

Cross-reactivity between *Parietaria* species using the major rParj1 and rParj2 allergens

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ABSTRACT

Parietaria pollen is one of the most important outdoor allergenic sources in all the Mediterranean countries, with a large number of subjects showing a positive skin prick test to both *P. judaica* and *P. officinalis* pollen extracts. A cross-reactivity between the two species has been already reported although few data are known at the molecular level. Twenty-five consecutive patients with *Parietaria* pollen allergy were selected on the basis of their clinical history. Skin prick test to *P. judaica* and *officinalis* extracts was performed. In vitro IgE measurement to both allergenic sources was performed by using a quantitative assay. ELISA inhibition experiments were made by using the rParj1 and rParj2 allergens. All the patients showed a positive skin prick test to both *Parietaria* species. Quantitative IgE measurement showed similar antibody concentration for *P. judaica* and *P. officinalis* extracts. ELISA inhibition experiments demonstrated that the cross-reactivity between the two species was due to the presence of the Parj1 and Parj2 allergens in the extracts with a high conserved IgE epitopes content. We conclude that *P. judaica* and *P. officinalis* pollens contain highly cross-reactive species-specific major allergens useful for the diagnosis and therapy of both allergenic sources.

(Allergy Asthma Proc 27:378–382, 2006; doi: 10.2500/aap.2006.27.2927)

Parietaria pollen is one of the most common outdoor allergenic sources in the Mediterranean area.¹ Epidemiologic studies have shown that almost 30% of all the allergic subjects in the Southern part of Italy presented a skin prick test (SPT) reactivity toward this pollen, and that more than 50% of these allergic subjects experienced bronchial asthma with high levels of bronchial hyper-responsiveness.² The genus *Parietaria* is composed of a few species with different immunologic importance. *P. judaica* (Pj) and *P. officinalis* (Po) represent the most relevant allergenic species.³ By means of DNA recombinant technology, we were able to isolate and characterize the two major allergens of the Pj pollen, named Parj1 and Parj2.³ Parj1 and Parj2 allergens are polypeptides of 14.726 and 11.344 Daltons, respectively, with a few common structural similarities.^{4–6} In fact, data bank analysis showed that both allergens belong to a widespread family of plant proteins named non-specific lipid transfer

proteins (nsLTP).⁷ The nsLTP are very stable allergens, as shown by their extreme resistance to pepsin digestion and heat treatment.⁸ Their distribution among plant foods suggest a potential role of these proteins as plant panallergens.⁸ We expressed these two allergens in *Escherichia coli* and the allergenic activity of the recombinant molecules was studied by means of several techniques such as Western blot, histamine release assay, skin prick test, proliferation assays and so on^{4–6,9} showing a pattern of reactivity similar to the native counterpart.

Whereas the allergens of the *P. judaica* pollen have been well described, few data are available for other *Parietaria* species. The relevance of this weed as a major cause of allergic disease in the Mediterranean countries underlines the importance of the identification and analysis of the allergen extracts. This will allow us to design test systems for the diagnosis and for a correct formulation of vaccines useful for the *Parietaria* allergic patients. In this paper we described the use of purified rParj1 and rParj2 allergens to compare the allergenic composition of the Pj and Po natural extracts at the molecular level.

MATERIALS AND METHODS

Study Population and Skin Prick Testing

Patients enrolled in this study fulfilled the following criteria: 1) seasonal rhinitis or bronchial asthma during *Parietaria* pollen season; 2) no previous *Parietaria*-spe-

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cific immunotherapy; 3) no undergoing glucocorticosteroid or antihistamine treatments. Twenty-five consecutive patients with positive SPT to *Parietaria* extract (13 females and 12 males, aged 15–66 years, mean age 28.68) were selected during pollen season between May and July 2003 from the Unità di Allergologia dell'Ospedale Civico, Palermo, Italy. Skin prick test was performed on the patients' forearms using *P. officinalis* and *P. judaica* extracts (Alyostal prick test, 100 IR/mL, Stallergenes, France). Histamine dihydrochloride (10 mg/mL) was used as a positive control, saline solution (NaCl 0.9%) as a negative control. Wheals and flares were marked with a demographic pen on the allergic patients' forearms 20 minutes after the prick and the size expressed in millimeters. Sera were collected and stored at -20°C . Pj and Po IgE measurements were made by using the Pharmacia CAP System.

Crude Extracts of *P. judaica* and *P. officinalis* Pollen

Pollens from *P. judaica* and *P. officinalis* were purchased from Allergon AB (Valinge, Sweden). Aqueous extracts were prepared as previously described¹⁰ and checked for quantity and quality by SDS-PAGE and Coomassie brilliant blue staining. Total protein amount was also measured by using the Bradford protein assay.

Recombinant Allergens

Recombinant Pj allergens (Parj1 and Parj2) were expressed in *E. coli* as His-tagged fusion proteins (pQE30 vector, QIAGEN, Rawley, U.K.). Purification was performed as previously described⁹ and the concentration of the proteins determined by SDS-PAGE and Coomassie brilliant blue staining followed by densitometric analysis (BIORAD Chemidoc, Quantity One software Version 4.2.1, Milano, Italy).

ELISA Detection

ELISA was performed by adding 200 μL of a solution containing Pj or Po extracts (15 μg /well) in coating buffer (sodium carbonate buffer pH 9.5) to each well of polystyrene plates, overnight, at room temperature. After several washes in solution A (1 $\times$ PBS, 0.1% Tween 20), plates were saturated with a solution containing 3% BSA and 0.5% Tween 20 in coating buffer, for 1 hour, at room temperature. After washing, 200 μL of sera (1:5 dilution) were incubated for 5 hours at room temperature. After three washing steps in solution A, bound IgE antibodies were detected with a goat antihuman IgE-HRP conjugate (Biosource International, Nivelles, Belgium) diluted 1 to 1000 in solution A. Colorimetric reaction was developed by adding 200 μL /well of substrate solution (0.4 mg/mL o-phenyl-

enediamine in 0.1 M citrate buffer). Optical density was read at 450 nm in a BIORAD micro-plate reader.

Inhibition experiments were performed pretreating the sera with an increasing concentration of the rParj1 and rParj2 allergens (from 0.1 to 10 μg /mL), overnight at room temperature, in duplicate. The reduction of human IgE binding after preincubation with the wild-type allergens was determined according to the formula: % inhibition of IgE binding = $100 - [\text{ODP} / \text{ODU} \times 100]$, where ODP and ODU represent the absorbance after preincubation with allergens and with no competitor, respectively. Data reported in TAB.2 represent the highest percentage of inhibition achieved. Residual IgE binding activity was detected as above described.

RESULTS

In Vivo and *in Vitro* Analysis of IgE Response

All the patients enrolled in this study showed a double positive SPT toward the Pj and Po extracts and detectable *Parietaria* specific-IgE antibodies. All of them displayed comparable wheal size (mean wheal diameter $\pm$ SD: 11.36 ± 3.21 for the Pj extract and 10.72 ± 2.87 for the Po extract) and elevated specific IgE (mean value $\pm$ SD) with values of 31.05 ± 20.16 kUA/L for the Pj extract and of 39.67 ± 17.15 kUA/L for the Po extract. Only 3 out of 25 subjects (patient numbers 3, 5, and 16) (12%) with very high concentration of Pj- and Po-specific IgE (>31 kUA/L) showed some discrepancies (see Table 1 for details).

ELISA Inhibition

Eight patients (4 males and 4 females) were studied in an ELISA inhibition experiment to determine the percentage of inhibition of the binding to the Pj- and Po-specific IgE by the rParj1 and rParj2 allergens. We selected patients with very high IgE concentration (numbers 4 and 8), with high IgE concentration (9, 22, and 24) and lower IgE concentration (11, 12, and 25). The pattern of inhibition showed an almost identical reactivity for the two allergenic sources (mean value $\pm$ SD). All the sera reacted against both molecules; rParj2 was able to bind $58.62\% \pm 15.08\%$ of the Pj-specific IgE and $58.25\% \pm 13.80\%$ of the Po-specific IgE. The rParj1 allergen inhibited to a lower extend the binding of the *Parietaria*-specific IgE antibodies to the Pj extract ($40.62\% \pm 18.26\%$) and Po extract ($41.62\% \pm 11.00\%$) (Table 2). In addition, when the same sera were preincubated with a solution containing equimolar amounts of the rParj1 and rParj2 polypeptides, we observed a similar percentage of inhibition to both extracts ($71.87\% \pm 9.87\%$ for the Pj extract and $71.25\% \pm 10.13\%$ for the Po extract) showing that Parj1 and Parj2 are major allergens for both species (Table 3).

Table 1 Skin prick test and IgE measurement to *P. judaica* and *P. officinalis* extracts

Patient number	Sex	Age	Symptoms	<i>Pj</i> IgE (kUA/L)	<i>Po</i> IgE (kUA/L)	<i>Pj</i> SPT mean wheal diameter (mm)	<i>Po</i> SPT mean wheal diameter (mm)
1	f	24	R/A	48.6	47.7	13	12
2	f	42	R/A	>100	>100	13	12
3	m	28	A	68.5	31.8	13	14
4	f	28	R/A	>100	>100	10	12
5	f	42	R/A	>100	72	12	8
6	f	37	R	7.69	10.1	6	6
7	f	15	R/A	5.56	5.60	5	6
8	f	29	R/A	>100	>100	16	9
9	m	66	A	33	24	18	18
10	m	17	R	0.57	0.77	9	8
11	f	15	R/A	n.d.	n.d.	12	13
12	m	38	R/A	19	18.16	9	12
13	m	40	R	44.8	29.5	12	13
14	f	16	RC	10.5	10.6	7	7
15	f	31	R/A	2.13	2.57	10	9
16	m	19	R	66.3	>100	13	13
17	m	22	R	28.5	35.2	7	8
18	f	18	A	17.4	15.9	15	11
19	f	18	R	28.4	27.4	8	8
20	m	25	R/A	11.7	13	11	10
21	m	34	R	20.8	18.1	14	14
22	f	21	R	36.7	31.6	11	10
23	m	22	R/A	>100	>100	12	12
24	m	28	R/A	42.3	37.1	15	10
25	m	42	A	19.8	21.1	13	13
Mean $\pm$ SD				31.05 $\pm$ 20.16	39.67 $\pm$ 17.15	11.36 $\pm$ 3.21	10.72 $\pm$ 2.87

R = rhinitis; A = asthma; RC = rhinoconjunctivitis; SPT = skin-prick test; *Pj* = *Parietaria judaica*; *Po* = *Parietaria officinalis*.

Table 2 ELISA inhibition of the IgE binding activity of the *Parietaria judaica* and *Parietaria officinalis* extracts after preincubation with the recombinant Parj1 and Parj2 allergens

Patient number	Competitor and antigen			
	rParj1 (%)		rParj2 (%)	
	<i>P. judaica</i>	<i>P. officinalis</i>	<i>P. judaica</i>	<i>P. officinalis</i>
4	20	27	48	55
8	33	38	85	84
9	30	45	50	63
11	60	41	69	52
12	38	45	46	53
22	20	24	44	35
24	59	58	54	61
25	65	55	73	63
Mean $\pm$ SD	40.62 $\pm$ 18.26	41.62 $\pm$ 11.00	58.62 $\pm$ 15.08	58.25 $\pm$ 13.80

DISCUSSION

The diagnosis of allergic reactions in practice is based on both clinical history and presence of specific IgE in

the serum and/or on skin mast cells. In the last 20 years, a great boost of information has been achieved about the composition of the allergenic extracts by

Table 3 Inhibition of IgE binding to ELISA plate-bound *Parietaria judaica* or *Parietaria officinalis* extracts after preincubation with a mixture containing the Parj1 and Parj2 allergens

Competitor antigen	rParj1 + rParj2 (%)	
	<i>P. judaica</i>	<i>P. officinalis</i>
4	56	60
8	75	78
9	71	72
11	70	59
12	78	83
22	60	63
24	85	85
25	80	70
Mean $\pm$ SD	71.87 $\pm$ 9.87%	71.25 $\pm$ 10.13%

means of the cloning of recombinant allergens.¹¹ This kind of analysis has shown that natural allergen extracts are a heterogeneous mixture of proteins where only a few components are responsible for the allergenic activity. Furthermore, the use of extract-based tests for the diagnosis of allergic disease is not enough to identify the specific molecules inducing the allergic response and, in some cases, is not able to distinguish between co-sensitization to different allergenic molecules and cross-reactivity between allergens.¹² Therefore, the use of purified allergens represents a powerful tool for a more precise diagnosis.

Parietaria is a family of dicotyledonous weeds of the Urticaceae family, including several genera growing in urban and rural areas. Among them, *P. officinalis* and *P. judaica* represent relevant allergenic source in geographical areas with temperate climate.³

Molecular biology techniques and immunologic studies have demonstrated that ~85% of the Pj allergic patients present high levels of Parj1- and Parj2-specific IgE in their sera.^{4–6,12} By means of immunoabsorption studies, we were also able to demonstrate that this pollen contains highly cross-reactive allergens (i.e., Ca²⁺ binding proteins, profilin) present in some other pollens and therefore the use of the whole *Parietaria* extract may lead to misleading diagnosis.¹² In addition, IgE measurement performed by the purified rParj2 allergen, showed that this allergen is a species-specific marker gene able to predict a primary sensitization toward the Pj pollen.¹² For these reasons, a genuine sensitization against the *P. judaica* pollen can be monitored *in vitro* looking for Parj1- or Parj2-specific IgE antibodies.

Several publications have dealt with the allergological characterization of other *Parietaria* species, describing an IgE cross-reactivity between the Po and Pj pollens.^{10,13} The Po major allergens were characterized by

N-terminal sequencing of mAbs affinity-purified proteins from pollen extract but no data on the full-length molecules are yet available.^{14–16} By means of ELISA inhibition experiments performed on a randomly selected number of subjects, we were able to prove that both allergenic sources contain two major allergens with highly cross-reactive IgE epitopes. All the allergic subjects recruited in this study presented similar IgE reactivity when analyzed for their Pj- and Po-specific IgE concentration (IgE quantitative assay or skin reactivity). By using recombinant purified Parj1 and Parj2 molecules, we were able to demonstrate that the *P. judaica* major allergens were able to inhibit the majority of the *P. judaica*- and *P. officinalis*-specific IgE to a similar extent, in all the tested sera. In conclusion, we were able to demonstrate that these nsLTPs represent major allergens for the *Parietaria* family with a high conserved IgE epitope and that the simultaneous reactivity to the *Parietaria* species is due to the cross-reactivity to Parj1 and Parj2. These data provide *in vitro* evidences that both rParj1 and rParj2 represent a powerful tool for the diagnosis¹² and future therapeutical options⁹ of the allergic reaction toward the *Parietaria* species.

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