

Allergenic Cross-Reactivity among Pollens of *Urticaceae*A.L. Corbí^a, C. Cortes^a, J. Bousquet^b, A. Basomba^c, A. Cistero^d, J. Garcia-Selles^e, G. d'Amato^f, J. Carreira^a^a Departamento de Investigación Biológica, Alergia e Inmunología Abello, Madrid, España; ^b Centre Hospitalier Régional de Montpellier, France; ^c Ciudad Sanitaria La Fe, Valencia, España; ^d Hospital Santa Cruz y San Pablo, Barcelona, España; ^e Ciudad Sanitaria Virgen de la Arrixaca, Murcia, España; and ^f Ospedale A. Cardarelli, Napoli, Italia

Abstract. Common antigenic determinants have been observed between *Parietaria* and *Urtica dioica* pollen. The four *Parietaria* pollens selected (*P. judaica*, *P. officinalis*, *P. lusitanica* and *P. mauritanica*) are shown to possess a high allergenic homology. IgE-binding structures, homologous to the *P. judaica* main allergenic polypeptide (Pj10), were found in the other species by immunodetection. Monoclonal antibodies specific to the Pj10 polypeptide recognized proteins from the four *Parietaria* pollens. Skin prick test and RAST inhibition yielded results that also indicated a high allergenic cross-reactivity among these pollens, with homologous peptides bearing common antigenic and allergenic determinants. On the other hand, *U. dioica* pollen showed only a slight allergenic similarity to *Parietaria*. The potential allergenic activity of these pollens is discussed.

Introduction

The finding of common polypeptides in different allergenic sources has become an important subject both for the knowledge of the IgE-binding structures they present and for the precise diagnosis and correct therapy of allergic diseases. In this way, studies about cross-reactivity between allergy-inducing pollens have been reported [Howlett et al., 1982; Weber et al., 1978; Martin et al., 1980].

Parietaria judaica pollen is known to be a common cause of allergy in the Mediterranean regions, and although some other pollens of the same family (*Urticaceae*) are also spread in the same area, they have a more restricted distribution [Raffaelli, 1977]. This plant family has been reported to be of great clinical importance in southern and western Europe [Charpin et al., 1977].

On the other hand, allergy to *Urtica* pollens still remains a disputable matter. Some authors have reported positive cutaneous reactions to this pollen, others described it as a potential source of allergy disorders [Charpin et al., 1974; Solomon and Cathey, 1970]. However, neither clinically demonstrated human allergy nor specific IgE-binding polypeptides have been so far assigned to any component of *Urtica dioica* pollen.

Trying to find out the potential allergenic cross-reactivity between pollens of plants from this family, four different *Parietaria* pollens were selected for study (*P. judaica*, *P. officinalis*, *P. lusitanica* and *P. mauritanica*) together with *U. dioica* pollen, which is widely distributed around western Europe.

In the present paper, we have used the *P. judaica* main allergenic structures (Pj10 polypeptide and CIE precipitation bands A1 and A2) [Corbí and Carreira, 1984a, b] as reference preparations for comparison among the antigenic and allergenic components from these five pollens. A high degree of allergenic homology has been found in the four *Parietaria* species. Conversely, only antigenic cross-reactivity has been observed between *Urtica* and *Parietaria* pollens. The allergological relevance of these pollens is discussed.

Materials and Methods

Pollen Extraction and Protein Determination

P. judaica and *U. dioica* pollens were purchased from Allergon AB (Vällinge, Sweden). *P. officinalis* was provided by Dr. Bousquet. *P. lusitanica* and *P. mauritanica* were collected in the west of Spain by Dr. J.A. Jimenez and Dr. J. Jimenez. All the pollens were tested for 70% purity, at least. The five pollens were extracted in an ammonium bicarbonate-buffered solution as described [Corbí and

Carreira, 1984a]. Protein measurements were done according to the method of Lowry et al. [1951].

Polyacrylamide Gel Electrophoresis, Isoelectric Focusing and Immunodetection

Polyacrylamide gel electrophoresis (PAGE) was done in presence of sodium dodecyl sulfate (SDS) following the method described by Laemmli [1970], under reducing conditions. Isoelectric focusing (IEF) was performed as previously described [Corbi and Carreira, 1984b], with 0.5% NP-40 in the gel. For immunodetection, SDS-PAGE or IEF separated proteins were electrophoretically transferred onto nitrocellulose sheets [Towbin et al., 1979] and after blocking of paper adsorptive sites with PBS 1% BSA, the sheets were successively incubated in serum containing IgE against *P. judaica*, washed and incubated in ¹²⁵I-labeled anti-IgE polyclonal antiserum (Pharmacia, Sweden). Then, the nitrocellulose membranes were washed, dried and autoradiographed with intensifying screens at -70°C.

RAST Inhibition and Skin Prick Test

RAST inhibition was performed according to the procedure of Yman et al. [1975]. Each pollen extract was assayed for inhibition of the binding of IgE from a serum pool of *P. judaica* allergic patients to *P. judaica* pollen extract previously coupled to CNBr-activated paper discs [Ceska et al., 1972].

Two pollen extract dilutions, containing 350 and 35 µg/ml of protein, were used for skin prick testing on the forearm of 52 patients. Wheal size areas were measured 20 min after the prick was done. Prick-tested patients were selected in areas where *P. judaica* was mainly found, in an attempt to avoid the possibility of sensitization to the other *Parietaria* pollens.

Crossed Immunoelectrophoresis and Crossed Radioimmunoelectrophoresis

Crossed immunoelectrophoresis (CIE) of the five pollen extracts [Weeke 1973] were done with the second dimension gel containing anti-*P. judaica* rabbit serum. For crossed radioimmunoelectrophoresis (CRIE) [Weeke and Lowenstein, 1973], CIE plates were incubated with anti-*P. judaica* human serum and ¹²⁵I-labeled anti-human IgE.

Antisera and Monoclonal Antibodies

Rabbit serum against *P. judaica* pollen extract was raised as described [Corbi and Carreira, 1984a]. Human sera from *P. judaica*-sensitive patients were collected and pooled in areas where only *P. judaica* could be found. The serum pool was maintained at -80°C until use.

Monoclonal antibodies against *P. judaica* pollen proteins AC/1.1 (γ₁, κ; anti-Pj10) and AC/9.1 (γ_{2b}, κ) were produced in our laboratory and will be described elsewhere [Corbi et al., submitted for publication].

The 187-1 MAb, a rat anti-mouse Kappa chain monoclonal antibody (MAb), was kindly provided by Dr. M. Scharff (Albert Einstein Medical School, N.Y.) and it was radioiodinated in solution with chloroglycoluril [Fraker and Speck, 1978].

Immunodetection on CIE Precipitation Bands

The identification of the precipitation bands recognized by anti-*P. judaica* monoclonal antibodies was carried out by incubation of the CIE plates with the MAb diluted 1:100 in 50 mM PBS, pH 7.4, followed by incubation with ¹²⁵I-labeled 187-1 MAb.

Results

Protein Yield and Allergenicity 'in vivo' and 'in vitro'

After extraction, protein concentration of the extracts was measured, and the protein yield calculated as milligrams of protein per gram of pollen (table I). *U. dioica* showed the highest ratio, while *P. lusitanica* and *P. mauritanica* rendered the lowest protein yield.

When these five pollens were tested by RAST inhibition at 100 µg/ml, all the *Parietaria* pollens were able to inhibit the binding of *P. judaica*-specific IgE to *P. judaica* pollen extract coupled to paper discs (table I). *U. dioica* failed to do so, even when higher amounts of protein were assayed. This fact proved that common allergenic determinants were present in the four *Parietaria* pollens. To support this finding with 'in vivo' studies, patients allergic to *P. judaica* were skin-prick tested with the five *Urticaceae* pollen extracts and similar results to those described above were obtained. *P. judaica* was found to be the pollen that produced the highest wheal size. It was followed by *P. officinalis* and *P. lusitanica* which rendered the same wheal area (table II). *P. mauritanica* induced slightly lower-sized wheals, while *U. dioica* gave no reactions, except for a few patients whose allergy to *U. dioica* could not be ruled out.

Antigenic Cross-Reactivity

The four *Parietaria* extracts were analyzed by CIE and they showed similar patterns of precipitation bands, indicating that common antigenic determi-

Table I. Protein yield and percentage of inhibitory capacity of each extract measured by RAST inhibition

	Protein yield g protein/ 100 g pollen	Amount of protein giving 50% inhibition, µg	In vitro relative allergenicity
<i>P. judaica</i>	6.9	0.3	100
<i>P. officinalis</i>	3.22	1.6	18.7
<i>P. lusitanica</i>	1.06	2.9	10.3
<i>P. mauritanica</i>	1.44	9.45	3.1
<i>U. dioica</i>	11.06	-	-

Relative allergenicity was calculated as percentage of the inhibitory ability of *P. judaica*, which was considered as 100%.

nants were present in their components. On the contrary, *U. dioica* only produced a very anodic precipitation curve, suggesting that the antigenic cross-reactivity between *U. dioica* and *Parietaria* genus pollens was very low (fig. 1). In addition, the two IgE-binding precipitation bands of *P. judaica*, previously reported to contain the major allergenic polypeptide Pj10 [Corbi and Carreira, 1984a, b], were also detected in the other *Parietaria* pollens studied.

For a better antigenic comparison, the five *Urticaceae* CIE plates were incubated with two different monoclonal antibodies raised against *P. judaica* pollen extract [Corbi et al., submitted for publication]. One of them, AC/1.1 MAb, which is directed against the Pj10 polypeptide, was able to recognize two different precipitation bands in the four *Parietaria* pollens (fig. 2), but failed to bind to components from the pollen of *U. dioica*. On the contrary, AC/9.1 MAb, which binds to a non-allergenic anodic band from *P. judaica* pollen, was found to recognize a homologous component in the five pollens assayed (fig. 2). These results demonstrated a very close antigenic homology among *Parietaria* pollens, whereas *U. dioica* did not seem to be allergenically related.

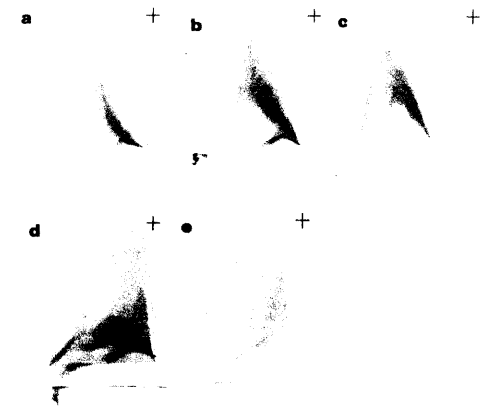


Fig. 1. Crossed immunoelectrophoresis (CIE) patterns of *P. judaica* (a), *P. officinalis* (b), *P. lusitanica* (c), *P. mauritanica* (d) and *U. dioica* (e) pollen extracts. The first dimension was performed at 400 V for 30 min. The second dimension against an anti-*P. judaica* rabbit antiserum-containing gel was accomplished at 150 V for 20 h. CIE plates were then washed, dried and stained with Coomassie Blue.

Table II. Arithmetic mean of the wheal area (mm²) produced when the five extracts were used in prick tests at two different (350 and 35 µg/ml) protein concentrations

	<i>P. judaica</i>		<i>P. officinalis</i>		<i>P. lusitanica</i>		<i>P. mauritanica</i>		<i>U. dioica</i>	
	350 µg/ml	35 µg/ml	350 µg/ml	35 µg/ml	350 µg/ml	35 µg/ml	350 µg/ml	35 µg/ml	350 µg/ml	35 µg/ml
Murcia 10 patients	not tested	42.7	85.1	36.8	69.6	32.6	49.0	29.1	0	0
Barcelona 12 patients	65.3	34.4	38.5	23.8	45	24.5	41.0	18.5	1.58	0.25
Valencia 10 patients	110.1	32.3	54	26	66.2	35.9	62.7	28.5	14.2	11.6
Montpellier 10 patients	24.2	7.6	17.3	7.4	22.9	5.8	13.1	6.6	4.4	1.8
Naples 10 patients	60.6	26.0	41.0	17.3	41.8	17.2	21.8	10.1	1.6	1.3
Total arithmetic mean	65.5	28.6	47.18	22.4	49.1	23.2	37.6	18.56	4.35	2.99

Wheal areas obtained in France were smaller due to incorrect transport conditions, which caused loss of activity.

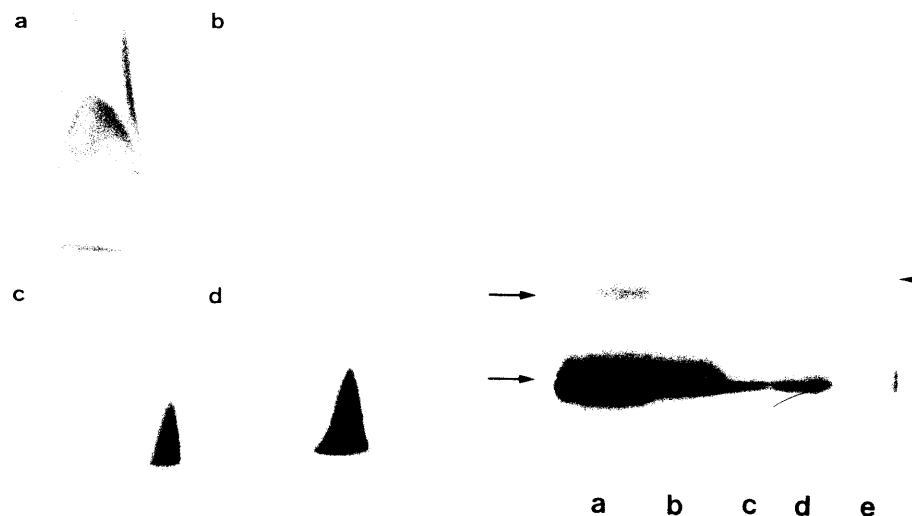


Fig. 2. Recognition of *Urticaceae* pollen proteins by anti-*P. judaica* MAb. The CIE plates of *P. officinalis* (a, c) and *U. dioica* (b, d) were incubated with AC/1.1 MAb (a, b) or AC/9.1 MAb (c, d), diluted 1:100 in 50 mM PBS, pH 7.4. After washing they were incubated with ^{125} I-labeled 187-1 MAb (100,000 cpm/plate). Finally, plates were washed and developed for autoradiography. The same technique was applied to *P. lusitanica* and *P. mauritanica* pollen extracts, which rendered the same results as *P. officinalis*.

Allergenic Cross-Reactivity

In a previous paper, we have reported the presence of the main *P. judaica* IgE-binding polypeptide Pj10 in two allergenic CIE bands A1 and A2 [Corbi and Carreira, 1984b]. Since the anti-Pj10 MAb AC/1.1 was also able to detect two distinct precipitation bands in the four *Parietaria* pollens, it follows that antigenic determinants found in the major *P. judaica* allergenic polypeptide were also present in pollens of the *Parietaria* species investigated. So, it is suggested that a close allergenic homology exists among them. In order to clearly demonstrate common allergenic proteins in the five pollens, immunodetection of IgE-binding proteins was achieved using a human serum pool with IgE antibodies to *P. judaica* (fig. 3). The

four *Parietaria* extracts exhibited a 10,000-dalton polypeptide with *P. judaica*-specific IgE-binding ability. In addition, some other higher molecular weight allergenic polypeptides were demonstrated in the four pollens. On the other hand, *U. dioica* did not show the 10,000-dalton allergenic component. However, a higher molecular weight polypeptide with low IgE-binding ability was detected. On comparing all the allergenic patterns, *P. judaica* and *P. officinalis* were those exhibiting the closest relationship.

The comparison of their allergenic components under non-denaturing conditions was accomplished by immunodetection after analytical IEF (fig. 4). The four *Parietaria* pollens exhibited proteins which were recognized by *P. judaica*-specific IgE although no



Fig. 4. Immunodetection of IgE-binding proteins after isoelectric focusing (IEF) of the pollen extracts. The five extracts (0.4 mg of pollen protein) were subjected to IEF in the presence of 0.5% NP-40, and then transferred to nitrocellulose membranes and incubated as described in figure 3. Each extract is represented by the same letter as in figure 1.

binding was detected with the *U. dioica* proteins. Moreover, the highest degree of homology was observed between *P. judaica* and *P. officinalis*.

As Pj10 had been previously reported to be component of A1 and A2 [Corbi and Carreira, 1984a] CRIE bands, the five pollens were subjected to the same technique in order to find out whether *P. judaica*-specific IgE was able to recognize the precipitation bands produced by the different *Urticaceae* pollens (fig. 5). Again, results clearly indicated the presence of common allergenic determinants, although no *U. dioica* precipitation band was recognized. Thus, no common allergenic determinant of *Parietaria* and *Urtica* could be found by CRIE, while A1 and A2 bands were detected in the four *Parietaria* pollens.

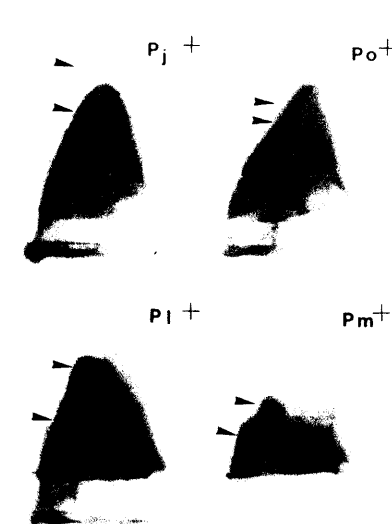


Fig. 5. Crossed radioimmunoelectrophoresis (CRIE) of the five extracts. After CIE, the immunoplates were dried under cold air and incubated with human anti-*P. judaica* serum (specific IgE) and ^{125} I-labeled anti-IgE polyclonal rabbit serum. The immunoplates were then exposed to X-ray medical film for 2 days at -70°C with intensifying screens. The arrows show the two major allergenic precipitation bands rendered by *P. judaica* (Pj), *P. officinalis* (Po), *P. lusitanica* (Pl) and *P. mauritanica* (Pm).

Discussion

In the present paper, four *Parietaria* pollens have been shown to exhibit common antigenic and allergenic determinants. An IgE-binding polypeptide of 10,000 daltons, previously described to be the major allergenic polypeptide of *P. judaica* pollen, has also been found in the other three species. *P. officinalis* and *P. lusitanica* have shown a very close allergenic homology to *P. judaica* by CIE and immunodetection.

The AC/1.1 MAb, directed against the allergenic polypeptide, has been very useful for the primary demonstration of allergenic cross-reactivity. In this way, antigenic determinants born by the major IgE-binding polypeptides of *P. judaica* have been detected in

the other three pollens of this genus by incubation of the CIE plates with the MAB. Furthermore, this MAB also binds to a 10,000-dalton polypeptide in all the *Parietaria* pollens, after SDS-PAGE (data not shown). This fact, together with the finding of IgE-binding proteins with the same molecular weight in all these extracts, strongly suggest that a homologous polypeptide is contained in these four pollen extracts. The ability of IgE and AC/1.1 MAB to bind to these polypeptides after SDS treatment seems to show that the recognized epitopes are sequential determinants non-dependent on the structure. Immunodetection of allergenic proteins in a group of pollens also supports this idea [Lombardero et al., in preparation]. This has been demonstrated to be the case in the only allergenic determinant that has been sequenced to the present time [Elsayed and Apold, 1983].

Data provided when *U. dioica* was studied deserve special consideration. Although CIE and CRIE analysis indicated only common antigenic determinants with *P. judaica*, immunodetection showed an *U. dioica* polypeptide with a slight ability to bind *P. judaica*-specific IgE, thus revealing a weak allergenic cross-reactivity. This poor allergenic homology can explain the lack of inhibition observed in RAST, even when high amounts of *U. dioica* proteins were assayed. On the other hand, a small percentage of individual patients, diagnosed to be allergic to *P. judaica*, were found to be sensitive to *U. dioica* also. Probably, these represented a group of patients especially sensitive to the cross-reacting 20,000-dalton polypeptide immunodetected in *U. dioica* (fig. 3). The non-precipitation of this protein or the absence of rabbit antibodies against it can explain the lack of allergenic bands when the pollen extract was subjected to CRIE.

It is a well-known fact that phylogenetically related families differ greatly in their allergenic relevance. This has led some authors to point out the possibility that the allergenic structures are family- (or genus-) specific antigens for the recognition of the pollen by the plant it reaches [Knox et al., 1972]. In this sense, AC/9.1 MAB defines an antigenic determinant which is common to the *Parietaria* and *Urtica* genera. However, this protein has no allergenic significance. Conversely, the main allergenic structure of *Parietaria*, which is Pj10 (or A1 and A2 CIE precipitation bands) seems to be a genus-specific antigen, as it is not detected in other genera of the same family.

All the above results suggest that the antigenically cross-reactive proteins of *Parietaria* also retained

common allergenic determinants. This indicates that some of the patients now diagnosed as *P. judaica* sensitive can be actually sensitized to other *Parietaria* pollens or even to *U. dioica*, though this has still to be proven. In spite of this, their allergenic activities have been shown to be quite different. This fact, together with the low protein yield rendered by some of them (*P. lusitanica* and *P. mauritanica*), is a great enough reason to explain that up to the present time they have not been considered to be allergologically relevant.

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Correspondence to: A. L. Corbi Lopez,
Departamento de Investigación Biológica,
Alergia e Inmunología Abello,
Julian Camarillo, 8,
Madrid-17 (Spain)