

Mercurialis annua pollen: A new source of allergic sensitization and respiratory disease

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Sensitization to Mercurialis annua pollen in 13 patients admitted for allergy study of asthma, rhinitis, or rhinoconjunctivitis is described. Twelve of these patients were also sensitized to other common aeroallergens. In five patients, a relationship was found between exposure to M. annua pollen and elicitation of symptoms. All patients were prick test and RAST positive to an extract of M. annua pollen. Nasal provocation test proved positive in 10 of 12 patients, and bronchial provocation test was positive in the only patient in whom it was performed. Two late responses were recorded. Immunoblotting of the 13 sera revealed two different groups of relevant allergens: one of isoelectric point 10.2, reacting with 12 of the 13 sera, and the other allergen of isoelectric point 5.0 to 5.5, reacting with 11 of the 13 sera. M. annua pollen is able to induce both sensitization and clinical disease in atopic patients. Since sensitization to this pollen accounts for 8.5% of total positive skin tested patients in the same period, we believe M. annua pollen should be considered as a relevant allergen and thus included in skin test batteries. Some patients labeled as having "intrinsic" asthma or rhinitis might be sensitized to this and other previously unknown allergens. (J ALLERGY CLIN IMMUNOL 1992;89:987-93.)

Key words: Mercurialis annua, weed pollens, sensitization to unknown allergens, allergic asthma, allergic rhinitis, immunoblotting

Mercurialis annua, a 40 to 60 cm high weed, of the Euphorbiaceae family (Fig. 1, A and B), is widespread in both rural and urban areas of Europe, North America, and north of Africa, from the sea level up to 2000 m.^{1,2} It is wind pollinated, and the flowering period lasts from October to June in our geographic area.³ Its pollen is tricolporated, reticulated, and small sized (25 by 22 μ m).⁴

In 1980, Lelong et al.⁵ suspected the possible allergenic implications of pollens of the genus *Mercurialis*, but they failed to demonstrate sensitization in a clinical trial. More recently, Ariano et al.⁶ reported prick test and RAST positivity to *M. annua* in a patient suffering from bronchial asthma.

Abbreviations used

HEP: Histamine-equivalent prick
TBS: Tris-buffered saline
pI: Isoelectric point
PRU: Phadebas RAST unit

In February 1990, one of our patients, who was sensitized to several spring and summer pollens, complained of allergic rhinitis during the winter. Pollen from flowering *M. annua* was collected during 2 months, and in March 1990, the patient was skin tested with a crude extract of this pollen, and a prick test proved positive.

A prospective study was undertaken to assess the allergenic role of *M. annua* pollen in our area and to investigate its immunochemical characteristics.

PATIENTS AND METHODS

Patients

Patients were selected from the outpatient allergy clinic of a general hospital in Tarragona (Mediterranean town 98 km from Barcelona). Between March 1990 and March 1991, 377 patients admitted for allergy study of asthma, rhinitis, or conjunctivitis were skin tested with a 1:20 wt/vol crude

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FIG. 1. A, Detail of flowering *M. annua*. B, Plants of *M. annua* in an urban environment.

extract of *M. annua* pollen. Routine prick tests were performed with three grass pollens (*Phleum pratense*, *Cynodon dactylon*, and *Phragmites communis*), two prevalent tree pollens (*Corylus avellana*, and *Olea europaea*), six weed pollens (*Parietaria judaica*, *Amaranthus retroflexus*, *Artemisia vulgaris*, *Chenopodium album*, *Plantago lanceolata*, and *Salsola kali*), two house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), six common molds (*Alternaria alternata*, *Cladosporium herbarum*, *Penicillium notatum*, *Aspergillus fumigatus*, *Fusarium* spp, and *Rhizopus nigricans*) and two animal danders (dog and cat). Of the

152 patients sensitized to some aeroallergen, 13 were sensitized to the crude extract of *M. annua* pollen. The characteristics and clinical features of these 13 patients, who form the study group, are summarized in Table I.

Methods

Preparation of *M. annua* pollen extract. *M. annua* specimens were collected in the Barcelona and Tarragona areas between November 1990 and March 1991. Defatted pollen from the above-mentioned specimens was extracted by magnetic stirring (24 hours at 4° C) in 0.1 mol/L of phosphate

TABLE I. Characteristics of 13 patients sensitized to *M. annua* pollen

Patient No.	Age (yr)	Sex	Symptoms	Other sensitizations*	Other features	
1	17	M	A, R	All-year round	<i>Artemisia</i> <i>Amaranthus</i> Grass Plantain	Previous 4-year grass pollen immunotherapy unsuccessful
2	20	F	A, R	All-year round	Dust mites <i>Alternaria</i> <i>Parietaria</i>	Occupational risk: working in a bakery
3	38	F	A, R, C	All-year round	Dust mites <i>Parietaria</i>	Previous 3-year <i>Parietaria</i> pollen immunotherapy partially successful
4	35	F	R	Winter, spring	Cat dander	Cat contact denied for the previous 5 years
5	20	M	R, C	Spring, summer	<i>Artemisia</i> Grass <i>Parietaria</i>	
6	24	M	A, R, C	All-year round	<i>Artemisia</i> <i>Parietaria</i> <i>Amaranthus</i> Plantain <i>Alternaria</i>	Bronchospasm while walking in a <i>M. annua</i> flowering area
7	16	M	A, R	Summer	<i>Parietaria</i>	
8	34	M	R, C	Winter, spring	<i>Parietaria</i> Olive	Previous 3-year <i>Parietaria</i> pollen immunotherapy unsuccessful
9	29	F	A, R, C	Spring, summer, autumn	Dust mites	Symptoms elicited by exposure to house dust
10	35	M	A, R, C	Spring	Olive Grass Hazelnut tree <i>Parietaria</i>	Previous 3-year grass pollen immunotherapy unsuccessful
11	51	M	R, C	Spring	<i>Parietaria</i> Olive Grass Hazelnut tree <i>Parietaria</i>	Previous 2-year <i>Parietaria</i> pollen immunotherapy partially successful
12	26	F	A, R, C	Spring	Olive Grass	
13	33	F	A	Winter	None	Admissions in emergency room in February 1989, 1990, 1991

A, Asthma; R, rhinitis; C, conjunctivitis.

*By prick test and RAST.

buffer, pH 8.0, at 15% wt/vol. Extract was clarified by centrifugation at 5600 g for 30 minutes, filtered through a 0.45 μ m pore diameter membrane, and dialyzed by ultra-centrifugation in a Pellicon System (Millipore Corp., Milford, Mass.) with a 5000 dalton cutoff. The dialyzed extract was sterilized by filtration through a 0.22 μ m pore diameter membrane and freeze-dried.

Protein concentration was estimated according to the method of Lowry et al.⁷ Carbohydrate content was measured by the anthrone method⁸ and by the orcinol method⁹ to determinate hexoses and pentoses, respectively. Pollen col-

lection revealed 95% purity, and pollen extract yielded 2.8 gm of allergenic material per 100 gm of pollen. Pollen content was 55.4% with 10.6% hexoses and 15.4% pentoses.

Aliquots of lyophilized extract of the same batch were used for all in vitro and in vivo tests performed in the 13 patients identified as positive by the crude extract.

Skin tests

M. annua pollen was diluted in phenolated albumin at concentrations of 0.1, 0.5, 1, and 5 mg/ml. Standardized

lancets (Phazet uncoated lancet, Pharmacia Diagnostics AB, Uppsala, Sweden) were used for prick tests. Histamine chlorhydrate, 10 mg/ml, and phenolated albumin were used as positive and negative controls, respectively. After 15 minutes, translucent tape was applied over the wheals obtained, and the areas were transferred to a record sheet. Areas were expressed in square millimeters.

The mean wheal area 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 wheal produced by histamine, 10 mg/ml, was defined as 1 HEP unit.¹⁰ 1 HEP unit was found to be equivalent to 2 mg/ml of allergen extract. This concentration was used to establish the maximum dose of allergen to be used in provocation tests (0.1 HEP unit per milliliter).

RAST

Solid-phase antigen was obtained by coupling the extract solution (10 mg/ml) to the 6 mm diameter CN Br-activated paper disks according to the method of Ceska and Lundqvist.¹¹ RAST was performed according to the method of Wide et al.¹² with Phadenzym RAST reagents (Pharmacia Diagnostics AB), according to the manufacturer's instructions.

Immunoblotting

Isoelectric focusing was performed on Isogel agarose plates (FMC Bio Products, Rockland, Maine), pH 3 to 10, and 20 µg of protein was applied per lane. Transfer of proteins from agarose gel to membrane (Immobilon P Millipore) was achieved by capillary blotting and contact diffusion according to the method of Elkon.¹³ Immunochemical staining was performed by first blocking the Immobilon membrane with 9% defatted dry milk in TBS (1 hour at 37°C). After three washes with TBS, the blots were incubated with 2.5 ml of undiluted human serum (16 hours at 4°C). The blots were washed three 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% wt/vol of 4-chloro-1-naphthol solution (Bio-Rad Laboratories, Richmond, Calif.) and 0.01% H₂O₂ in TBS. After 30 minutes, the blots were rinsed in distilled water and air-dried. Antigen-antibody binding was identified by purple-stain deposition.¹⁴

Nasal provocation test

Nasal provocation tests were performed at 9 AM in the 12 patients with rhinitis. Active anterior rhinomanometry was performed according to the criteria of the Committee Report on Standardization of Rhinomanometry.¹⁵ A Rhinospir 64 (Sibel, Barcelona, Spain), programmed to perform nasal provocation tests, was used. Airflow and resistances were recorded in an X-Y mirror image.¹⁵ After spraying 0.2 ml of diluent, increasing concentrations of allergen were sprayed into the same nostril every 15 minutes until resistances doubled the resistances induced by the diluent. The

concentrations used were 0.001, 0.01, and 0.1 HEP units per milliliter (equivalent to 0.002, 0.02, and 0.2 mg/ml, respectively). To detect possible late responses, nasal resistances were measured 4 and 6 hours after the last administration of allergen.

Bronchial provocation test

Bronchial provocation test was performed in patient No. 13 according to general guidelines for standardization of bronchial challenges with nonspecific bronchoconstricting agents of the European Society for Clinical Respiratory Physiology.¹⁶ An ultrasonic nebulizer (Pulmosonic, DeVilbiss Co., Somerset, Pa.) and a homologated spirometer (Datospir 92, Sibel) were used. After FEV₁ recording, diluent and allergen administrations were scheduled at 15-minute intervals, with five complete inhalations of each concentration. Allergen concentrations, starting from 0.0001 to a maximum of 0.1 HEP units per milliliter were aerosolized. The test was considered positive when a sustained 20% decrease in FEV₁ with respect to a decrease obtained by inhalation of diluent was recorded. FEV₁ was recorded hourly during the next 8 hours to detect and treat any possible late response.

RESULTS

Results of skin tests were strongly positive, with the allergen wheal areas being larger than wheal areas of histamine, 10 mg/ml, in most patients (Table II). Serum-specific IgE levels to *M. annua* pollen (RAST) ranged from 0.38 to 4.79 PRU/ml. RAST class 3 was found in one serum, whereas five sera demonstrated RAST class 2 and seven sera, RAST class 1 (Table II). In general, the highest RAST scores occurred in patients with more positive skin tests. Individual differences in the degree of sensitization, as demonstrated by differences in the equivalence for 1 HEP unit in skin tests, were observed. Provocation tests with *M. annua* pollen extract were positive in 11 of the 13 patients, and in two of the 11, late reactions were recorded (Table II). Six patients had a positive nasal test with the lowest concentration administered. Sneezing, itching, and nose running occurred in nine patients at some time during the provocation test. Patient No. 13 had a 39% decrease in FEV₁ 15 minutes after inhalation of the 0.002 HEP unit per milliliter concentration and a 24% FEV₁ decrease 7 hours later. Wheezing was recorded in both immediate and late response in one patient, and the patient was treated with inhaled terbutaline and budesonide before discharge.

Immunoblotting of the sera of 13 patients is illustrated in Fig. 2, A, and the characteristics (pI) of IgE-binding fractions for each serum are illustrated in Fig. 2, B. One IgE-binding fraction (pI, 10.2) was present in 12 of the 13 sera, two IgE-binding fractions (pI, 5.2 and 5.1) were observed in all the sera, and two

TABLE II. RAST, prick test, and provocation test in 13 patients sensitized to *M. annua* pollen

Patient No.	RAST		Prick test			Provocation test*	
	PRU/ml	Class	Allergen wheal† (mm ²)	Histamine wheal (mm ²)	HEP (mg/ml)	Immediate response‡ (mg/ml)	Late response
1	2.73	2	61	50	0.74	0.02	Neg
2	4.79	3	49	30	0.56	0.002	Neg
3	1.11	2	101	64	0.52	0.02	Neg
4	0.45	1	22	48	>50	0.02	Pos
5	0.55	1	33	116	>50	0.2	Neg
6	0.92	2	152	80	0.41	0.002	Neg
7	0.52	1	21	72	>50	Neg	Neg
8	0.47	1	38	49	5.25	0.002	Neg
9	0.38	1	34	36	2.57	0.002	Neg
10	1.38	2	69	30	0.12	0.002	Neg
11	1.42	2	74	40	0.29	Neg	Neg
12	0.48	1	82	48	0.18	0.002	Neg
13	0.59	1	77	64	0.85	0.002	Pos

Neg, Negative; Pos, positive.

*Patients Nos. 1 to 12, nasal provocation test; patient No. 13, bronchial provocation test.

†Allergen concentration, 2 mg/ml.

‡Concentration of allergen inducing positivity of provocation tests.

more IgE-binding fractions (pI, 5.3 and 5.5) were observed in 11 of the 13 sera.

DISCUSSION

The pollination period of *M. annua* in our geographic area is long lasting during fall, the complete winter, and spring (from October to June).³ High concentrations of this pollen have been recorded in Tarragona, reaching 9% of the total annual pollen count.³ This percentage is similar to that of more active allergenic pollens in our area, namely, *Parietaria judaica* and grass pollen. Despite its high prevalence and the characteristics of its pollen, *M. annua* has been underestimated as an allergenic source until now.

To our knowledge, only one prospective study has been performed, but failed to detect, significant sensitization.⁵ This finding may be due to the age of the population studied (children) and the fact that the pollen extract used was probably obtained from *M. perenne*, a species with a short summer-flowering period (August to October). Recently, the case of one patient suffering from asthma and sensitized to *M. annua* pollen has been described, but details of other possible concomitant sensitizations and challenge by provocation tests are lacking.⁶

The present study was designed as a prospective trial to detect sensitization to the pollen of *M. annua*. Sensitization to this pollen was detected in 13 (8.5%) of the 152 positive skin test patients detected during a 1-year period in our unit and accounted for the 17%

of the 78 patients sensitized to some kind of pollen during the same period. As with any other allergen, sensitization does not signify clinical symptoms on natural exposure. Nevertheless, we were able to establish a reasonable relationship between exposure and symptoms in patients Nos. 1, 4, 6, 8, and 13. Patients Nos. 1, 4, and 6 complained of acute allergic rhinitis in the vicinity of flowering *M. annua* and indicated that this plant was a probable cause of their symptoms. Patient No. 8 reported moderate rhinitis while he was in the open air during the winter. Patient No. 13 had begun to suffer asthma attacks 3 years earlier, which began in October and lasted until March. She remained asymptomatic the rest of the year and demonstrated no sensitization to any of the routine battery of allergens. She had been diagnosed as having "intrinsic" asthma with crises related to winter influenza-like illnesses. In the remaining eight patients, allergic symptoms could be fully accounted for by other concomitant sensitizations (mainly mites and *Parietaria judaica* pollen).

Positive provocation tests in 11 of the 13 patients suggest a close relationship between target organ exposure to *M. annua* pollen and clinical symptoms. Two patients (patient No. 4 in nasal provocation test and No. 13 in bronchial provocation test) demonstrated late reactions, which suggested the IgE-mediated nature of the process.

The pattern of IgE-binding proteins, obtained after isoelectric focusing, and subsequent capillary transfer

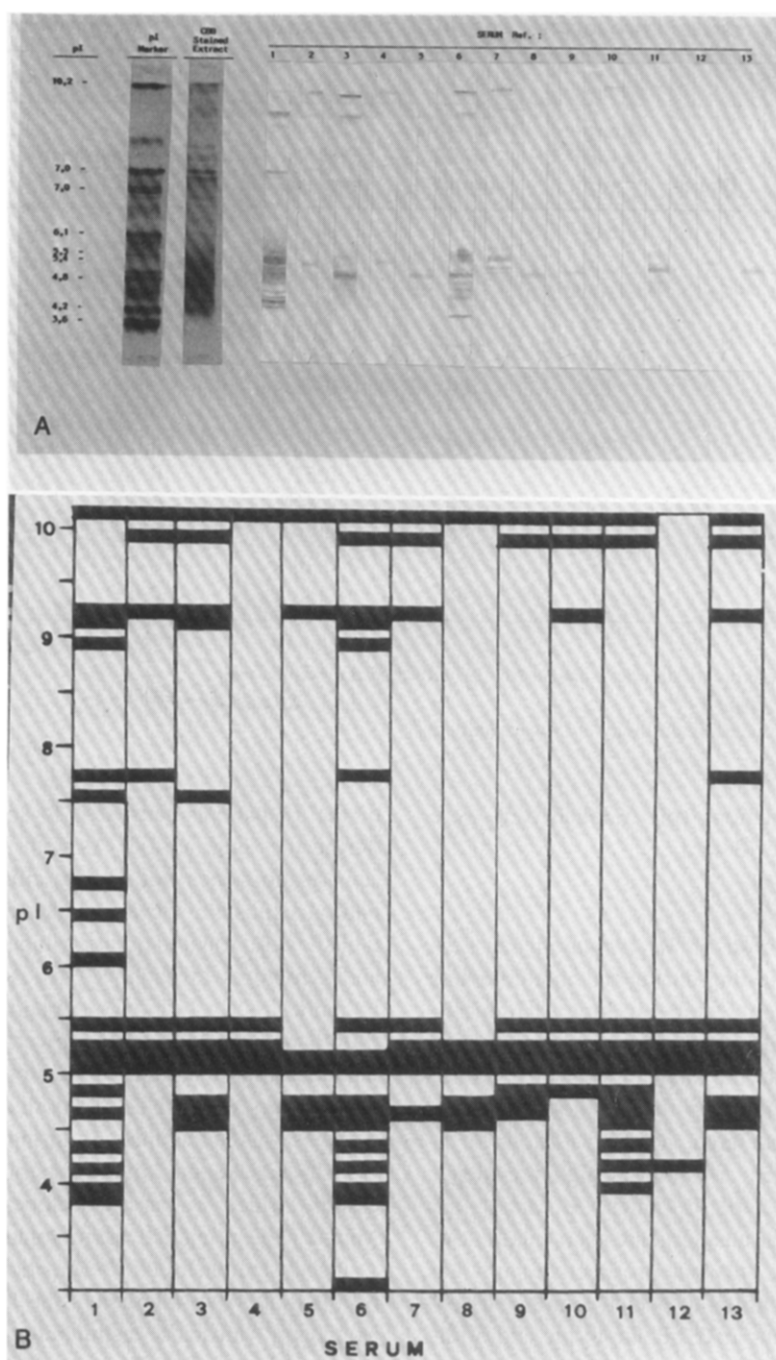


FIG. 2. A, Agarose isoelectric focusing immunoblotting from 13 patients sensitized to *M. annua* pollen. Protein profiles of pI marker and of Coomassie brilliant blue (CBB)-stained pollen extract are illustrated on left. B, Allergogram obtained by isoelectric focusing immunoblotting of *M. annua* pollen extract illustrating characteristics (pI) of IgE-binding proteins from patients' sera.

to Immobilon membranes, indicates the presence of two main groups of allergenic proteins: one of a basic nature (pI, 10.2) and the other protein composed of four close bands of acidic nature (pI, 5.0 to 5.5). These components, reacting with >80% of the indi-

vidual sera included in this study, could be considered as clinically relevant allergens. The basic component exhibits a pI coinciding with that of cytochrome c, major allergen from *Poa pratense* and *Lolium perenne*.¹⁷ It would be worthwhile to conduct further

studies with this *M. annua* fraction (pI, 10.2) to establish the possible cytochrome nature of the mentioned protein.

The present study reveals *M. annua* pollen to be capable of inducing both sensitization and disease in natural conditions. Thus, this pollen should be taken into account as a cause of respiratory allergic disease in our area, since 8.5% of aeroallergen skin test-positive patients were positive to this pollen. As described recently with other allergens,¹⁸ some patients diagnosed as having "intrinsic" asthma or rhinitis may, in fact, be sensitized to unknown allergens not included in skin test batteries of common aeroallergens. This finding might explain the lack of basic differences between so-called extrinsic and intrinsic forms of asthma¹⁹ and encourage the research of hidden allergic factors in the etiology of rhinitis and asthma.²⁰

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