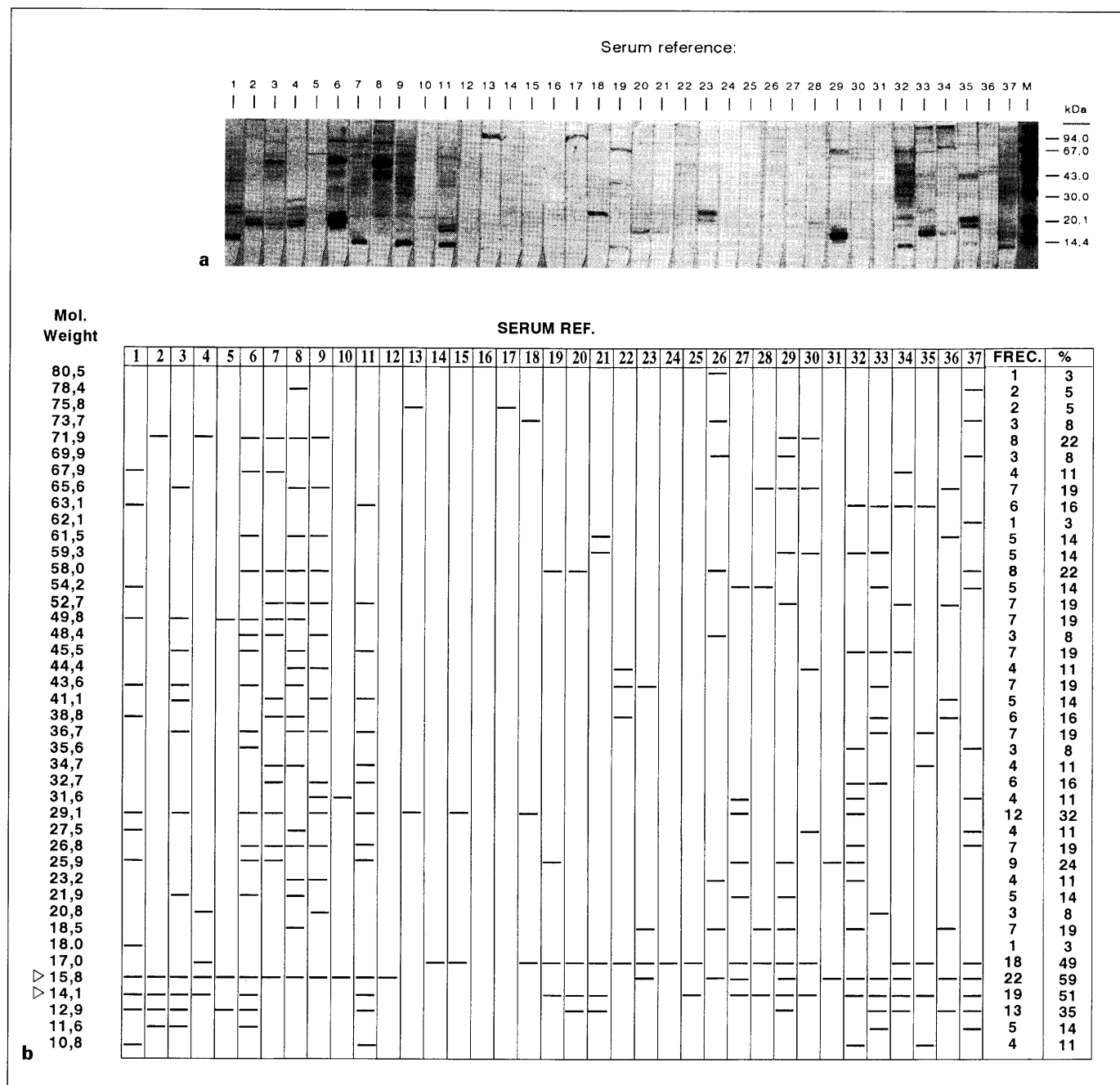


Fig. 2. a SDS-PAGE immunoblotting of *M. annua* pollen extract incubated with sera from 37 patients with RAST ≥ 3 . M = Molecular weight marker (kD). **b** Allergogram. Image analysis of SDS-PAGE immunoblotting by the Bio-Image system.

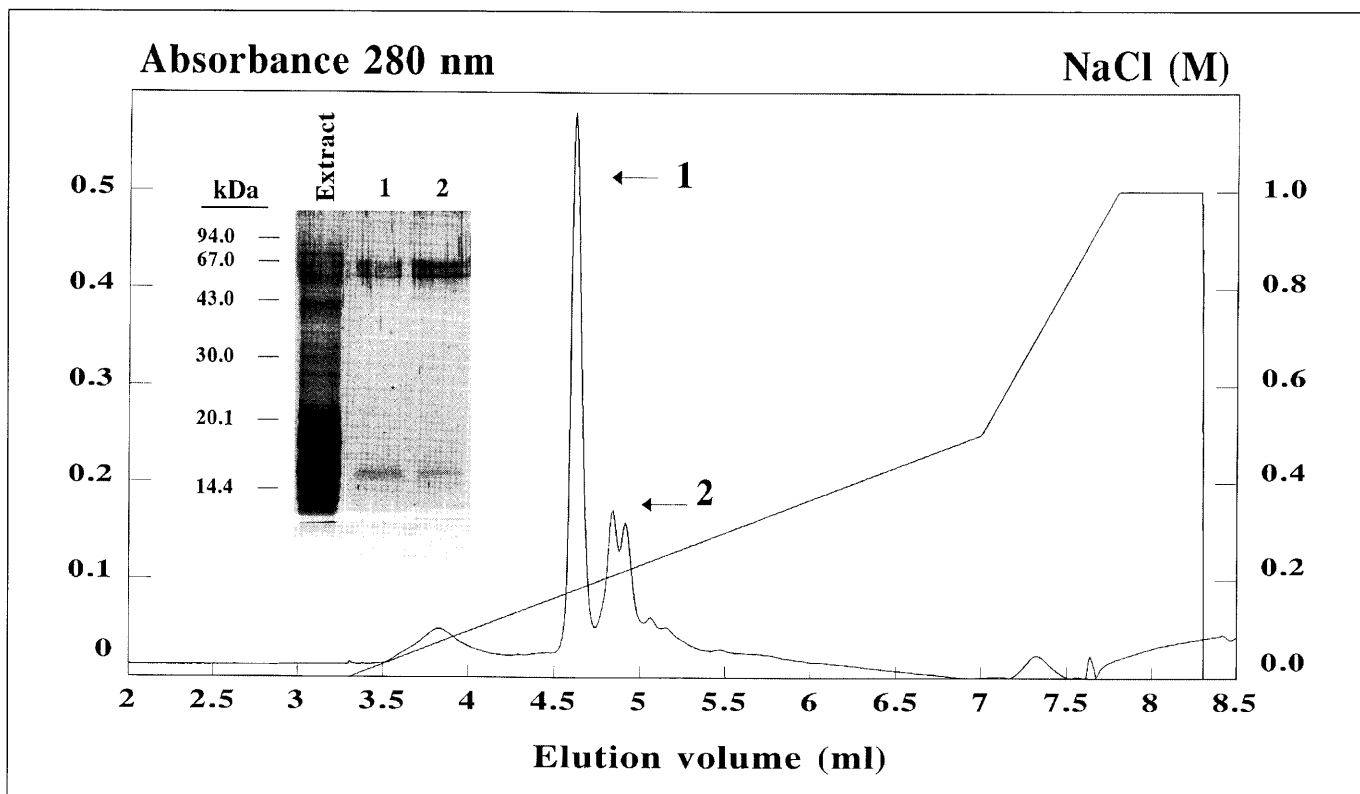


Fig. 3. Anion exchange chromatographic profile of the 17.5- to 19.7-kD gel filtration fraction from *M. annua* pollen. SDS-PAGE of fractions 1 and 2 were revealed by silver staining.

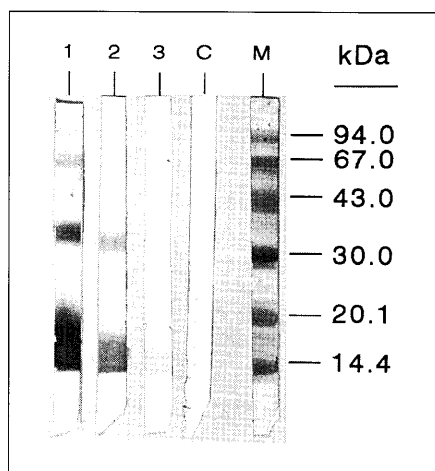


Fig. 4. SDS-PAGE immunoblotting of *M.-annua*-purified profilin, using rabbit profilin antiserum (anti-*M. annua* profilin) at three different dilutions. (1) 1:3,000, (2) 1:30,000, (3) 1:300,000; C = Membrane incubated with a control rabbits serum (not immunized animal); M = Molecular weight markers stained with Amido black B.

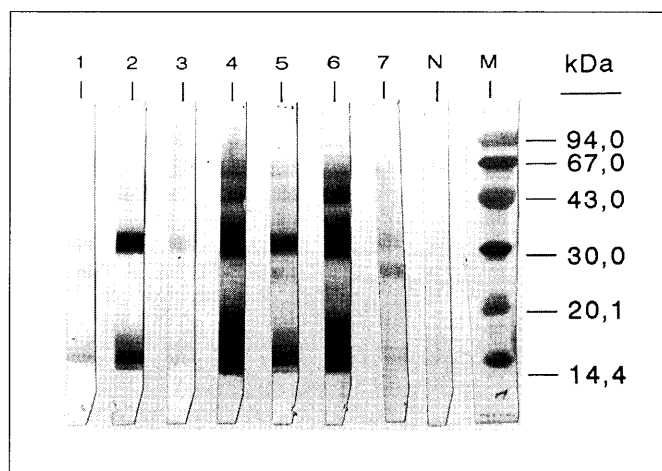


Fig. 5. SDS-PAGE immunoblotting of *M. annua* purified profilin, using sera from sensitized patients. Correspondence of lane numbers with serum references from figures 2a and b is the following (lane number/serum Reference): 1/1, 2/2, 3/4, 4/6, 5/11, 6/29, 7/32. N = *M. annua* strip incubated with negative control serum; M = molecular weight marker revealed with Amido black B.

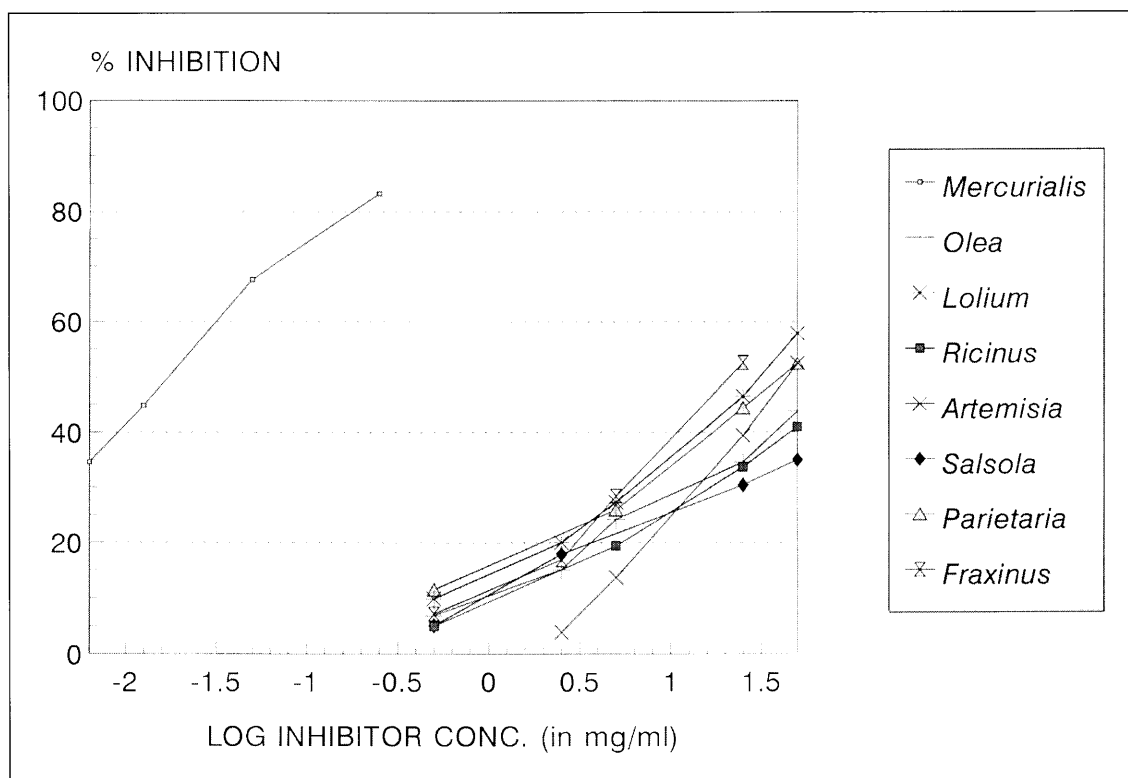


Fig. 6. ELISA inhibition curves of a pool of rabbit anti-*M. annua* sera using *M. annua* extract in solid-phase and different pollen extracts as inhibitors.

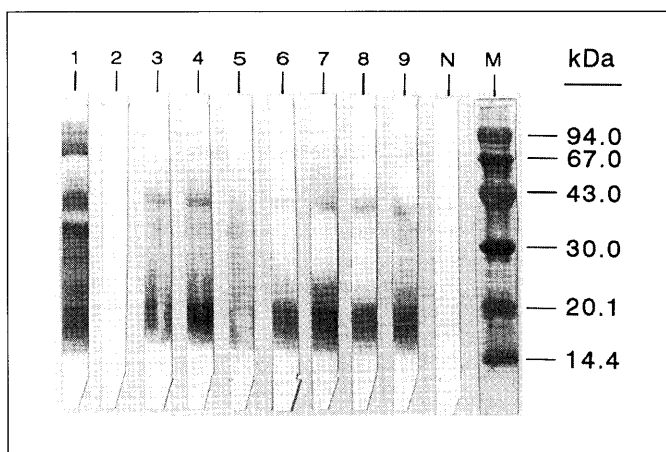


Fig. 7. SDS-PAGE immunoblotting inhibition using rabbit anti-*M. annua* serum and different pollen extracts. Lane 1 = Noninhibited control; lanes 2–9 = inhibition caused by *M. annua*, *O. europaea*, *L. perenne*, *P. judaica*, *S. kali*, *A. vulgaris*, *R. communis* and *F. excelsior*, respectively; lane N = *M. annua* blot incubated with nonatopic serum; M = molecular weight marker revealed with Amido black B.

Discussion

M. annua sensitization was prevalent in all climatic areas studied. In fact, sensitization to this pollen ranged from 28 to 54% of all patients sensitized to pollens in the areas studied. These results differ from those reported by Ariano et al. [5], who found a lower prevalence (0.9%) in their area.

SDS-PAGE immunoblotting performed with the sera of 37 *M. annua*-allergic patients revealed the presence of two bands in more than 50% of sera tested (fig. 2a, b) at 14.1 and 15.8 kD. Of note, an IgE binding band at 17 kD was detected in 49% of patients' sera, which was not investigated further. Apart from these three bands, thirty-nine were revealed with a lesser frequency.

In a previous attempt, the native proteins which gave rise to the 14.1- and 15.8-kD bands were satisfactorily purified by a combination of gel filtration and ion exchange chromatography in the SMART system (data not shown). However, once the relevant allergens were preliminarily identified as profilins, a more efficient method for their isolation was followed. The combination of a first affinity chromatography step, on a PLP-CNBr-Sepharose column, followed by

SMART gel filtration and anion exchange chromatography (fig. 3) permitted the isolation of the major allergens with a high degree of purity, as already achieved for other pollen allergens [22]. The bands observed at around 67.0 kD are contaminant artifacts which usually appear in silver staining of gels, presumably due to skin keratin contamination, and not to protein contaminants from the analyzed peaks [23]. Following international guidelines on the nomenclature of major allergens [24], we propose the denomination of Mer a 1A and Mer a 1B for the 15.8- and 14.1-kD protein components of *M. annua* pollen, respectively. Both proteins were demonstrated to be profilins according to three different criteria: specific binding to PLP peptides, IgG binding from a rabbit antiserum anti-sunflower profilin (fig. 4) and dissociated and native molecular parameters. SMART gel filtration results of these components showed a similar molecular weight in their native forms. Moreover, profilins purified as described above were incubated with sera from selected patients (fig. 5) demonstrating that the IgE binding showed at ~15 kD in the SDS-PAGE immunoblotting of *M. annua* whole extract was due to the identified and isolated profilin and not to other comigrating allergens. IgE-binding bands at around 30 and 60 kD are probably due to dimeric and tetrameric forms of profilin, according to recent data published by Babich et al. [25].

These authors analyzed affinity-purified profilins by SDS-PAGE, not only the band at 14.8 kD, but also extraneous higher-molecular-weight bands (primarily at 30 and 58.5 kD), which showed actin-binding capacity. Accordingly, they postulated the human platelet profilin self-association as a physiological way by which monomeric profilin forms tetramers which are the relevant high-affinity actin-binding form. Under this postulate the IgE-binding band found at ~43 kD in the *M. annua* profilin SDS-PAGE immunoblotting could also be due to association of three profilin molecules.

Profilins belong to the family of actin-binding proteins, which have recently been identified as allergens in various different sources, mainly vegetables and fruits, being involved in a large number of allergic cross-reactions [26–28]. In spite of its panallergenic character, profilin has not been considered a major allergen in any of the sources studied so far [27]. However, in the present study, profilin has been revealed, at least from an in vitro point of view, as a relevant allergen in *M. annua* pollen being detected as an IgE-binding component in more than 50% of the sera studied.

The chromatographic and electrophoretic behavior of the two copurified allergens indicated their physicochemical similarity in terms of molecular weight and net charge.

These results suggest that both components could be isoforms originated by differential glycosylation or by protease action – as already pointed out by Vallier et al. [29] – to explain the doublet band of celery profilin.

In a previous report [4], we identified a relevant allergen with pI 5.0, which could correspond to one of the profilin isoforms described in the present report.

ELISA inhibition and double-diffusion using experimental immunosera proved to be comparable, allowing us to observe a high degree of reactivity when the homologous system was used, and clearly lower cross-reactivities when heterologous systems were used. Results obtained by ELISA inhibition and double diffusion techniques indicate that there is a significant degree of cross-reactivity between *Mercurialis* and *Olea*, *Fraxinus*, *Ricinus*, *Salsola*, *Parietaria* and *Artemisia*, in spite of being species which belong to unrelated phylogenetically different families such as: Euphorbiaceae (*Mercurialis* and *Ricinus*), Oleaceae (*Olea* and *Fraxinus*), Chenopodiaceae (*Salsola*), Urticaceae (*Parietaria*) and Compositae (*Artemisia*). However, low inhibition percentages (not higher than 26%) and low Ag₅₀ values were shown by all the above-mentioned heterologous antigens. In fact, more than thousandfold higher concentrations are needed for 50% inhibition in heterologous experiments as compared to the homologous one. A very low degree of antigenic cross-reactivity was demonstrated between *M. annua* and *L. perenne*. These results partially differ from those reported by Ariano et al. [5], which showed a high degree of allergenic cross-reactivity between *Mercurialis* and Gramineae or *Olea*, by using sera from 7 subjects that were polysensitized to pollens such as *O. europaea* and grasses which showed RAST scores being equal to or higher than those to *M. annua*. In the present study, we analyzed the structural homology between the different pollen extracts using rabbit antiserum raised against *M. annua* allergenic extracts in a close system, to avoid possible interferences due to polysensitized sera.

The results obtained by inhibition of the *M. annua* SDS-PAGE immunoblotting ratify those observed by ELISA and double diffusion, that is, there is a significant but low extent of inhibition of the IgG binding to *M. annua* extract by the heterologous systems with respect to the one produced by the homologous one (*Mercurialis*), being attributable to the 94.0- to 30.0-kD interval (fig. 7), a molecular weight range in which the most relevant *M. annua* allergens are not present. From the results obtained with the three above-mentioned techniques, it can be concluded that some but not many common epitopes exist in the allergenic pollen extracts analyzed in the present study.

The results presented in this study strongly suggest that antigenic cross-reactivity between *M. annua* and other pollen extracts does not lie on the profilin molecule to a large extent, although it is probably present in the studied pollens, but on other allergens of higher molecular weights. Accordingly, further studies should be focused on the role of such *M. annua* proteins in cross-reactivity phenomena.

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