

Review article Series II

An update on allergens

Parietaria pollen allergens

Colombo P, Duro G, Costa MA, Izzo V, Mirisola M, Locorotondo G, Cocchiara R, Geraci D. *Parietaria* pollen allergens. Allergy 1998; 53: 917-921. © Munksgaard 1998.

**P. Colombo, G. Duro, M. A. Costa,
V. Izzo, M. Mirisola, G. Locorotondo,
R. Cocchiara, D. Geraci**

Istituto di Biologia dello Sviluppo, CNR, Palermo, Italy

Key words: IgE epitopes; *Parietaria*; pollen allergens; recombinant allergens.

Dr Domenico Geraci
Istituto di Biologia dello Sviluppo
CNR
Via Ugo La Malfa 153
90146 Palermo
Italy

Accepted for publication 30 June 1998

Pollen allergens are multivalent proteins or glycoproteins capable of inducing IgE-mediated type I allergy in about 10-15% of the population of the industrialized countries (1). In those patients, the immune system causes a direct hypersensitivity reaction associated with a high concentration of circulating IgE and interleukin-4 (IL-4) and interleukin-5 (IL-5) cytokine production by allergen-specific Th2 cells.

Parietaria is a genus of dicotyledonous weeds of the Urticaceae family, including several species widely distributed in the Mediterranean area (2); among them, *Parietaria judaica* (*Pj*) and *P. officinalis* (*Po*) pollens have been reported to be the most common cause of allergy in this area. In fact, an epidemiologic study of 1787 allergic patients in southern Italy showed that 50% were skin test positive to *Pj* pollen (3). Therefore, considering that approximately 180 million people of Europe, Asia, and Africa live in this area (4), we estimate that a mean number of 10 million people may be allergic to *Parietaria* pollen. Moreover, the Mediterranean is not the only region affected by *Parietaria* pollen atopic disease, since the presence of this weed and related IgE have also been described in the southern UK, Austria, temperate regions of central and eastern Europe, Australia, and California (5-7).

Over the last 15 years, the *Parietaria* allergenic molecules have been analyzed by conventional biochemical methods (CIE, CRIE, HPLC, and Western blot) (8-12), and nine antigens of 10-80 kDa capable of binding human IgE were detected in *Pj* pollen extract. These probably account for all the important allergens present in the dialyzed *Pj* pollen solution, since rabbit anti-*Pj* extract antibodies absorbed all the RAST-inhibitory capacity of the dialyzed *Pj* extract (13). This was further confirmed by gel fractionation-electroblotting methods. Among the nine *Pj* allergens, four were classified as major; in particular, an immunoblot analysis of 52 sera from *Pj*-allergic patients showed that the fast-migrating components were recognized by most of the sera and that a large number of them recognized that area exclusively. In addition, SDS-PAGE analysis showed that the 10-14-kDa area separated into two very close bands, suggesting the presence of more than one component (14, 15). Common antigenic determinants have been observed in pollen extracts of *Pj* and *Po* (16, 17).

Po and *Pj* native major allergens

The major allergens of the *Po* and *Pj* pollens have been described. Par o 1 is a glycoprotein with a protein-to-carbohydrate ratio of 100:21 and an isoelectric point of 4.6, and the amino-acid

Table 1. N-terminal sequences of native *Parietaria* purified allergens. Panel A) Par o 1 isoform allergens; panel B) Par j 1 isoform allergens. Question marks indicate unsolved sequences. Underlined amino acids might replace amino acids in these positions. Numbers in parentheses indicate references

A <i>Parietaria officinalis</i>																																	
A	P	A	G	G	V	V	<u>P</u>	<u>I</u>	<u>I</u>	M	P	P?	?	F	F	(21)																	
A	A	A	G	G	V	V	<u>H</u>	H	I	M	P	P	L	(21)																			
A	<u>P</u>	<u>K</u>		<u>K</u>	V	V	V	A	A	M	P	P	P	L	(21)																		
G	P	V	G	G	V	V	H	<u>A</u>	H	M	M	<u>D</u>	L	L	(21)																		
<u>A</u>	E	X	<u>P</u>	G	V	V	<u>P</u>	<u>H</u>	<u>I</u>	M	P	P	L	L	(21)																		
G	<u>T</u>	<u>T</u>		<u>T</u>	V	V	G	<u>A</u>	<u>L</u>	M	<u>M</u>	P	L	F	V	Q	G	K	E	K	E	?	P	P	S	?	K	(21)					
<u>E</u>	<u>T</u>	<u>T</u>	G	<u>G</u>	V	V	G	A	L	M	P	P	<u>P</u>	(18)																			
<u>A</u>	T	G	K	<u>V</u>	V	Q	G	A	M	P	P	(18)																					
B <i>Parietaria judaica</i>																																	
A	D	G	K	V	V	Q	D	I	M	P	P	L	L	F	V	K	(22)																
G	<u>T</u>																																
G	<u>P</u>	?	G	K	V	V	?	D	I	M	P	P	L	L	F	V	K	G	(22)														

composition and sequence of the first 12 N-terminal amino-acid residues have been determined (18) (Table 1, panel A). Several reports have dealt with the size of Par o 1, for which SDS-PAGE revealed an approximate molecular mass of 10–15 kDa (19, 20). Recently, Kahlert et al. (21) reported the characterization of a few isoforms of the major *Po* allergen; they used a monoclonal antibody for their purification. These proteins were tested in ELISA inhibition with *Po* extract, a large binding inhibition of IgE from *Po*-allergic patients being achieved. The N-terminal sequence showed microheterogeneity in the amino-acid composition of the isoforms isolated (Table 1, panel A).

The first report on the sequence of *Pj* allergens was published by Ayuso et al., who used monoclonal antibodies and affinity chromatography for their purification (22). They isolated two independent isoforms of Par j 1 with differing molecular mass (13 and 10.5 kDa) and similar N-terminal amino-acid sequence (Table 1, panel B). The cross-reactivity between the purified allergens was determined by competition ELISA with a pool of sera from *Pj*-allergic patients. The two isoforms could strongly inhibit each other (Par j I A reached 80%; Par j I B, 90%), showing that most of the IgE epitopes are common to both proteins.

cDNA cloning

The molecular characterization of the allergens has received an exceptional boost by recombinant DNA technology, which has allowed the isolation of purified allergens. Costa et al. (23) and Duro et al.

(24) reported the isolation of cDNAs from a *Pj* expression library codifying for two major allergens (Par j 1.0101 and Par j 2.0101). Successively, a full-length isoform and one variant of the Par j 1.0101 allergen (Par j 1.0102 and Par j 1.0201, respectively) have been described (25). The Par j 1.0102 clone showed an insert of 794 nucleotides with a deduced amino-acid sequence of 176 aa and a molecular mass of 18 450 Da (25). Par j 1.0201 showed a 637-nucleotide insert with a deduced amino-acid sequence of 139 aa and a molecular mass of 14 400 Da (Fig. 1, panel A). The coding region of Par j 1.0102 and Par j 1.0201 showed 91% homology at the nucleotide level and 89% identity at the amino-acid level, with no homology in the 5' and 3' untranslated regions, suggesting that the two clones were derived by transcription of independent genes. In particular, the Par j 1.0201 clone can be considered a shorter variant of the major Par j 1.0101 allergen (25).

The Par j 2.0101 clone showed a 622-nucleotide insert with an open reading frame of 133 aa and a deduced molecular mass of 14 105 Da (Fig. 1, panel B). It showed homology of 45% with the coding sequence of Par j 1.0102 (25), and from aa E38 to G57, a region of significant homology with the N-terminal region, two native isoallergens (Par j 1 A and Par j 1 B) were isolated by Ayuso et al. (Table 1, panel B).

In addition, the hydrophobicity profile of the coding regions of all the *Pj* allergens isolated so far showed an N-terminal sequence of about 36 amino acids characteristic of the eukaryotic signal peptide of glycosylated proteins (Fig. 1, panels A and B). The putative mature processed

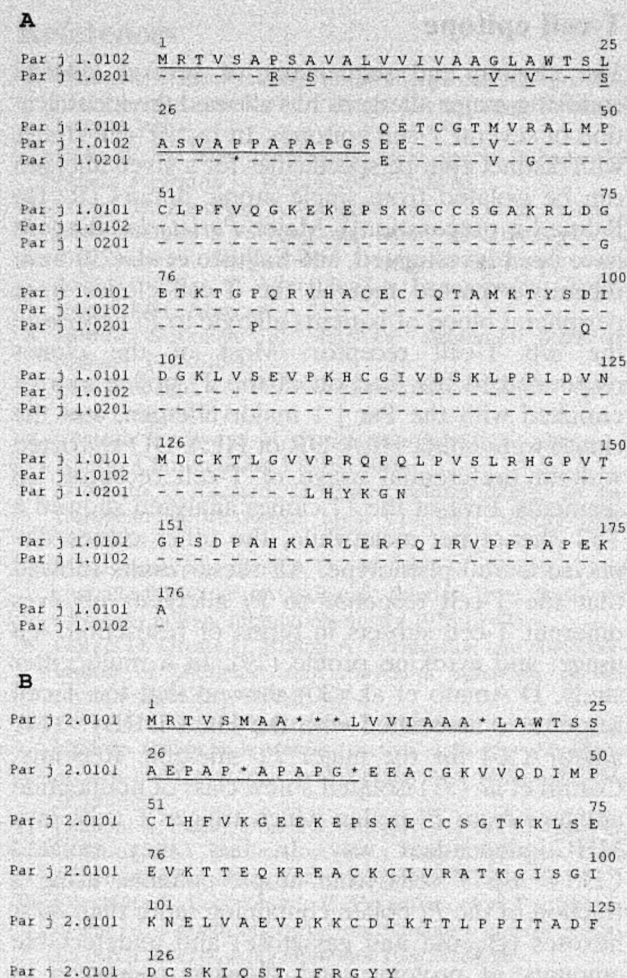


Fig. 1. Amino-acid alignment of *Parietaria judaica* major allergens. Panel A) amino-acid alignment of Par j 1 isoallergens; panel B) amino-acid sequence of Par j 2.0101 allergen. Numbers indicate relative position of amino acids in unprocessed proteins. Underlined amino acids indicate putative signal peptides. Asterisks indicate gaps introduced to reach maximum homology with Par j 1 sequences. Lines indicate homologous amino acids.

proteins contain 139 amino acids for the Par j 1.0102 clone and 103 amino acids for either the Par j 1.0201 or the Par j 2.0101 clone. The molecular mass of the mature processed proteins was 14 726 Da for Par j 1.0102, 10 677 Da for Par j 1.0201, and 11 344 Da for Par j 2.0101 (24, 25), in accordance with the size of the native major *Pj* allergens described by several authors. More interestingly, the three mature processed proteins showed a high degree of homology at the N-terminal region, with the native major allergens isolated from either *Pj* or *Po* confirming the previously reported data on the cross-reactivity between the two species.

In addition, database analysis showed that the *Pj* major allergens belong to a large family of plant proteins capable of transferring lipid molecules across membranes and therefore termed non-

specific lipid transfer proteins (nsLTP). nsLTP exist in plant and animal tissues, but while there is no sequence similarity to animal nsLTP, similarity among the plant proteins is high. All of them belong to multigene families, show low molecular mass, relevant homology at the amino-acid level, and eight cysteine residues capable of forming four disulfide bridges (26).

Immunologic characterization of the recombinant allergens

The immunologic properties of the recombinant allergens Par j 1.0101 and Par j 2.0101 were determined by cloning them in a pMalC2 expression vector and testing the recombinant fused proteins. Dot-blot experiments with the rPar j 1.0101 and rPar j 2.0101 proteins were performed to study the immunologic relevance of the two allergens with single sera from patients allergic to *Pj* pollen. The Par j 1.0101 allergen was capable of binding 95% of the tested sera and blocked approximately 40% of all the *Pj*-specific antibodies in ELISA (23). Par j 2.0101 inhibited 35% of the specific IgE for the 10–14-kDa native allergens and was capable of binding IgE in 82% (23/28) of the sera of *Pj*-allergic subjects (24). These observations confirmed that they can be classified as major allergens. For study of the presence of common IgE epitopes, the two proteins were spotted onto nitrocellulose membranes and tested in a cross-absorption test. Preincubation of a serum pool from *Pj*-allergic patients with increasing amounts of Par j 1.0101 and Par j 2.0101, respectively, did not show a relevant cross-reactivity, suggesting a different IgE-epitope composition (24). Both recombinant proteins were capable of inducing histamine release (25) (G. Duro, personal communication); more interestingly, preincubation of a pool of allergic sera with both rPar j 1.0101 and rPar j 2.0101 proteins was capable of totally inhibiting the binding of the 10–14-kDa native allergenic region on Western blot, suggesting that this region is formed exclusively by the two allergens. The latter experimental result agrees with the previously reported observation that the fast-migrating component of the *Pj* pollen crude extract separated into at least two components.

B-cell epitope and 3-D structure

The homology between the *Pj* allergens and the nsLTP proteins has been used to build a three-dimensional (3-D) structure by homology with the already published soybean nsLTP crystal. Colombo et al. (27) reported a 3-D model giving an α - α - α - α - β secondary structure with a subset of specific amino

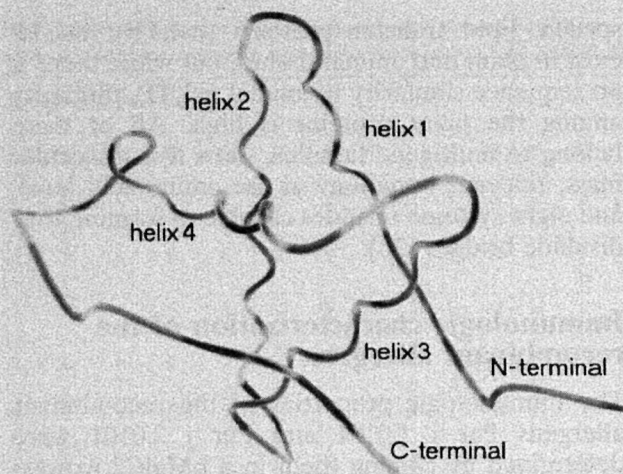


Fig. 2. 3-D modeling of Par j 1.0101 allergen. Schematic representation of Par j 1.0101 allergen, indicating position of four alpha helices detected by structural homology.

acids required to maintain a tertiary structure, including eight cysteine residues forming four disulfide bonds that compact the four helices. The overall effect of the secondary structures is to form a right-hand winding of the alpha helices with a hydrophobic pocket for lipid interaction (Fig. 2). Knowledge of the 3-D structure of the Par j 1.0101 molecule allowed the identification of residues presenting side-chains exposed to the solvent surface and involved in the epitope structure. In particular, the region of the first 30 amino acids of Par j 1.0101 was investigated, since it shares high homology with the corresponding region on Par j 2.0101 and possesses the properties to form an epitope. This region could bind about 30% of all the human IgE to the native major *Pj* allergens, did not induce release of inflammatory mediators in a histamine-release assay, and is a common IgE epitope shared by the Par j 1.0101 and Par j 2.0101 allergens.

In addition, site-directed mutagenesis analysis was performed, and the amino acids involved in the antibody recognition were determined. Consequently, the 1–30 region peptide may represent a useful tool to develop new immunotherapy strategies (27).

A different strategy of characterization was used for the Par o 1 allergen. Scotto D'Abusco et al. (28) screened a cDNA expression library with a serum pool from allergic patients and isolated a linear eight-amino-acid epitope fused to the β -galactosidase protein (peptide 6a). The fused protein specifically inhibited Par o 1 and Par j 1 binding of a serum pool from allergic patients, and an antiserum obtained in rabbit specifically precipitated the purified Par o 1. The presence of specific human IgE against peptide 6a was determined in the sera of 33 patients by inhibition assays, which showed a high percentage of response.

T-cell epitope

The cloning and sequencing of several cDNAs codifying major allergens has allowed the identification of several T-cell epitopes. In fact, T-cell clones with distinct epitope specificities for a given allergen can be isolated from single atopic donors (R. De Palma [in preparation]). Major *Parietaria* allergens have been investigated, and Sallusto et al. (29) have recently reported that all the T-cell clones from peripheral blood of patients allergic to *Pj* expressed the α/β T-cell receptor. Most of the clones responded to the fraction of the *Pj* pollen extract enriched with the Par j 1 major allergen, and the clones were either HLA-DR or HLA-DQ restricted without preferential usage of T-cell receptor V β segments. Five of the 17 clones analyzed showed a Th2 phenotype; meanwhile, the other clones displayed a Th0 phenotype. All these results showed that the T-cell response to *Pj* allergens involves different T-cell subsets in terms of restriction, V β usage, and cytokine profile (29). In a multicenter study, D'Amato et al. (30) showed that the T-cell response is associated with the HLA-DRB1* 1101 and/or 1104 for the major *Po* allergen. Recently, Corinti et al. (31) isolated a new class of nonpeptide antigens from *Pj* pollen which trigger T cells in a MHC-independent way. In fact, they isolated CD8+ α/β T cells from atopic patients, using a fraction of the *Pj* pollen containing more than 80% hexoses (glucose and galactose) and undetectable amounts of proteins, and showing resistance to proteinase K and alkaline digestion. Analysis of the TCR V β transcripts showed six independent specific T-cell clones carrying V β 8 segments with a conserved motif in the CDR3 region.

Concluding remarks

The isolation and characterization of allergenic molecules have offered the following aids to immunotherapeutic research:

- 1) the means to produce large amounts of highly purified allergens for desensitization studies
- 2) the means to isolate B-cell epitopes capable of binding selectively either IgG or IgE antibodies
- 3) the elucidation of T-cell-specific recognition and the design of modified allergens capable of activating Th1 responses.

Acknowledgments

We thank Drs Raffaele De Palma (Fondazione Salvatore Maugeri, Pavia) and Gabriella Di Felice (Istituto Superiore di Sanità, Rome) for critical reading of the manuscript. This work was partially supported by Grant no. 712 from the Italian Ministero del Lavoro.

References

1. Wüthrich B. Epidemiology of the allergic diseases: are they really on the increase? *Int Arch Allergy Appl Immunol* 1989;90:3-10.
2. Raffaelli M. Note corologiche sulle specie italiane del genere *Parietaria* L. *Webbia* 1977;31:49-68.
3. Geraci D, Amoroso S, Capotummino D, et al. Asma e rinite allergica a Palermo: uno studio epidemiologico su 1787 pazienti allergici. XV Congresso Nazionale della Società Italiana di Allergologia e Immunologia Clinica. Rome, 1981, Abstract, p. 115.
4. Istituto Nazionale di Statistica. *Annuario Statistico Italiano*, 1996:610-15.
5. Bass D, Baldo BA. *Parietaria* as a cause of asthma. *Med J Aust* 1984;140:511-14.
6. Charpin J, Charpin H. *Parietaria officinalis* L. allergy. *J Allergy Clin Immunol* 1980;65:80-1.
7. D'Amato G, Liccardi G. Pollen related allergy in the European Mediterranean area. *Clin Exp Allergy* 1994;24:210-18.
8. Menegozzo M, Geraci D, Ruffilli A. Isolation and characterisation of an allergenic fraction from *Parietaria officinalis* pollen. *Immunochemistry* 1976;13:475-6.
9. Geraci D, Oreste U, Ruffilli A. Purification and characterisation of allergens from *Parietaria officinalis* pollen. *Immunochemistry* 1978;15:491-8.
10. Corbi AL, Ayuso R, Carreira J. Identification of the IgE binding polypeptides cross-reactive with the *Parietaria judaica* main allergenic polypeptide. *Mol Immunol* 1986;23:1357-63.
11. Giuliani A, Pini C, Bonini S, Mucci N, Ferroni L, Vicari G. Isolation and purification of a major allergen from *Parietaria officinalis* pollen. *Allergy* 1987;42:434-40.
12. Cocchiara R, Locorotondo G, Parlato A, et al. Purification of Par j 1, a major allergen from *Parietaria judaica* pollen. *Int Arch Allergy Appl Immunol* 1989;90:84-90.
13. Geraci D, Bache-Billesbolle K, Cocchiara R, Løwenstein H, Ipsen H. Immunochemical characterisation of antigens of *Parietaria judaica* pollen: identification of allergens by means of crossed radioimmuno-electrophoresis. *Int Arch Allergy Appl Immunol* 1985;78:421-8.
14. Corbi AL, Carreira J. Identification and characterisation of *Parietaria judaica* allergens. *Int Arch Allergy Appl Immunol* 1984;74:318-23.
15. Ford SA, Baldo BA, Geraci D, Bass D. Identification of *Parietaria judaica* pollen allergens. *Int Arch Allergy Appl Immunol* 1986;79:120-6.
16. Corbi AL, Pelaez A, Errigo E, Carreira J. Cross-reactivity between *Parietaria judaica* and *Parietaria officinalis*. *Ann Allergy* 1985;54:142-7.
17. Bousquet J, Hewitt B, Guerin B, Dhivert H, Michel FB. Allergy in the Mediterranean area. II. Cross-allergenicity among Urticaceae pollens (*Parietaria* and *Urtica*). *Clin Allergy* 1986;16:57-64.
18. Oreste U, Coscia MR, Scotto d'Abusco A, Santonastaso V, Ruffilli A. Purification and characterization of Par o 1, major allergen of *Parietaria officinalis* pollen. *Int Arch Allergy Appl Immunol* 1991;96:19-27.
19. Giuliani A, Pini C, Bonini S, Mucci N, Ferroni L, Vicari G. Isolation and purification of a major allergen from *P. officinalis*. *Allergy* 1987;42:434-40.
20. Corbi AL, Pelaez A, Arrigo E, Carreira J. Cross-reactivity between *Parietaria judaica* and *Parietaria officinalis*. *Ann Allergy* 1985;54:142-7.
21. Kahlert H, Weber B, Teppke M, Wahl R, Cromwell O, Fiebig H. Characterisation of major allergens of *Parietaria officinalis*. *Int Arch Allergy Appl Immunol* 1996;109:141-9.
22. Ayuso R, Carreira J, Lombardero M, et al. Isolation by mAb based affinity chromatography of two Par j I isoallergens. Comparison of their physicochemical, immunochemical and allergenic properties. *Mol Immunol* 1993;30:1347-54.
23. Costa MA, Colombo P, Izzo V, et al. cDNA cloning, expression and primary structure of Par j 1, a major allergen of *Parietaria judaica* pollen. *FEBS Lett* 1994;341:182-6.
24. Duro G, Colombo P, Costa MA, et al. cDNA cloning, sequence analysis and allergological characterisation of Par j 2.0101, a new major allergen of *Parietaria judaica* pollen. *FEBS Lett* 1996;399:295-8.
25. Duro G, Colombo P, Costa MA, et al. Isolation and characterisation of two cDNA clones coding for isoforms of the *Parietaria judaica* major allergen Par j 1.0101. *Int Arch Allergy Appl Immunol* 1997;112:348-55.
26. Shin DH, Lee JY, Hwang KY, Kim KK, Suh SW. High-resolution crystal structure of the non-specific lipid-transfer protein from maize seedlings. *Structure* 1995;3:189-99.
27. Colombo P, Kennedy D, Ramsdale T, et al. Identification of an immunodominant IgE epitope of the *Parietaria judaica* major allergen. *J Immunol* 1998;160:2780-5.
28. Scotto d'Abusco A, De Santo C, Menna T, et al. Characterisation of a dominant antigenic determinant of Par o I encoded by recombinant DNA. *Clin Exp Allergy* 1996;26:223-31.
29. Sallusto F, Corinti S, Pini C, Biocca MM, Bruno G, Di Felice G. *Parietaria judaica*-specific T-cell clones: heterogeneity in restriction, Vb usage and cytokine profile. *J Allergy Clin Immunol* 1996;97:627-37.
30. D'Amato M, Scotto d'Abusco A, Maggi E, et al. Association of responsiveness to the major pollen allergen of *Parietaria officinalis* with HLA-DRB1* alleles: a multicenter study. *Hum Immunol* 1996;46:100-6.
31. Corinti S, De Palma RD, Fontana A, Gagliardi C, Pini C, Sallusto F. Major histocompatibility complex-independent recognition of a distinctive pollen antigen, most likely a carbohydrate, by human CD8+ alpha/beta T cells. *J Exp Med* 1997;186:899-908.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.