

The Allergens of *Parietaria*

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Key Words

Parietaria pollen · Pollinosis · IgE · Natural allergens ·
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Hypoallergenic derivatives

Abstract

Parietaria is a genus of dicotyledonous weeds of the Urticaceae family including several species and its pollen grain is one of the most important allergenic sources in the Mediterranean area. Species belonging to this genus induce IgE responses in approximately 10 million people. Identification of allergens by means of independent strategies suggest that the allergens of the two more common species, *Parietaria judaica* and *Parietaria officinalis*, show molecular weights ranging between 10 and 14 kD and that the allergens of the two extracts are highly cross-reactive. Biochemical analysis and molecular cloning allowed the isolation and immunological characterization of the two major allergens of the *P. judaica* pollen, Par j 1 and Par j 2. Sequence comparison suggests that the P j major allergens of *P. judaica* belong to the non-specific lipid transfer protein family, and three-dimensional modeling by homology has revealed that both proteins present a very conserved structural motif composed of four α -helices. Immunological analysis has shown that Par j 1 and Par j 2 are able to bind most of the *P. judaica*-specific IgE and some of their IgE determi-

nants have been mapped. Recombinant Par j 1 and Par j 2 allergens have been shown to possess immunological properties equivalent to their natural counterpart and their availability represents a fundamental tool for the diagnosis and therapy of *Parietaria* pollen allergy.

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Introduction

Allergy is an IgE-mediated hypersensitivity reaction that represents a major health problem affecting almost 25% of the population in western countries. It is mainly due to inhalation of allergenic molecules from dust mite, animal dander and plant pollen. *Parietaria* are dicotyledonous weeds belonging to the Urticaceae family that are commonly found in the countryside and urban areas, growing on walls and soils with a high nitrogen content. Several reports have shown that *Parietaria* pollen is one of the most common causes of pollinosis in the Mediterranean area [1–6] with a very long period of pollination, reaching very high peaks (1,000 grains/m³ of air) between May and June [7] (fig. 1).

In particular, immunological studies have shown that almost 50% of the allergic patients in the southern part of Italy are allergic to this pollen and that more than 50% of these patients experienced asthma with severe bronchial hyperresponsiveness [7, 8]. Moreover, the presence of

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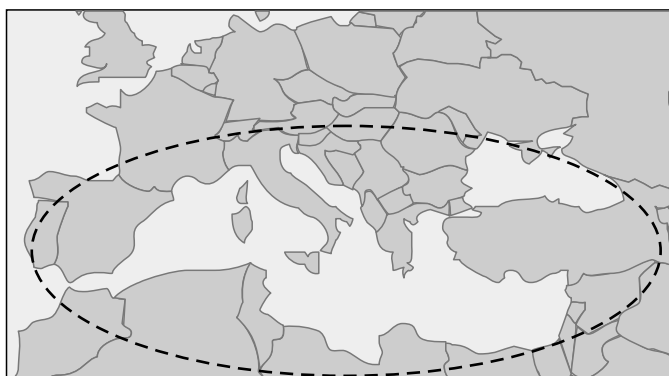


Fig. 1. Geographical distribution of the *Parietaria* species. Regions inside the oval represent areas where *Parietaria* pollen is a major allergenic source.

these weeds and related IgE has also been described in the temperate regions of Europe [9], Australia [10] and California [11]. A combination of transmission electron microscopy and immunocytochemical methods was used to localize the allergenic proteins in *Parietaria judaica* pollen that may become released explosively from the elastic filaments of the anther and become wind-borne [12]. Germination of pollen grains was induced in vitro for 10 and 30 min to activate proteins in the intine and in material exuding from the pollen; the labeling observed with human IgE was localized more specifically in the intine (oncus) at random points near the apertures. In the case of *P. judaica*, the existence of a thickening of the intine below the apertures (oncus) causes a release of its contents in an explosive manner [13]. The Urticaceae family is composed of several allergenic species including *P. judaica*, *P. officinalis*, *P. lusitanica*, *P. cretica* and *P. mauritanica*. Immunologically, *P. judaica* and *P. officinalis* represent the most relevant species with *P. judaica* growing mainly on the coasts of Spain, southern France, Italy, Greece, Mediterranean Asia and North Africa and *P. officinalis* in northern Italy, central France and central and eastern Europe.

Identification and Characterization of *Parietaria* Allergens

Several studies have reported on the purification and characterization of allergenic fractions from *Parietaria* pollen of the two more common species, *P. judaica* and *P. officinalis*.

Parietaria allergens were biologically characterized by means of independent strategies, such as CIE, CRIE, HPLC, Western blot and affinity chromatography, suggesting that the major allergens of both species show molecular weights ranging between 10 and 14 kD depending on the procedures employed [10, 14–19]. The first report on the major allergen of *P. judaica* pollen showed that nPar j 1 allergen is a glycopeptide with a molecular weight of about 10 kD, responsible for most of the allergenic activity of the whole extract [20, 21]. Its carbohydrate composition has been determined to show a certain degree of heterogeneity in the sugar moiety by absorption on a column of concanavalin A-Sepharose [21]. However, the presence of glucidic residues does not interfere with the allergenic activity [22]; both natural and deglycosylated Par j 1 inhibited the binding of specific human IgE to *P. judaica*-coated wells to a similar extent in an ELISA assay. Reduction and alkylation caused complete loss of the allergenic activity, suggesting that disulfide bridges are essential for the IgE-binding ability of Par j 1 [23]. In addition, several data from the literature quite consistently describe an unusual resistance of *Parietaria* allergens to heat treatment and enzymatic digestion [17, 18]. In addition to the carbohydrate heterogeneity, the existence of distinct isoforms of Par j 1 has been proposed by several authors since immunoblot analysis shows two clear bands when crude extract of *P. judaica* is fractionated by SDS-PAGE [10, 14].

Ayuso et al. [15] reported the first amino acid determination and partial N-terminal sequencing of two nPar j 1 isoallergens using monoclonal antibodies and affinity chromatography. This strategy allowed the isolation of two independent highly cross-reactive isoforms (named Par j 1A and Par j 1B) with molecular weights of 13 and 10.5 kD, respectively. The results of an amino acid composition analysis showed that Par j 1A and Par j 1B were distinct proteins although with a high degree of homology (table 1B). Immunological analysis made by Western blots and skin prick tests (SPT) performed on individual patients allergic to *P. judaica* showed that all of them recognized both isoforms and that allergenic epitopes present in Par j 1A and 1B allergens were responsible for most of the allergenic activity of the whole extract. This was the first biochemical characterization confirming the observation that the fast-migrating component of the *P. judaica* extract separated into at least two independent components. Proteins homologous to nPar j 1 were also detected in *P. officinalis*, *P. lusitanica* and *P. mauritanica*. Monoclonal antibodies specific to the nPar j 1 major allergen recognized proteins from the three *Parietaria* pollens [24].

SPT and radioallergosorbent test (RAST) inhibition yielded results supporting high allergenic cross-reactivity among these pollen extracts [25]. In addition, nPar o 1 and nPar m 1 were purified by affinity chromatography using a monoclonal antibody against the nPar j 1 allergen. Cross-inhibition experiments demonstrated that nPar j 1, nPar o 1, and nPar m 1 competed for the binding of specific IgE from a serum pool of patients sensitive to *P. judaica* [26].

The major *P. officinalis* allergen was characterized by several authors [17, 18, 27–29]. Oreste et al. [30] were the first to describe the isolation of a glycoprotein with a molecular weight between 13.5 and 14.5 kD and to assay its allergenicity in vivo by SPT and in vitro by RAST. The amino acid composition and the sequence of the first twelve N-terminal residues were determined showing a strong coincidence with the Par j 1A sequence reported by Ayuso et al. [15] (table 1). nPar o 1 was able to inhibit up to 85% of the *P. officinalis*-specific IgE and the RAST inhibitory activity was not abolished by deglycosylation [30]. Similar results were obtained by Kahlert et al. [31] who reported the isolation of a few isoforms of the major *P. officinalis* allergen using a panel of nine monoclonal antibodies. The affinity-purified proteins were subjected to N-terminal amino acid sequence analysis. The sequences of all the proteins contained highly conserved regions: GGVV (positions 4–7) and MPPLL (positions 11–15) and a high percentage of homology with the nPar j o 1 described by Oreste et al. [30] and nPar j 1 isoforms described by Ayuso et al. [15] (table 1). All the affinity-purified proteins represent major IgE-reactive peptides since they strongly inhibit (80–93%) the binding of IgE to allergen extract on a solid phase.

Molecular Cloning and Immunological Characterization

The development of the DNA recombinant technology enabled the molecular characterization of the allergens of *Parietaria* pollen. Screening of a cDNA expression library with a pool of sera from patients allergic to *P. judaica* pollen allowed the isolation of the two major allergens named Par j 1 [32] and Par j 2 [33] and some other isoforms [34]. Sequence analysis indicated that Par j 1.0102 cDNA clone contained an insert of 622 nucleotides and a deduced open reading frame of 176 amino acids. This polypeptide contains an amino-terminal region of 37 amino acids with characteristics of a signal peptide of glycosylated proteins giving a mature processed protein of 139

Table 1. Sequence alignments of the NH₂-terminal regions of the natural and recombinant *Parietaria* allergens

A				
1	30			
QETCGTMVRALMPCLPFVQGEKEPSKGGCC		rPar j 1.01	[32]	
***** * *****				
EETCGTVVGALMPCLPFVQGEKEPSKGGCC		rPar j 1.02	[34]	
* ***** * *****				
GTT-GTVVGALMPPL-FVQGEKE		nPar o 1	[31]	
* ** ***				
ATGKVVQGAMPP		nPar o 1	[30]	
B				
1	30			
EEACGKVVQDIMPCLHFKGEEKEPSKECC		rPar j 2.01	[33]	
***** * ***				
DGKVVQDIMPPLLFVK		nPar j 1A	[15]	
*** *****				
?GKVV?DIMPPLLFVKG		nPar j 1B	[15]	

Panel **A** and **B** show the homologies between the recombinant Par j 1 and Par j 2 allergens and some of the affinity-purified *Parietaria* allergens. Numbers indicate the relative position of the amino acids referred to the Par j 1 and Par j 2 sequences. Asterisks indicate homologous residues. Question marks are for unsolved sequences.

amino acids and a molecular weight of 14,726 D [32]. Using the same strategy, a shorter variant of the major Par j 1 allergen (Par j 1.0201) expressing a polypeptide of 139 amino acids with an amino-terminal region of 37 amino acids giving a mature protein of 102 amino acids and a molecular weight of 10,677 D has been described. The coding regions of the Par j 1.0102 and Par j 1.0201 allergens show 89% homology at the amino acid level (table 2) and their cDNAs did not present any homology in the 5' and 3' untranslated regions, suggesting that both polypeptides are the product of independent genes belonging to a multigene family [34].

The Par j 2 allergen (Par j 2.0101) is a polypeptide of 133 amino acids with an amino-terminal signal peptide of 31 amino acids giving a mature processed protein of 102 amino acids and a deduced molecular weight of 11,344 D (table 2). It showed a homology of 45% with the coding sequence of the Par j 1 as a consequence of their common evolutionary origin [33]. In fact, a data bank analysis has shown that the Par j 1 and Par j 2 allergens belong to the same family of plant proteins named nonspecific lipid transfer proteins (nsLTP) whose function is not completely understood. They are ubiquitous proteins implicated in the transport of fatty acids across membranes and defense against pathogens and environmental stress [35]. All the

Table 2. Amino acid comparison of the major allergens of *P. judaica*

1	EETCGTVVGA	LMPCLPFVQG	KEKEPSKGCC	SGAKRLDGET	KTGPQRVHAC	50	
	*****	*	*	*****	*****	*****	Par j 1.02
	QETCGTMVRA	LMPCLPFVQG	KEKEPSKGCC	SGAKRLDGET	KTGPQRVHAC		Par j 1.01
	* * *	*	*	*****	*	*	Par j 2.01
	EEACGKVVQD	IMPCLHVFVG	EEKEPSKECC	SGTKKLSEEV	KTTEQKREAC		
51	ECIQTAMKTY	SDIDGKLVSE	VPKHCIVDS	KLPPIDVNMD	CKTLGVLYHK	100	
	*****	*****	*****	*****	*****		Par j 1.02
	ECIQTAMKTY	SDIDGKLVSE	VPKHCIVDS	KLPPIDVNMD	CKTVGVVPRQ		Par j 1.01
	** *	*	*	*****	*		Par j 2.01
	KCIVRATKGI	SGIKNELVAE	VPKKCDIKTT	LPPITADFDC	SKIQTSTIFRG		
101	GN					139	Par j 1.02
	PQLPVSLRHG	PVTGSPDPAH	KARLERPQIR	VPPPAPEKA			Par j 1.01
	YY						Par j 2.01

Numbers indicate the relative position of the amino acids on the mature processed proteins. Asterisks indicate homologous residues.

members of this group belong to multigene families, possess short 5' and 3' untranslated regions and contain eight cysteine residues able to form four disulfide bonds according to an invariant order [36]. This family of proteins has been recently described as a new pan-allergen with a very high degree of cross-reactivity between vegetable foods comparable to profilin [37].

To study the immunological properties of the major allergens of *P. judaica* pollen, the Par j 1 and Par j 2 molecules were expressed in a prokaryotic system and the purified recombinant proteins were used to test the allergologic significance of these allergens. In a dot blot assay using single sera from atopic patients, the rPar j 1 and rPar j 2 allergens were able to bind 95 and 82% of the tested sera. In particular, the rPar j 1 protein was able to induce histamine release from basophils of patients allergic to *P. judaica* pollen in a way comparable to the release observed with crude extract from natural *P. judaica*. Similarly, it was able to bind 40% of the *P. judaica*-specific IgE in an ELISA assay confirming that it is a major allergen. rPar j 2, which was able to inhibit about 35% of the *P. judaica*-specific IgE, behaved similarly. Despite their structural similarities, Par j 1 and Par j 2 are two independent allergens since cross-inhibition experiments showed that they possess an independent repertoire of IgE epitopes [33]. Finally, preincubation of a pool of sera from patients allergic to *P. judaica* with a large excess of both rPar j 1 and rPar j 2 inhibited the binding of human IgE with the 10- to 14-kD area to a large extent, thus support-

ing the idea that those proteins are the main allergenic components of this region [33].

A similar strategy was employed by Asturias et al. [38], who recently isolated complete cDNA clones coding for profilin genes from *P. judaica*, a ubiquitous protein described in several other allergenic sources [39] and in part responsible for cross-reactivities between pollen and food [40]. The authors isolated two isoforms of 131 and 132 amino acids and a molecular weight of 13,784 and 13,920 D, respectively. The deduced amino acid sequences share high identity with other plant profilins.

Three-Dimensional Structure and B Cell Epitopes

The identification of the IgE epitopes of an allergenic molecule is a key step in the understanding of the allergic reaction since they represent the effectors of the immune reaction. In this strategy, knowledge of the structure of an allergen may be of paramount importance and lead to the identification of regions that are exposed to the solvent and for this reason probably contacted by the antibodies. The three-dimensional model of the Par j 1 allergen has been solved by homology against an X-ray crystal of a soybean nsLTP. In fact, this family of proteins contains a conserved three-dimensional structure forming an α - α - α - β -fold with a subset of specific amino acids required to maintain a tertiary structure including eight cysteine resi-

dues forming four disulfide bonds that compact the four α -helices.

The overall effect of this structure is to form a right-hand winding of the α -helices with a hydrophobic pocket for lipid interactions while the C-terminal may form a cap to the pocket. As a member of the plant nsLTP family, the Par j 1 major allergen has been shown to adopt the same structural fold with four disulfide bridges in the following order: Cys4–Cys52, Cys14–Cys29, Cys30–Cys75 and Cys50–Cys91 [the same folding has been shown for Par j 2; D. Kennedy, pers. commun., and 41] (fig. 2). In the same paper, the authors mapped an immunodominant IgE epitope in the loop1 region located between α -helix1 and α -helix2 (region from amino acids 1 to 30, see table 2). In particular, gene fragmentation and site-specific mutational analysis of the amino acids K21, K23, E24 and K27 showed that those residues are essential for IgE recognition and that the disulfide bond between Cys14 and 29 is fundamental to maintain this structural domain (see table 2 for the relative position of the amino acids). Cross-inhibition experiments have shown that this peptide binds a large proportion of the *P. judaica*-specific IgE, and is an epitope shared by the Par j 1 and Par j 2 allergens. In fact, the NH₂-terminal region of the Par j 1 and Par j 2 allergens is the region of maximum homology between the two proteins [41]. Still more stimulating, sequence comparison between the amino-terminal regions of rPar j 1 and nPar o 1 isolated by Kahlert et al. [31] showed extensive homology (83% identity) and the conservation of this *P. judaica* IgE epitope in the *P. officinalis* major allergen sequence confirming the high cross-reactivity between the two species (see table 2A for details). A different strategy of characterization was used for the *P. officinalis* major allergen. The immunological screening of a cDNA library yielded the isolation of a linear eight amino acid peptide that showed the ability to bind a large proportion of sera from patients allergic to *P. officinalis*. This peptide was also able to inhibit the binding of specific IgE to nPar j 1 allergen [42].

A more detailed analysis of the IgE-binding regions of the *P. judaica* allergens was performed by Western blot using five recombinant peptides obtained by gene fragmentation of the rPar j 2 sequence and single sera from patients allergic to *P. judaica*. At least four putative IgE epitopes were identified and the analysis of their diffusion suggested that the Par j 2 allergen contains IgE determinants spread all over the molecule and heterogeneously recognized by sensitive patients suggesting that the therapeutic approach based on the use of haptenic peptides needs careful evaluation [43].

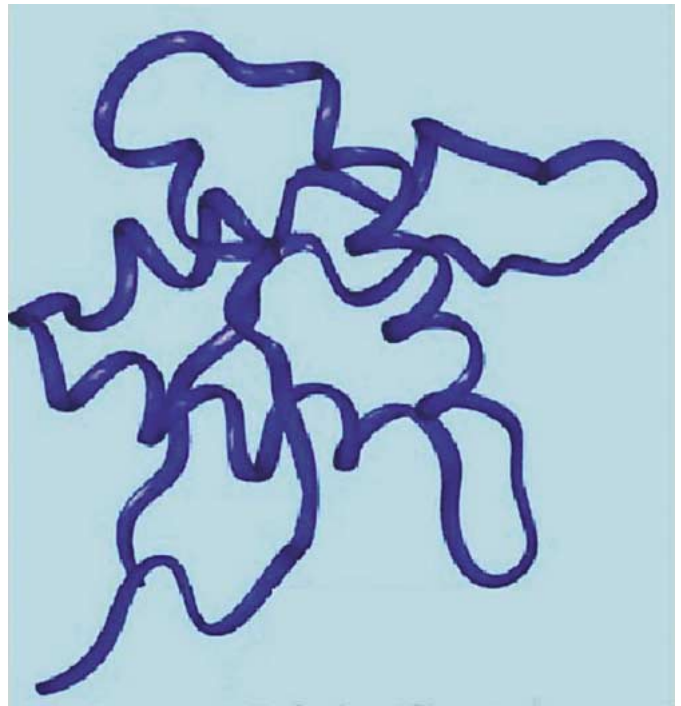


Fig. 2. Three-dimensional model of the Par j 1 allergen.

Starting from these observations, Bonura et al. [44] reported the immunological characterization of a family of hypoallergenic derivatives of the Par j 1 allergen with reduced allergenicity but retained T cell recognition. By means of site-directed mutagenesis, the authors decided to target cysteine residues designing four full-length variants lacking independent disulfide bridges. Recombinant hypoallergens were expressed in *E. coli* and their IgE-binding activity was measured in vitro by ELISA and ELISA inhibition and in vivo by SPT. This kind of analysis indicated a strong correlation between IgE-binding activity and the folding of the protein and, in particular, the importance of the Cys14–Cys29 and Cys30–Cys75 bridges on overall allergenicity. The loss of any additional disulfide bond leads to the absence of any IgE reactivity in vitro and in vivo. On the contrary, disruption of the Cys50–Cys91 and Cys4–Cys52 bridges seems to have a minimal effect of the antigen–IgE interaction. This kind of strategy did not interfere with T cell recognition since all the variants maintained T cell reactivity when tested in a peripheral blood mononuclear cell proliferation assay and for these reasons those molecules have been suggested as a new tool for an improved specific immunotherapy of *Parietaria* pollen allergy.

In conclusion, this review would like to emphasize a few points:

- Pollen extract of *Parietaria* species present a very high degree of cross-reactivity;
- biochemical purification and molecular cloning have demonstrated that the major allergens of *Parietaria* pollen are small glycoproteins with similar molecular

weights and belong to the same multigene family (nsLTP) whose glucidic residues are not important for the IgE-binding activity;

- rPar j 1 and rPar j 2 present an immunological behavior similar to their natural counterpart and represent powerful tools for the diagnosis and therapy of the *Parietaria* pollen allergy.

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