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Sensitization to Sunflower Pollen: Only an Occupational Allergy?

Abstract

Sunflower (*Helianthus annuus*) pollen sensitization has been reported as an occupational allergy. In this report, the sensitization of the general population living in sunflower-growing areas to *Helianthus* pollen was studied. Both RAST results in 32 adults with summer symptoms previously diagnosed as allergic to *Artemisia* pollen, and cross-reactivity studies between *H. annuus* and other Compositae suggested that *H. annuus* pollen was the main allergen involved in the hypersensitivity reaction of those patients. Good correlation was found between RAST and SPT to *Helianthus* and between RAST and conjunctival provocation test to *Helianthus*. Bronchial challenge tests performed on 8 of the 32 patients confirmed the clinical implication of *Helianthus* pollen in suspected subjects. Five workers, handling sunflower pollen, who suffered from related symptoms were subjected to the same study, showing lesser wheal areas and lesser specific IgE levels than a non-worker group. Thirteen patients with RAST values $\geq$ class 2 showed 2 IgE-binding fractions at 34.0 and 42.8 kD in 65% of sera and 3 IgE-binding fractions at pI 4.9, 9.6 and 10.2 in 54% of sera. By means of micropreparative high-resolution chromatography, it was possible to purify a 34-kD major allergen. Analysis performed by RAST inhibition with sera from atopic patients and ELISA inhibition with experimental anti-*Helianthus* rabbit sera demonstrated a cross-reactivity between *Helianthus* and other Compositae, but low affinity of specific anti-*Helianthus* antibodies for heterologous antigens. Taking into account the above-mentioned data, and the high prevalence of *Helianthus* pollen in the atmosphere during harvesting (in spite of its entomophilous character), *Helianthus* pollen should be considered as an allergenic source to be investigated in the general population living in sunflower-growing regions suffering from seasonal summer allergy.

Key Words

Helianthus annuus
 Compositae pollens
 Occupational allergy
 Summer seasonal allergy
 Biological standardization of allergens
 Sunflower pollen sensitization
 Relevant allergens
 Cross-reactivity

Introduction

Due to the commercial interest in the sunflower (*Helianthus annuus*), this composite is an expanding crop throughout the world [1].

In spite of the large amount of pollen grain produced in areas dealing with surrounding crops and the risk of occupational allergy among people handling sunflower pollen grains [2], there have been no reported studies dealing with sensitization in the general population.

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Table 1. Characteristics and clinical features of the groups included in the study

Group	Degree of relation with sunflower pollen	Symptoms	Inclusion criteria
I (32) Ma = 29.5 m (15); f (17)	Non-workers	Rhinoconjunctivitis (28) Asthma (16) Angioedema (7) Urticaria (6) Cough (1)	Adults Symptoms during summer season Living in sunflower pollen influence areas Sensitivity to <i>Artemisia</i> pollen No IT to any Compositae
II (5) Ma = 36.2 m (3); f (2)	Workers	Rhinoconjunctivitis (5) Asthma (2) Itching (2)	Symptoms during exposition time No IT to any Compositae Living in sunflower influence areas for more than 3 years
III (20) Ma = 32.8 m (11); f (9)	Non-workers (control group)	Rhinitis due to mites (10) Healthy (10)	Symptoms during exposition time No IT to any Compositae Living in sunflower influence areas for more than 3 years
IV (7) Ma = 31.6 m (7)	Workers (control group)	Healthy (7)	Symptoms during exposition time No IT to any Compositae Living in sunflower influence areas for more than 3 years

Number of subjects shown in parentheses; Ma = mean age; m = males; f = females. Group II: 3 subjects worked at same place and 2 left due to intolerance; group IV: all subjects working at least 3 years at sunflower crop fields.

This work was intended to elucidate (1) the ability of *H. annuus* pollen to sensitize sunflower workers, as well as the general (non-working) population living in sunflower-growing areas; (2) the cross-reactivity between pollens of *H. annuus* and other Compositae, and (3) the characteristics of the main allergens involved.

Material and Methods

Subjects

Table 1 shows the characteristics and clinical features of different groups included in this study.

Preparation of *H. annuus* Pollen extract

H. annuus pollen grains were collected in Córdoba, during the pollination period, in spring 1990. The examination of purity revealed 99% of pollen grains from *H. annuus* species.

Defatted pollen was extracted by magnetic stirring (24 h at 4°C) in 0.1 mol/l of phosphate buffer, pH 8 at 15% w/v. The extract was clarified by centrifugation at 5,600 g for 30 min, filtered through 0.45-µm pore diameter membranes and dialyzed by ultrafiltration in a Pellicon System (Millipore Corp. Milford, Mass., USA) with a 5,000 dalton cut-off. The dialyzed extract was sterilized by filtration through a 0.22-µm pore diameter membrane and freeze-dried.

Protein concentration was estimated according to the method of Lowry et al. [3]. Carbohydrate content was measured by the anthrone method [4] and by the orcinol method [5] to determine hexoses and

pentoses, respectively. Protein content was 32.5% with 16.5% hexoses and 41.2% pentoses, in relation to raw material.

The yield of the extraction process was 10.9 g of freeze-dried material per 100 g of pollen.

Aliquots of lyophilized extract from the same batch were used for all in vitro and in vivo tests.

Skin Prick Test

Skin prick test was performed according to the Subcommittee on Skin Tests of the European Academy of Allergology and Clinical Immunology [6].

H. annuus was diluted in phenolated and glycerinated saline solution at concentrations of: 0.08, 0.2 and 2 mg/ml. Standardized lancets (Dome Hollister, Leverkusen, Germany) were used for prick tests. Histamine chlorhydrate at 10 mg/ml and phenolated glycerol saline solution were used as positive and negative controls, respectively. After 15 min, translucent tape was applied over the wheals obtained, and the outlines were transferred to a record sheet. Areas were expressed in square millimeters. The mean wheal areas produced by each allergen concentration was plotted as a function of the allergen concentration in a log-log system, and linear regression was performed. The allergen concentration eliciting a wheal equal to the one produced by histamine, 10 mg/ml, was defined as 1 histamine-equivalent prick (HEP) [7]. 1 HEP unit was found to be equivalent to 1.8 mg/ml of allergen extract. Values of skin prick test expressed in results were calculated as the ratio between extract wheal area and histamine wheal area.

Commercially available prick tests of *Artemisia vulgaris*, *Chrysanthemum leucanthemum*, *Taraxacum officinalis* and *Ambrosia elatior* were also tested.

Conjunctival Provocation Test

This test was performed according to Möller et al. [8] by administering one drop of the allergen dilution in the conjunctival sac. If no reaction was observed within 20 min, the next concentration (0.005; 0.05 and 0.5 HEP/ml) of aqueous extract was applied in the other eye.

Criterion for positive test was congestion of conjunctival mucosa, itching and watering of the eye and nose.

Negative control was carried out by administering one drop of the diluent in the conjunctival sac of both eyes.

Bronchial Provocation Test

This test was performed on 10 subjects (8 from group I and 2 from group II). The rest (up to 18 asthmatics) had contraindications or were not collaborative. All of 10 tested subjects remained in the hospital during 24 h. The test was carried out according to general guidelines for nonspecific bronchoconstricting tests of the European Society for Clinical Respiratory Physiology [9], recording vital capacity, FEV₁ and FEV₂₅₋₇₅ values in a Datospir 2000 Admate DP-130 (Sibel, SA) set, after basal, diluent *Helianthus* and terbutaline inhalation with an Ultraneb-99 (DeVilbiss), respectively. The patients inhaled 2 ml of each allergen concentration, starting at 0.0001 HEP to a maximum of 1 HEP. The spirometric records were also carried out at 4, 6, 8 and 24 h after terbutaline administration (or the last *Helianthus* concentration in the negative tests) and peak flow was recorded by means of a peak flow meter after 12 h, to check any possible late response. The challenges were considered as positive when a 20% decrease in FEV₁ and/or a 35% decrease in FEV₂₅₋₂₇ could be registered. PC₂₀ indicates the concentration of *H. annuus* extract, expressed as HEP, which produces a FEV₁ decrease of 20%. BPT was considered negative when 1 HEP did not produce a FEV₁ and FEV₂₅₋₂₇ significant fall.

In vivo tests were performed during asymptomatic phases of disease, without interference treatment.

RAST

Solid-phase antigen was obtained by coupling the extract solution (1 mg/ml) to the 6-mm diameter CNBr-activated paper discs by the method of Ceska and Lundqvist [10]. RAST was performed by the method of Wide et al. [11] with Phadezym RAST reagents (Pharmacia Diagnostics AB), according to the manufacturer's instruction.

For *Artemisia*, *Taraxacum*, *Chrysanthemum* and *Ambrosia*, solid phases were prepared in the same manner.

Isoelectric Focusing (IEF)-Immunoblotting

Isoelectric focusing (IEF) was performed on Isogel agarose plates (FMC Bio Products, Rockland, Me., USA), pH 3–10, and 20 µg of protein was applied per lane. Transfer of proteins from agarose gel to PVDF membrane (Immobilon-P, Millipore) was achieved by capillary blotting and contact diffusion according to the method of Elkon [12]. Immunochemical staining was performed by first blocking the Immobilon membrane with 90% defatted dry milk in Tris-buffered saline (TBS) (1 h at 37°C). After three washes with TBS, the blots were incubated with 2.5 ml of undiluted human serum (16 h at 4°C). The blots were washed 3 times with TBS and incubated with 2.5 ml of 1:25 peroxidase-conjugated rabbit immunoglobulins to human ε-chains (Dako, Glostrup, Denmark). 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 Laboratories, Richmond, Calif., USA) and 0.01% H₂O₂ in TBS. After 30 min, the blots were rinsed in distilled water and air-dried. Antigen-antibody binding was identified by purple stain deposition [13].

SDS-PAGE Immunoblotting

SDS-PAGE was carried out by the method of Laemmli [14]. 12.5% and 3% polyacrylamide concentrations were used for separating and stacking gels, respectively. Proteins dissolved in 0.125 M HCl-Tris, pH 6.8 were dissociated with 0.1% SDS and 5% β-mercaptoethanol by treatment at 100°C during 5 min 20 µg protein, estimated by the Bradford [15] method, was applied per lane.

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 Immobilon-P-membranes, essentially by the method of Towbin et al. [16].

Then, IgE-binding proteins were immunochemically revealed as described for IEF Immunoblotting.

Allergen Purification

The chromatographic steps were achieved using the SMART™ system (Pharmacia, Sweden). Size exclusion chromatography was performed in a Superdex 75 PC 3.2/30 column using 0.05 M phosphate buffer, 0.15 M NaCl, pH 7.0, at a flow rate of 10 µl/min and 8°C. Samples were injected with a 20 µl loop. The ion-exchange chromatography was performed in a Mono Q PC 1.6/5 column using 20 mM Tris-HCl buffer, pH 8.0 and 20 mM Tris-HCl buffer, 1 M NaCl, pH 8.0, with a flow rate of 100 µl/min at 25°C. Samples were injected with a 1 ml loop.

RAST Inhibition

RAST inhibition was done according to Yman et al. [17]. Fifty microliters of sera from patients sensitive to *H. annuus* pollen plus 50 µl of 14 dilutions (0.01–10 µg) of allergenic extracts (inhibitors) were added to solid-phase antigen.

The following allergenic extracts were used as inhibitors: *H. annuus*, *Artemisia*, *Ambrosia*, *Chrysanthemum* and *Taraxacum*. The mentioned extracts were obtained as described for *H. annuus*.

Elaboration of Experimental Immunosera

Immunization was performed according to Gallart et al. [18] in adult male white New Zealand rabbits. Weekly injections of 10 mg extract with Freund's complete adjuvant were given subcutaneously. After eight injections, the animals were bled and the serum collected.

ELISA Inhibition

ELISA inhibition was done according to Martínez et al. [19]. The *H. annuus* allergen was dissolved to an optimal solid-phase concentration [20] (1.2 mg/ml) in 0.1 M carbonate-bicarbonate buffer, pH 9.6 and dispensed (100 µl/well) in polystyrene microdilution plates (Maxisorp, NUNC, Denmark). The plates were incubated overnight (room temperature, moist chamber), supernatant discarded and PBS 0.05% Tween 20–1% BSA (PBS-T-S) was added (200 µl/well) and incubated for 11 h (room temperature, moist chamber). After washing with PBS, the allergen extracts (*H. annuus*, *Artemisia*, *Ambrosia*, *Chrysanthemum* and *Taraxacum*) at different concentrations (20,000–0.6 µg/ml) dissolved in PBS-T-S were dispensed (50 µl/well) followed by rabbit serum anti-*H. annuus* pollen extract diluted 1/500 (50 µg/well). These plates were incubated for 3 h (room temperature, moist chamber), supernatants removed and washed as before. Swine gammaglobulin fraction anti-rabbit immunoglobulins, affinity purified and conjugated to horseradish peroxidase (Dako, Denmark), appropriately diluted in PBS-T-S was dispensed (100 µl/well) and incubated overnight (4°C,

Table 2. Group I results: values of SPT and provocation tests

Subjects	Skin prick test					Provocation test (<i>Helianthus</i>)		
	<i>Helianthus</i>	<i>Ambrosia</i>	<i>Artemisia</i>	<i>Chrysanthemum</i>	<i>Taraxacum</i>	conjunctival	bronchial	
							PC ₂₀	late response
1	16	4.5	21	4	7	P		
2	8	1.4	4	3	5	P	0.0006	no
3	7	2	1.8	1.2	1	P		
4	7	1	4	1.4	4	P	0.009	no
5	4	0.6	9	0.5	9	P	0.69	no
6	3	0.1	0.8	0.2	0	P		
7	3	0.6	3	2	0.6	P	> 1	no
8	3	1	6	1.4	2	P	0.57	no
9	6.5	1.6	14	5	4.5	P		
10	1.3	0.5	1.8	0.9	0.8	P		
11	2	0.4	2	1.4	3	P		
12	1.6	0.3	2.5	1.4	0.8	P	0.74	yes
13	1.3	0.5	5.5	1.3	3	P		
14	1.4	0.5	4	2	0.9	N	> 1	no
15	1.7	0.5	0.7	0.3	0.2	N		
16	1	0.7	1	0.7	0.5	P		
17	0.4	0.1	0.8	0.5	0.3	P	> 1	no
18	0.6	0	0	0.6	0.3	N		
19	0.3	0.3	3	1.2	0.7	P		
20	0.3	0.2	3	1.2	0.6	N		
21	0	0	2.8	1.6	0	N		
22	0	0	1.1	0.5	0	N		
23	0	0	3	0	1.8	P		
24	0	0	2.8	0.4	0	N		
25	0	0	0.5	0	0	N		
26	0	0	1.6	0	0	N		
27	0	0	0.7	0	0	N		
28	0	0	0	0	0	N		
29	0	0	0.7	0	0	P		
30	0	0	0.5	0.3	0	N		
31	0	0	0.2	0.2	0	N		
32	0	0	0.2	0	0	N		

Skin prick test values are expressed as extract wheal area/histamine wheal area. PC₂₀ values are expressed in HEP; P = positive; N = negative.

Table 3. Group II results: values of SPT and provocation tests

Subjects	Skin prick test					Provocation test (<i>Helianthus</i>)		
	<i>Helianthus</i>	<i>Ambrosia</i>	<i>Artemisia</i>	<i>Chrysanthemum</i>	<i>Taraxacum</i>	conjunctival	bronchial	
							PC ₂₀	late response
1	3	15	1	9	1	P	0.1	yes
2	4	0	5	3	2	P		
3	3	0	0	0	0	N	0.83	no
4	8	6	15	0	0	P		
5	22	5	2	0	0	P		

P = positive; N = negative. Skin prick test values are expressed as extract wheal area/histamine wheal area. PC₂₀ values are expressed in HEP.

moist chamber). The wells were washed, then peroxidase activity was measured with MBTH-DMAB substrate solution [21]. OD₆₀₀ and concentrations of inhibitor allergens were correlated by linear regression. Inhibition percentages were calculated on this concentration basis, and the antigen concentrations needed for inhibition (Ag₅₀) were calculated.

Double Diffusion

Double diffusion was performed according to the Ouchterlony method [22], using a support of 1% agarose in barbital buffer 0.05 M pH 8.6.

Fifty milligrams of allergen (*H. annuus*, *Artemisia*, *Chrysanthemum*) and undiluted rabbit immunosera were used.

Passive immunodiffusion was carried out during 48 h (4°C, moist chamber) and afterwards, the plates were first washed with 5% Trisodium citrate and then with saline.

After drying the agarose plate, the proteins were stained with amido black.

Results

The results of in vivo tests from subjects included in group I are shown in table 2. In this group, skin prick test results showed mean values of 2.17, 3.10, 1.44, 1.05 and 0.52 for *Helianthus* (5 HEP), *Artemisia* (100 BU/ml), *Taraxacum* (5%, w/v), *Chrysanthemum* (5% w/v) and *Ambrosia* (5% w/v), respectively. Correlation between skin prick test with values ≥ 1 and conjunctival provocation test positive values to *Helianthus* was statistically significant ($p < 0.05$, $r = 0.63$).

RAST results of subjects from group I are shown in figure 1. Among the five Compositae species studied, *Helianthus* was the one that showed higher values. Correlation between skin prick test values ≥ 1 and RAST values > 0.35 PRU/ml to *Helianthus* was statistically significant ($p < 0.05$, $r = 0.67$). Skin prick test and provocation test results corresponding to subjects of group II are presented in table 3.

RAST results of subjects from group II are shown in table 4.

In vivo and in vitro tests of control groups (groups III and IV) produced negative results (no response in skin prick test or conjunctival provocation tests and RAST values < 0.35 PRU/ml).

Figures 2a, b show IgE binding fractions to *H. annuus* pollen extract based on the MW. Fourteen patient sera with RAST values ≥ 3.5 PRU/ml belonging to groups I and II were used. Two IgE binding fractions of 34.0 and 42.8 kD were present in 61.5% of the sera.

Figures 3a, b show IgE-binding fractions to *H. annuus* pollen extract based on the pI. Thirteen patient sera with RAST values ≥ 3.5 PRU/ml belonging to groups I and II

were used. Three IgE binding fractions of pI 4.9, 10.2 and 9.6 were present in 61.5, 54 and 54% of tested sera, respectively.

Figure 4 shows the inhibition curves using *H. annuus* allergen in solid-phase and other Compositae allergens as inhibitors, when ELISA inhibition was applied using experimental rabbit anti-*H. annuus* sera.

Figure 5 shows the inhibition curves using *H. annuus* allergen in solid-phase and other Compositae allergens as inhibitors, when RAST inhibition was applied using a pool serum from sensitized patients to sunflower pollen.

Figure 6 shows the pattern of precipitation lines demonstrated by double diffusion when comparing *Helianthus*, *Artemisia* and *Chrysanthemum* extracts using homologous and heterologous experimental anti-sera. When rabbit serum anti-*Helianthus* was used, four precipitating systems could be demonstrated with *Helianthus* antigen, two with *Artemisia* and one with *Chrysanthemum*. When rabbit serum anti-*Chrysanthemum* was used, five lines could be demonstrated with the homologous antigen, four with *Artemisia* and two with *Helianthus*. When rabbit serum anti-*Artemisia* was used, five bands could be demonstrated with *Artemisia* extract, two with *Chrysanthemum* and two with *Helianthus*.

Fractions collected from gel filtration chromatography, corresponding to elution of molecules with 30–35 kD and containing *Hel a* I, were chromatographed again using anion exchange columns (fig. 7a). The peak eluted at 32 mM NaCl revealed after SDS-PAGE one 34-kD band with 100% purity measured by densitometry with the BIO-IMAGE SYSTEM, Millipore (Fig. 7b). Immunoblotting of this protein showed one IgE-binding fraction of 34 kD (fig. 7b).

Discussion

In our media, Compositae of the genera *Artemisia*, *Chrysanthemum* and *Taraxacum* are usually tested as common allergens to screen general pollen-sensitive population. Airborne counts demonstrated low concentrations of pollen grains from the mentioned species and large amounts of *Helianthus* pollen grains [23]. These data suggested that this last species could be a cause of sensitization to Compositae in our region.

The aim of this study was to determine the role of *Helianthus* as an occupational and non-occupational sensitizing agent.

The results showed sensitization in sunflower pollen handlers, employed in laboratories or processing plants, where they worked under clouds of dust containing sun-

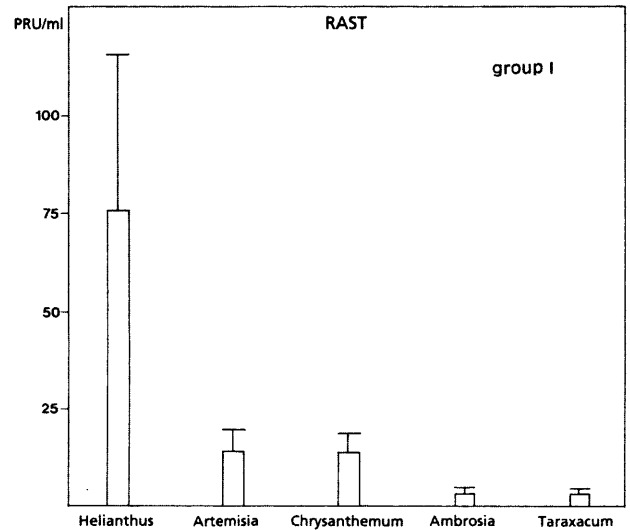
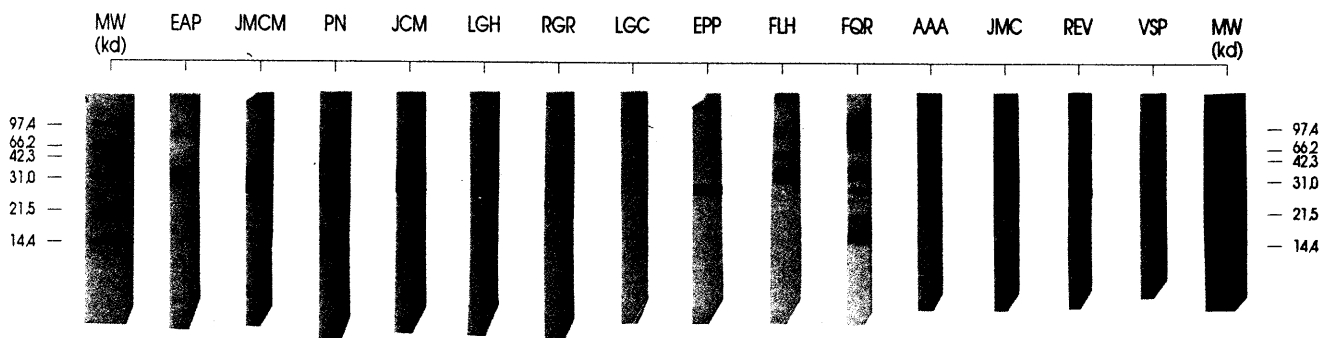
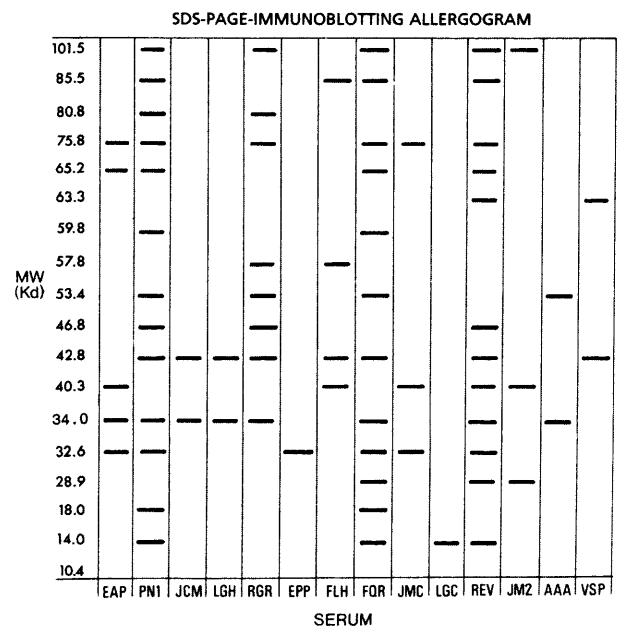


Fig. 1. RAST values of sera from subjects included in group I. Results are expressed as arithmetic means $\pm$ SE.

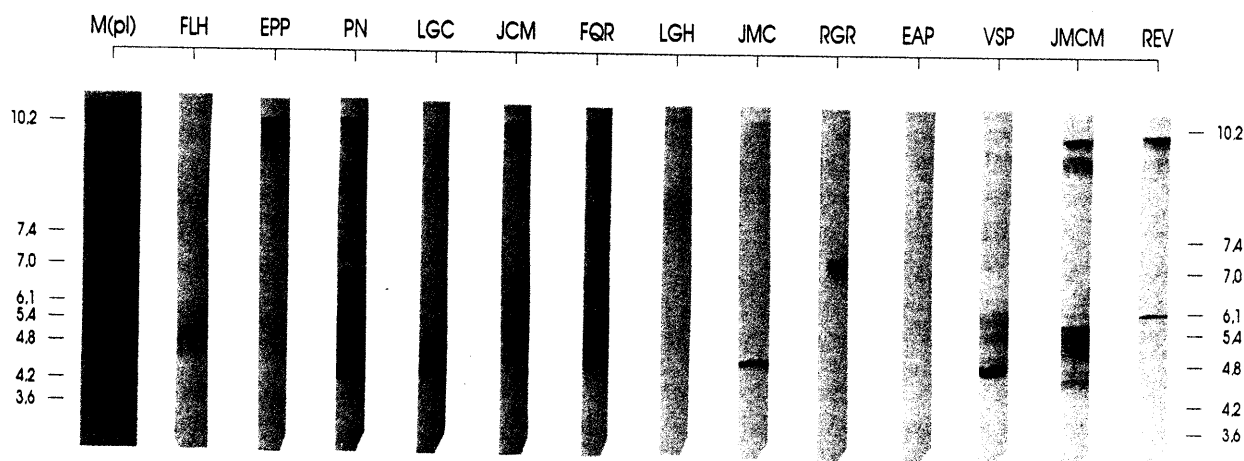


a

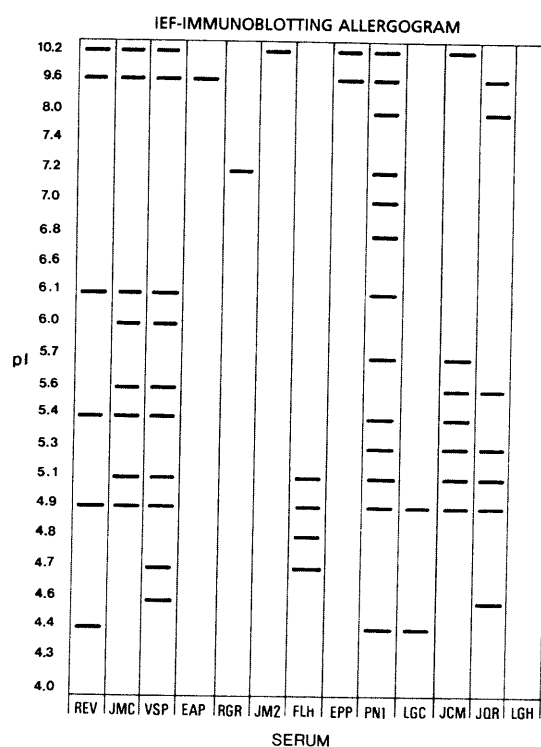


b

Fig. 2. a SDS-PAGE Immunoblotting to *H. annuus* incubated with sera from 14 subjects with RAST class ≥ 3 included in groups I and II. MW: molecular weight marker (kD). **b** Schematic representation of SDS-PAGE immunoblotting.



a



b

Fig. 3. a IEF-Immunoblotting to *H. annuus* incubated with sera from 13 subjects with RAST class ≥ 3 included in groups I and II. **b** Schematic representation of IEF-immunoblotting.

Table 4. Group II results: RAST values (PRU/ml)

Subjects	<i>Helianthus</i>	<i>Ambrosia</i>	<i>Artemisia</i>	<i>Chrysanthemum</i>	<i>Taraxacum</i>
1	34.73	<0.35	2.1	4.7	0.97
2	1.09	<0.35	<0.35	<0.35	<0.35
3	<0.35	<0.35	<0.35	<0.35	<0.35
4	15.06	0.75	0.59	n.d.	<0.35
5	12.18	46	0.59	n.d.	0.59

n.d. = Not done.

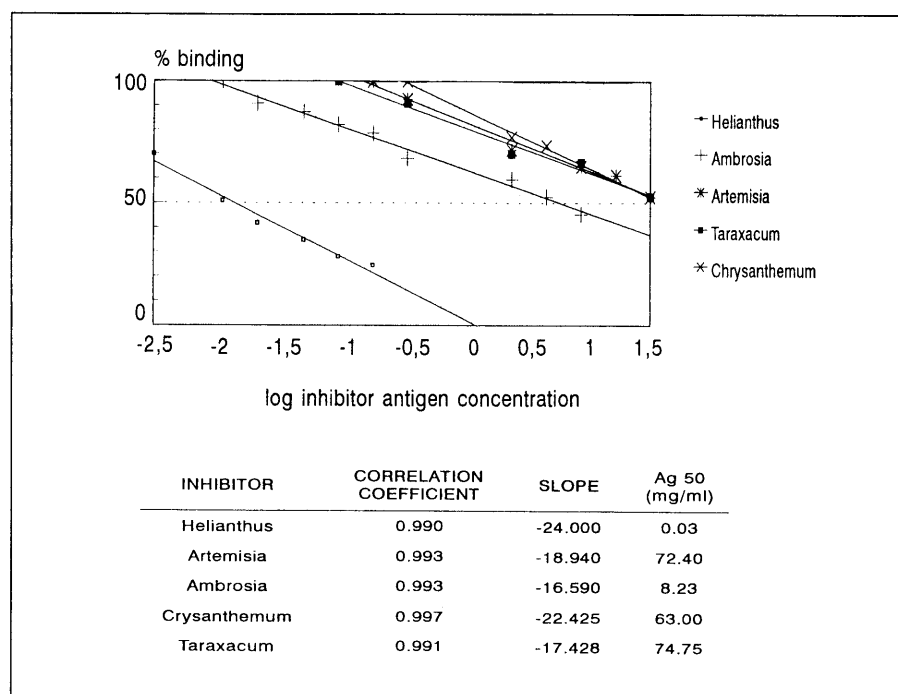


Fig. 4. ELISA inhibition curves using *H. annuus* pollen extract in solid phase.

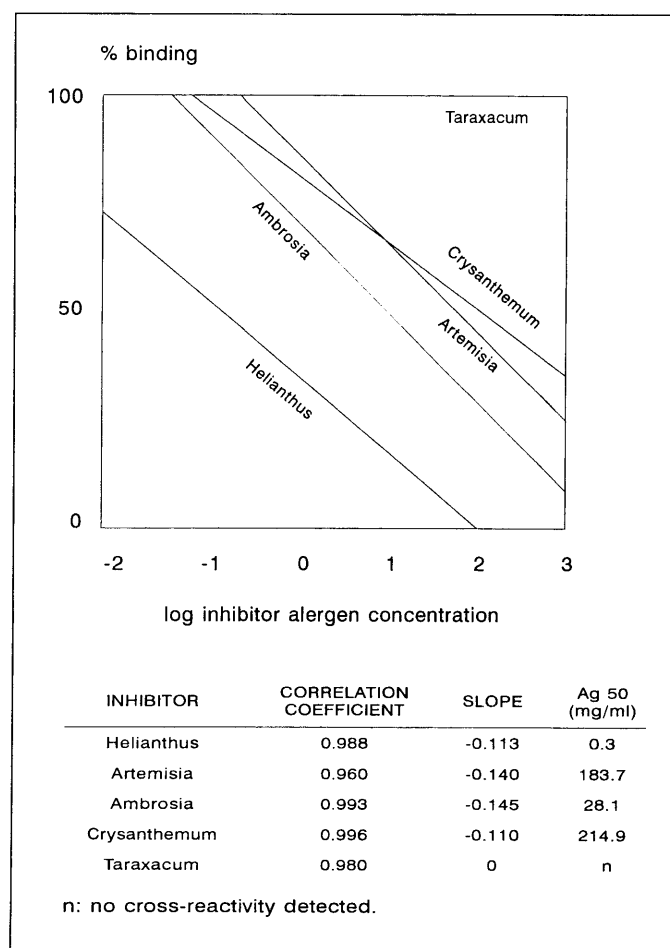


Fig. 5. RAST inhibition curves using *H. annuus* pollen extract in solid phase.

flower pollen grains. This could be the cause of specific sensitization in atopic workers (all the controls coming from sunflower-pollen-related work were negative), as well as irritating symptoms in hyperreactive subjects, as was to be expected from results of subject 3 from group II.

Results of group I show sensitivity to *Helianthus* of the general population living in sunflower-growing areas. From these data, *Helianthus* pollen could be considered as the most important allergen eliciting clinical symptoms among the Compositae in our region. In fact, *Helianthus* had the best prick/RAST correlation out of the 5 species, and the number of positive RAST patients to *Helianthus* was the highest. Furthermore, the clinical symptoms in group I are expressed more intensely during summer which does not correspond to sunflower pollination, but to sunflower harvesting time, when detection of sunflower pollen grains by volumetric samplers is the largest among the Compositae, much larger than *Artemisia* counts, a plant scarcely found in our region [23]. These sunflower pollen grains are trapped at pollination time, on the stems, on the leaves and the ground during the summer months, being airborne when the mechanical procedures for harvesting take place. Then, the atopic subjects living in these regions are likely to be sensitized to *Helianthus* allergenic pollen, as suggested by the results presented in this paper. These results showed a good correlation between clinical symptoms, cutaneous response, serum specific IgE levels and challenge test to *Helianthus*.

Considering the source of the extracts and the methodology used, both for RAST and skin prick tests, it seems evident that in vitro tests provide more reliable results for the different species evaluated in this study, and therefore, it can be considered that *Helianthus* pollen is the main cause of IgE-mediated hypersensitivity in the subjects of groups I and II.

Analysis of RAST and skin prick test results and the lack of correlation obtained for Compositae other than *Helianthus* suggest that the latter and not *Artemisia* is the source of clinical findings, and these should be taken into consideration when evaluating skin prick test results.

From these clinical findings, backed up by the laboratory studies, it could be suggested that further research may be required in order to ascertain the epidemiological relevance of *Helianthus* allergy in people living in areas with strong environmental pressure from sunflower crops.

Once demonstrated, the involvement of *H. annuus* pollen in occupational and non-occupational allergy, the subsequent aim was to study the cross-reactivity between *H. annuus* and other Compositae.

The similarity of slope values corresponding to the different linear regressions, when using RAST and ELISA in-

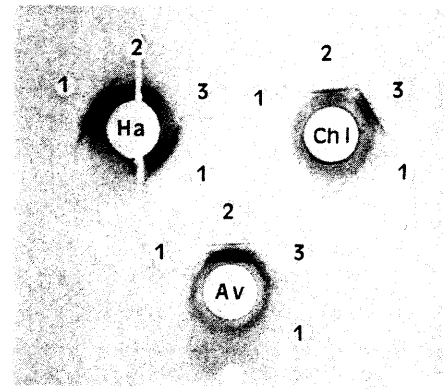


Fig. 6. Double-diffusion immunoprecipitation pattern. Ha = Rabbit serum anti-*H. annuus* pollen extract; Chl = rabbit serum anti-*Chrysanthemum leucanthemum* pollen extract; Av = rabbit serum anti-*Artemisia vulgaris* pollen extract; 1 = *H. annuus* pollen extract (100 mg/ml); 2 = *A. vulgaris* pollen extract (100 mg/ml); 3 = *C. leucanthemum* pollen extract (100 mg/ml).

hibition, demonstrated the homology among the different Compositae pollen extracts studied. However, the great differences found between *H. annuus* Ag₅₀ values and other Compositae by EIA inhibition methods suggest the low affinity of heterologous antibodies in spite of antigenic resemblance.

Fernández et al. [24] suggested a high degree of allergenic homology between sunflower and mugwort and indicated that in most cases primary sensitization occurred through exposure to mugwort pollen. In contrast, our results demonstrate homology between sunflower and other Compositae, but with Ag₅₀ values 100 times lower or less for *Helianthus*, using both EIA inhibition methods. Furthermore, the results obtained in this report suggest that in this population primary sensitization occurs through exposure to *H. annuus* pollen. When immunodiffusion was used, using anti-sunflower pollen rabbit sera, the results demonstrated 2 faint immunoprecipitation lines with *Artemisia* antigen and 1 with *Chrysanthemum* antigen with partial and total identity, respectively, corroborating the EIA inhibition results.

Analysis of IgE binding fractions of *Helianthus* by means of immunoblotting of 13 sera with RAST values ≥ 2 demonstrated the presence of two relevant fractions with molecular weights of 34.0 and 42.8 kD and three relevant fractions with pI of 4.9, 9.6 and 10.2.

Complementary studies were performed in order to purify the relevant allergen 34-kD fraction, demonstrated to be a monomeric protein that was eluted with 32 mM NaCl in a Mono Q anion exchange column.

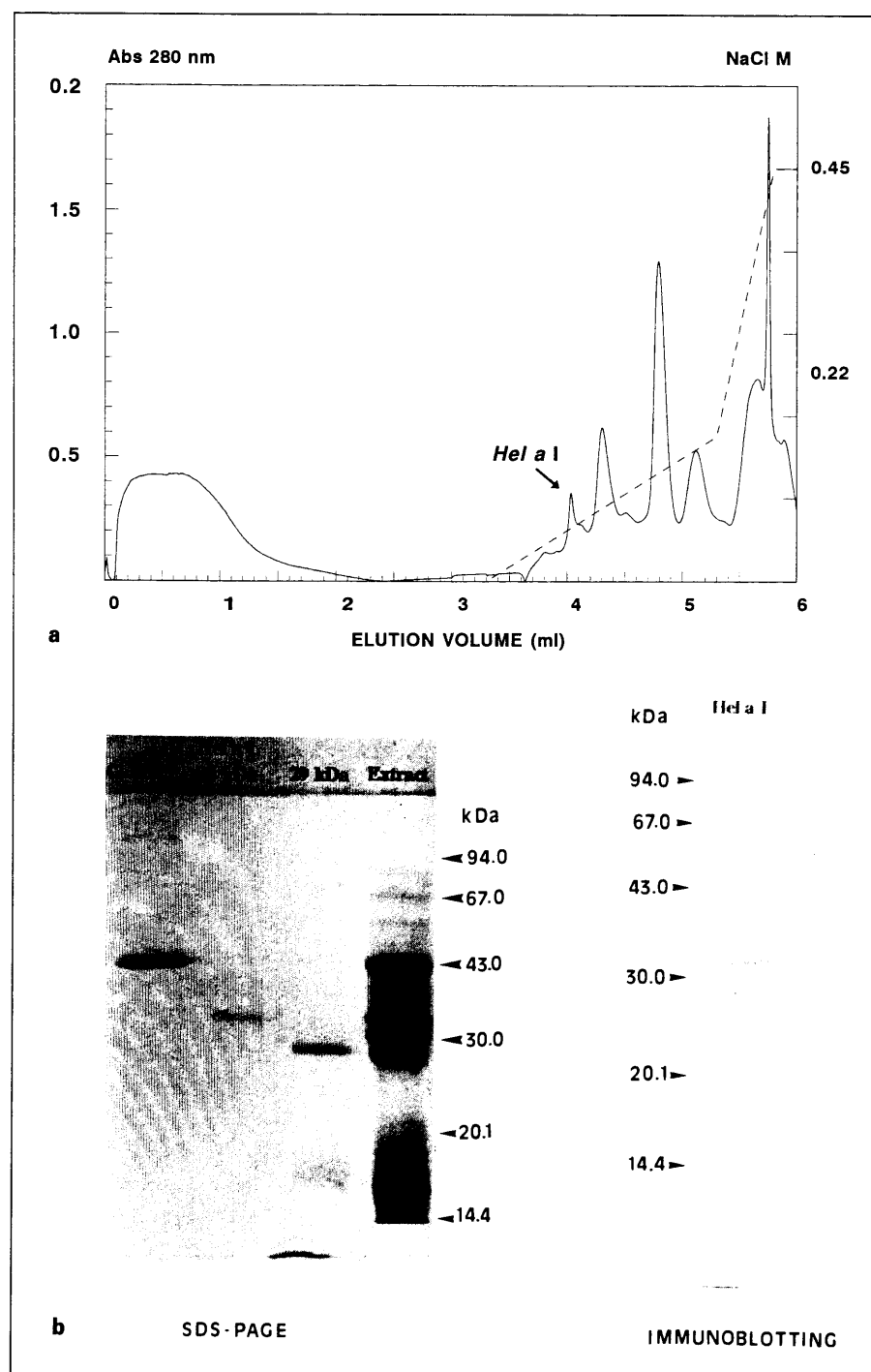


Fig. 7. a Anion exchange chromatographic profile of 30- to 35-kD gel filtration fraction of *H. annuus* extract. **b** SDS-PAGE and SDS-PAGE immunoblotting pattern of chromatographic peaks.

The allergen was isolated with more than 95% purity measured in a BIO-IMAGE analyser. Those results allow us to initiate further works to study the composition of the protein and DNA patterns in order to elucidate the possible degree of matching homology.

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