

Characterization of a dominant antigenic determinant of *Par o* I encoded by recombinant DNA

A. SCOTTO D'ABUSCO, C. DE SANTO, T. MENNA, M. R. COSCIA*, U. ORESTE*, C. GELLER-BERNSTEIN† and A. RUFFILLI

International Institute of Genetics and Biophysics and *Institute of Protein Biochemistry and Enzymology, National Research Council, Naples, Italy and †General Sick Fund, Zamenhof Allergy Clinic, Tel Aviv, Israel

Summary

Background The pollens from *Parietaria judaica* and *Parietaria officinalis* are a major cause of pollinosis in Europe. *Par o* I (13.5 kDa) and *Par j* I (12 kDa), the major allergens from these species, are highly crossreactive.

Methods We have immunoscreened a *P. judaica* pollen cDNA expression library with a rabbit antiserum specific for *Par j* I and with a serum pool from allergic patients. An immunopositive clone containing a 26 bp insert was further characterized. The insert sequence was determined and the beta-galactosidase fusion protein was partially purified by electroelution from sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) gels.

Results This fusion protein specifically and extensively inhibited *Par o* I and *Par j* I binding of a rabbit antiserum and of a serum pool obtained from allergic patients. The antifusion-protein antiserum obtained in a rabbit (anti 6a) specifically precipitated radioiodinated purified *Par o* I in the double antibody radioimmunoassay (DARIA) and competed with antibodies of sera from allergic patients for the binding to *Parietaria* pollen extract allergens by enzyme linked immunosorbent assay (ELISA). We investigated the prevalence of antibody response towards the 6a epitope in patients naturally sensitized to *Parietaria*. The presence of 6a specific IgE antibodies was assessed in the sera of 33 patients using inhibition assays. All sera had antibodies with this specificity: the extensive percentage of inhibition reached suggested that they dominated individual ab response.

Conclusion In conclusion, the antibody response induced by natural exposure to the pollen of *Parietaria* appears to be highly focused on a single linear antigenic determinant of the major allergens which may play a relevant role in the development of clinical allergy. This report is, to our knowledge, the first description of a dominant linear epitope of a major allergen.

Keywords: recombinant B cell epitope, IgE binding, *Parietaria*, cDNA clone

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Introduction

The cellular and molecular mechanisms underlying allergy are not completely understood. Allergenicity can

be only operationally defined as the ability displayed by some antigens to induce in predisposed individuals immunoglobulin (Ig) E antibody response. There is, however, increasing evidence that T- and B-cell response in allergy and in the response to viral and bacterial parasites are profoundly different [1]. An important step towards the dissection *in vitro* of the immune

Correspondence: Dr A. Ruffilli, IIGB, CNR, Via Marconi 10, Napoli 80125, Italy.

response in allergy is the identification of T- and B-cell epitopes which are relevant in natural sensitization. Whereas T-cell epitopes consist of peptidic segments of antigens, peptides representing B-cell dominant epitopes have been rarely described, as antibodies usually recognize tridimensional structures. As expected, B-cell epitopes of major allergens from several sources have been reported to be conformational [2–6].

We report here the isolation of a short cDNA sequence encoding a dominant antigenic determinant shared by the major allergens from two species of *Parietaria*. To assess the relevance of epitope-specific antibody response in natural immunization we analysed individual sera from *Parietaria* allergic patients.

Parietaria (pellitory), a member of the nettle family, is ubiquitous in Mediterranean Europe, where the species *P. judaica* L. (1756) and *P. officinalis* L. (1753) are a major cause of pollinosis [7]. In allergic patients, IgE antibody response is focused on the major allergens, *Par j* I (12 kDa) and *Par o* I (13.5 kDa). Both allergens have been isolated from the pollen extract and partially characterized: they share the N-terminal aminoacid sequence and are extensively crossreactive [8–10].

Materials and methods

Antisera and immunoglobulins

Rabbit antisera towards *Par o* I, *Par j* I and pollen extract antigens were raised as described [10]. An anti-serum specific for the 6a fusion protein (anti 6a FP) was obtained by three injections, on alternate days, of 80 µg of protein in 100 µL of borate buffered saline (BBS) emulsified with 100 µL of complete Freund's adjuvant, followed by a single injection (320 µg of protein) after a 4 day interval. Human and rabbit IgGs were affinity purified from sera by affinity chromatography on Protein A Sepharose.

Goat anti-human IgE was donated by D.G. Marsh or purchased from Kirkegaard and Perry Laboratories (USA). The isotype specificity of anti-IgE antibody (ab) was assessed by showing that the ability of anti-human IgE to precipitate allergen-antibody complexes in double antibody radioimmunoassay (DARIA) was abolished by absorption with an IgE but not with an IgG myeloma protein.

Radiolabelled rabbit anti-IgE (PRIST) was purchased from Pharmacia (Sweden). Alkaline phosphatase labelled goat anti-human and anti-rabbit IgG were purchased from Biorad (USA) and rabbit anti-*Escherichia coli* beta-galactosidase from Boehringer (Germany).

An IgE myeloma protein was donated by D.G. Marsh.

Human sera and pools

Serum donors were clinically allergic to *Parietaria*, had not received immunotherapy and had serum IgE antibodies specific for *P. officinalis* and *P. judaica* pollen extracts by radioallergosorbent test (RAST). All donors were informed volunteers. Serum pools were obtained from 10 allergic (allergic patients serum pool) and 10 non-allergic patients (control serum pool). Sera from olive-allergic patients were donated by C. Lahoz.

Allergen purification and radiolabelling

P. judaica and *P. officinalis* pollens were purchased from Allergon AB (Sweden). *Par o* I purification has been described [10]. The procedure followed for *Par j* I purification was essentially the same. For radioimmunoassays, 14–40 µg of protein was labelled using 2 mCi of ¹²⁵I (3.7 GBq/mL, Amersham, UK) [11]. Radiolabelled protein was 75% TCA precipitable; 100 µL aliquots (1 µg/mL; 10–20 10⁶ cpm) in BBS, 5% BSA were stored at –20°C. Purified *Ole e* I was donated by C. Lahoz and radiolabelled following the same procedure.

Enzyme linked immunosorbent assay (ELISA)

The ELISA was performed according to established protocols [12]. Typically, 5 µg of *P. judaica* or *P. officinalis* pollen extract proteins in 50 µL of coupling buffer (0.1 M bicarbonate buffer, pH 9.6), were added to each microtitre plate well for 16 h at 4°C. After washing with phosphate buffered saline (PBS) containing 0.1% BSA, 0.1% azide, 0.05% Tween 20, and blocking of the remaining binding sites with PBS containing 0.5% BSA, 0.1% Na azide, pH 7 (1 h at room temperature), 50 µL of serum were added to each well for 2 h at 4°C. Wells were washed with PBS containing 1.5 mM MgCl₂, 2 mM betamercaptoethanol, 0.1% Na azide, 0.1% BSA. For the measurement of human and rabbit IgG antibody, alkaline phosphatase linked goat anti-human or anti-rabbit IgG was added for 1 h at 4°C. Following the addition of substrate, absorbance at 405 nm was read by an automated ELISA reader. For the measurement of human IgE ab, radiolabelled (approximately 10 000 cpm) anti-human-IgE was added for 4 h at room temperature. After washing, bound cpm were measured by a gamma counter.

Double antibody radio immunoassay (DARIA)

The DARIA was carried out essentially as described [13]. For the determination of *Par o* I specific IgG, human or rabbit sera were diluted 1/10 serum in BBS (further

dilutions were made in 1/10 control rabbit serum or control human pool). For the determination of *Par o* I specific human IgE, 50 μ L of serum were mixed with 50 μ L of the appropriate dilution of an IgE myeloma protein. Some 100 μ L of serum dilution were incubated 4 h at room temperature with 100 μ L of a BBS containing 1 ng of radiolabelled *Par o* I (10–20 000 cpm). The antigen-antibody complexes were precipitated by addition of an appropriate amount of goat anti-human or anti-rabbit IgG or goat anti-human IgE for 16 h at 4 °C. The precipitates were collected on Whatman GF/A glass fibre paper filters and, after washing (10 mL of BBS), the radioactivity retained was measured by a gamma counter.

Antibody level was expressed in arbitrary units using as a standard the IgG binding curve obtained with a reference serum. Background value (obtained in assays performed with preimmune rabbit serum or human control pool) was subtracted from the reported values. Average background: IgG and IgE ELISA: 230 ± 30 OD at 405 nm and 160 ± 21 cpm, respectively; IgG and IgE DARIA: 250 ± 33 cpm and 190 ± 25 cpm, respectively.

Inhibition assays

For antibody competition in ELISA, serial amounts of rabbit antiserum were added to human serum prior to incubation with allergen-bound microtitre wells. Absorption of the human sera with 6a FP or control betagalactosidase was carried out for 1 h at room temperature. The inhibition was expressed as: $(1-(a-b)/(c-b))\%$, where a and b were values obtained in the presence and in the absence of the inhibitor tested, respectively, and c was the background value. Average background: IgG and IgE DARIA: 290 ± 38 cpm and 230 ± 30 cpm, respectively; IgE ELISA: 190 ± 29 cpm.

ELISA, DARIA and inhibition assays were run in duplicate: accepted coefficients of variation from the calculated arithmetic mean were 10% (DARIA) and 12% (ELISA). Average variation between experiments was 23% and 25%, respectively.

SDS-PAGE and Western blotting

SDS-PAGE and Western blotting were performed as described [10], using 8% or 12% polyacrylamide gel in 225 mM Tris-HCl (pH 8.8), 0.4% SDS.

cDNA library, nucleotide sequencing

A total of 3 μ g of *P. judaica* pollen poly (A) + RNA [14] was reverse transcribed (cDNA Synthesis kit, Pharmacia). cDNA was inserted into dephosphorylated lambda gt11 arms (EcoRI/NotI adaptors). *In vitro* packaged phages

(Gigapack Gold II packaging system, Stratagene) were propagated in *E. coli*. The library was immunoscreened using the Lambda lift expression detection kit (Bio-rad). Purified phage DNA [15] was subcloned in the plasmid vector pUC18 (pUC Cloning kit, Boehringer). Nucleotide sequencing was performed according to Sanger [12].

PCR amplification

Insert cDNA was amplified using lambda gt11 primers (Promega). A total of 150 ng of primer was used for 1 μ g of DNA in 30 amplification cycles (1' at 97 °C, 2' at 45 °C, 2' at 65 °C).

Results

Isolation and characterization of the 6a fusion protein

A cDNA library in lambda gt 11 was obtained from *P. judaica* pollen mRNA as described under Methods. Some 30 out of 300 000 clones screened were immunopositive with a rabbit anti-serum specific for *Par o* I and *Par j* I. Figure 1 shows the bands obtained in agarose gel electrophoresis by PCR amplification of DNA from seven immunopositive and one immunonegative clone. The immunoblot analysis of bacterial lysates using rabbit anti *Par j* I and a human serum with a high level of IgG

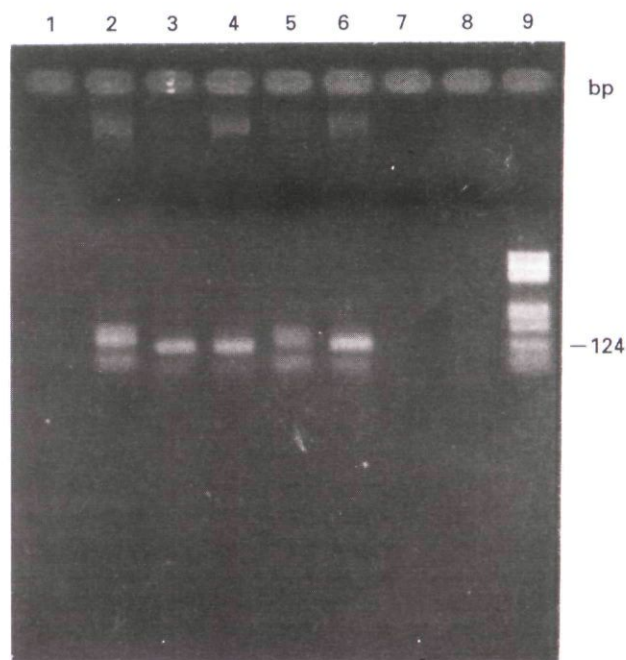


Fig. 1. Agarose gel electrophoresis of PCR amplified DNA from immunopositive (lane 1–7) and immunonegative clones (lane 8). lane 9: mw markers; lane 3: clone 6a.

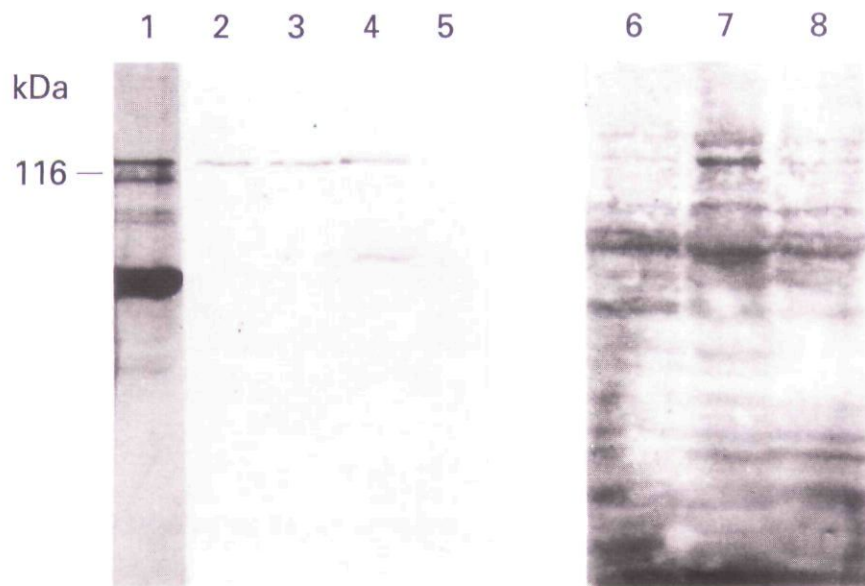


Fig. 2. Western blotting. Analysis of bacterial lysates from three immunopositive clones (lanes 1–4 and 6,7) and from an immunonegative clone (lanes 5 and 8) using IgG from rabbit anti *Par o I* antiserum (lanes 2–5) or from the serum of an allergic patients (lanes 6–8). The lysate from clone 6a yield a 116 kDa band with rabbit (lane 3) and with human IgG (lane 7); its reactivity with anti beta-galactosidase is shown in lane 1.

ab towards *Par o I* and *Par j I* is reported in Fig. 2. With the rabbit antiserum three immunopositive clones (lanes 2–4) but not an immunonegative clone (lane 5) yield a band with the mobility of beta-galactosidase; lane 7 shows the band obtained with clone 6a lysate and the human serum. The insert from clone 6a was sequenced and yield the following 26 bp sequence (6a sequence): GGCACGAGCTCGTGCCGACT CGTGCC.

The beta-galactosidase fusion protein expressed by clone 6a (6a FP) was electroeluted from a sector of SDS PAGE gel which corresponded, by molecular weight and by comparison with immunoblotting pattern, to the beta-galactosidase band (1 mL of 10X lysate yield 5 µg of protein) and used to immunize a rabbit. The antiserum obtained (anti 6a) crossreacted with *Parietaria* pollen allergens (Figs 3 and 4). In immunoblotting (Fig. 3), anti 6a reacted with beta-galactosidase from clone 6a (Fig. 3, lane 2) and with a components of *P. judaica* pollen extract having the electrophoretic mobility of *Par j I* (Fig. 3, lane 4), whereas no antibody binding was observed using the preimmune rabbit serum (Fig. 3, lanes 3 and 6). In the DARIA, radiolabelled *Par o I* (1 ng) was incubated with serial dilutions of anti 6a, or of a rabbit antiserum specific for *Par o I* or of a serum from an allergic patient. Antibody-bound allergen was precipitated by adding anti-rabbit or anti-human IgG. Figure 4 shows that anti 6a specifically bound *Par o I* according to a dose–response curve; 10 µL of anti 6a bound 0.47 ng of *Par o I*, whereas the same amount of preimmune rabbit serum bound 0.07 ng. In a control assay, performed with 1 ng of a radiolabelled human Bence Jones protein (4700 cpm), anti 6a bound less than 0.7% of added cpm.

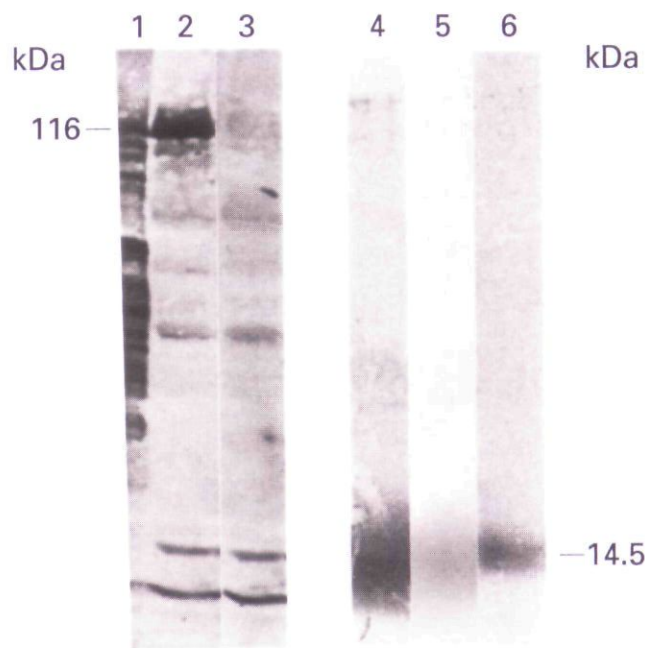


Fig. 3. Western blotting. Lane 1: reactivity of anti 6a with the partially purified beta-galactosidase obtained from clone 6a and used for immunization. Lanes 2,3: analysis of clone 6a lysate with anti 6a (lane 2) and preimmune rabbit serum (lane 3). Lanes 4–6: analysis of *P. judaica* pollen extract with anti 6a (lane 4), with preimmune rabbit serum (lane 5) and with anti *Par j I* rabbit antiserum (lane 6).

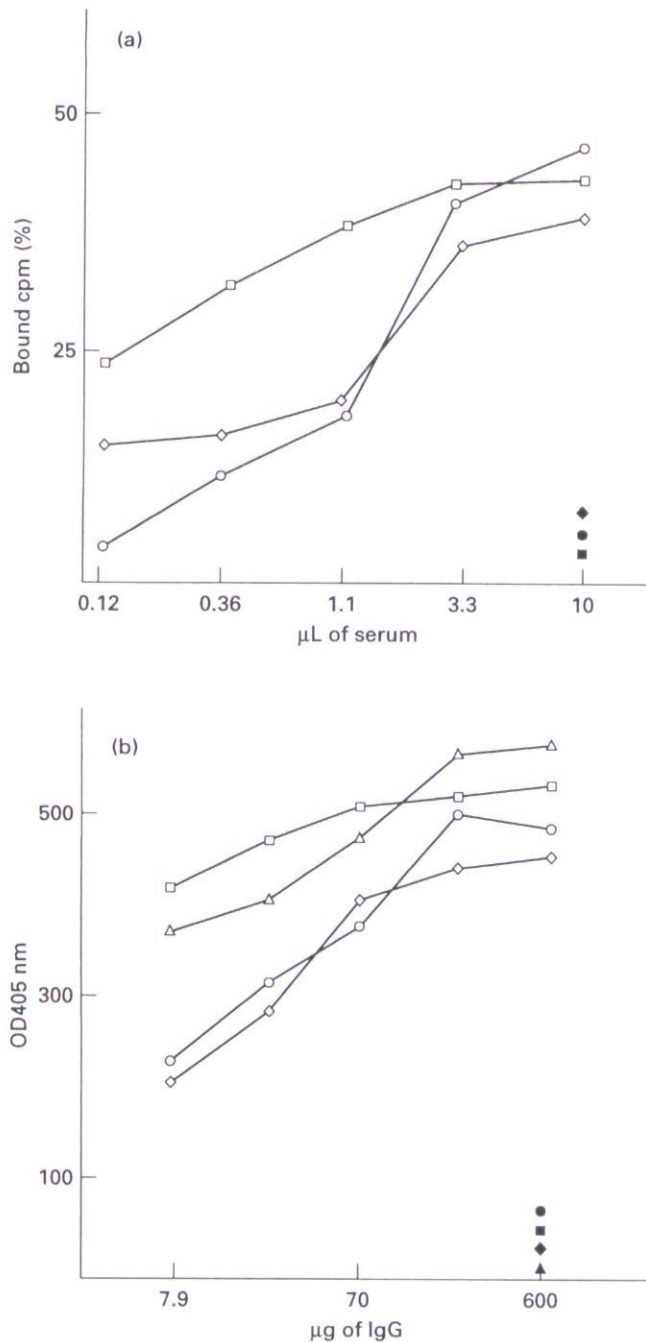


Fig. 4. (a): DARIA. 100 μ L (11 450 cpm) of *Par o I* (10 ng/mL in PBS) was incubated with serial dilutions of rabbit anti 6a FP (○) or of anti *Par o I* (□) or of a serum from an allergic patient (●) or with 1/10 dilution of the preimmune rabbits sera (■) or of a control human serum (◆). Allergen-antibody complexes were precipitated by addition of 70 μ L of goat anti rabbit IgG (□○■●) or goat anti human IgG (◆) IgG. (b): ELISA. *P. judaica* (□○■●) or *P. officinalis* (◆) pollen extract coated wells (5 μ g) were incubated with 50 μ L of serial dilutions of the IgG fraction from rabbit anti 6a FP (○), or from rabbit anti *P. officinalis* pollen extract (□) or of preimmunized rabbits sera (■●◆). Allergen bound IgG was revealed using alkaline phosphatase conjugated goat anti rabbit IgG.

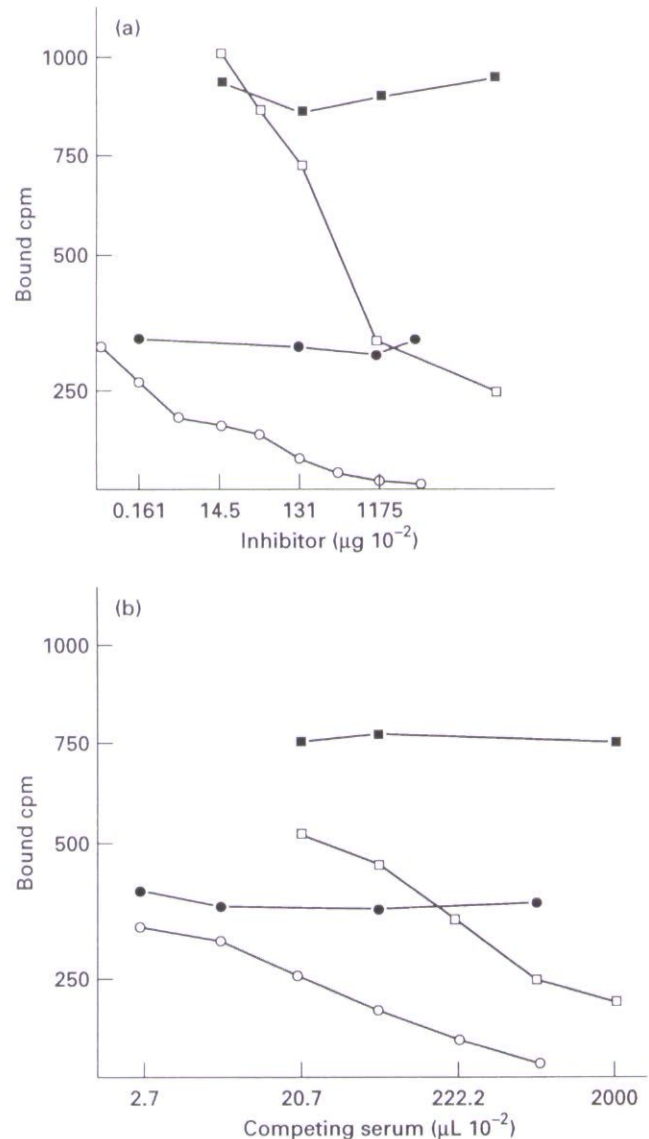


Fig. 5. Inhibition of IgE and IgG ab binding to *Parietaria* allergens. (a) 100 μ L of the allergic patients serum pool diluted 1/5 (IgG ab), or 1/2 (IgE ab), was absorbed with the amounts of 6a FP (□○) or of control beta-galactosidase (■●) indicated in the abscissae. After incubation with 100 μ L (11 450 cpm) of radiolabelled *Par o I* (10 ng/mL), bound cpm were measured following precipitation of ag-ab complexes with goat anti human IgG (□■) or anti human IgE (○●). (b) 10 μ L of serial dilutions of 40 μ L of rabbit anti 6a (■●), or of pre-immune rabbit serum (nl) were added to 40 μ L of the human pool (○●) or of 1/3 dilution of the serum from an allergic patient (□■) prior to incubation with *P. officinalis* pollen extract coated wells (5 μ g). Bound IgE was revealed using radiolabelled anti-human IgE.

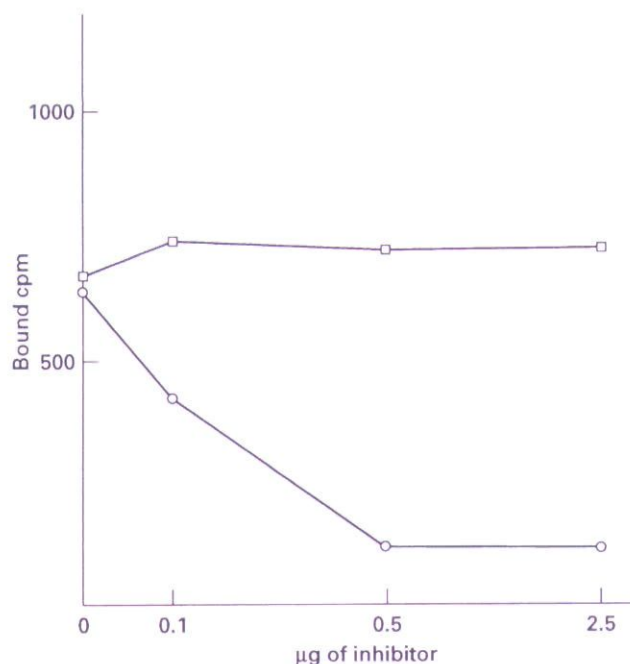


Fig. 6. DARIA. Five sera containing ab towards *Par o I* and *Ole e I* were pooled and samples of 50 μ L were incubated with 20 μ L of BBS containing increasing amount of 6a FP for 1 h at room temperature. After addition of 50 μ L of the appropriate dilution of the myeloma carrier, the mixtures were incubated with 100 μ L of BBS containing 1 ng (14 450 cpm) of *Par o I* (○) or 1 ng (10 110 cpm) of *Ole e I* (□) for 4 h at room temperature. Ag-ab complexes were precipitated by goat anti-human IgE (16 h at 4 °C).

IgG from anti 6a, from rabbit anti *P. officinalis* extract antiserum and from preimmune rabbit sera were affinity purified and assayed for reactivity with solid phase *P. officinalis* and *P. judaica* extracts in ELISA. Figure 4b represents the dose response curves obtained and shows that anti 6a specifically bound *Parietaria* pollen antigen.

Inhibition of human IgG and IgE antibody binding to Parietaria pollen allergens

The 6a fusion protein was assayed for its ability to absorb *Par o I* specific IgE and IgG antibodies from the human pool. Beta-galactosidase electroeluted from SDS-PAGE gels of the lysate from an immunonegative clone was used as a control. Figure 5a represents the inhibition curves obtained by absorbing allergic patients serum pool with serial amounts of 6a FP prior to incubation with purified radiolabelled *Par o I* (1 ng) in DARIA. Absorption with 6a FP, unlike absorption with control beta-galactosidase, extensively inhibited IgG and IgE antibody binding. Fifty per cent inhibition of IgE and IgG ab binding required 0.35 μ g and 8 μ g of 6a FP,

respectively. Maximum inhibition of IgG and IgE ab binding was 97 and 74%, respectively, and was achieved by preabsorption with 34 μ g of 6a FP. Preabsorption with the same amount of control beta-galactosidase yield 2% and 1% inhibition of IgG and IgE ab binding, respectively. The 6a FP absorbed anti *Par o I* but not anti *Ole e I* ab from a serum pool obtained from allergic patients who had ab towards *Ole e I*, the major pollen allergen from *Olea europea* as well as towards *Par o I* (Fig. 6).

The ability of rabbit anti 6a to compete with human antibodies for binding to *Parietaria* pollen allergens was assayed in ELISA. Figure 5b shows the dose-response inhibition curves obtained by adding serial dilutions of anti 6a to the human pool or to the serum from an individual patient prior to incubation with *P. officinalis* pollen extracts coated microtitre wells.

Prevalence of 6a specific antibody in individual sera

To investigate the prevalence of IgG and IgE antibody response specific for the 6a epitope in *Parietaria* allergic patients we used the inhibition assays described previously.

For five allergic patients and two pools, 100 μ L of serum were absorbed with 2.35 μ g of control betagalactosidase (Table 1 A, column b) or of 6aFP (Table 1 A, column c) prior to incubation with the radiolabelled allergen (1 ng). Pool I was formed from the sera of eight Israeli patients (I.01-I.25; Table 1 B) and pool T from the sera of eight Italian patients (T.70-T.91; Table 1 B). IgE and IgG ab binding was determined in *Par o I* specific DARIA. Preabsorption with 6a FP but not with the control protein in each case extensively inhibited antigen binding.

For 28 patients the serum was mixed with an equal amount of preimmune rabbit serum (Table 1B, column b) or of anti 6a (Table 1B, column c) prior to incubation with *P. officinalis* pollen extract coated microtitre wells in ELISA. Of these patients, eight (I.01-I.25) were from Israel, the remaining from Italy. Addition of anti 6a inhibited 61–100% (average 88%) of IgE ab binding in the sera from Italian patients and 29–73% (average 50%) in the sera from Israeli patients.

Discussion

The deduced aminoacid sequence of the antigenic determinant encoded by the 6a cDNA, in frame with beta-galactosidase is: gly thr ser ser cys arg leu val (pro). The focusing of antibody response to *Par o I* and *Par j I* on a linear epitope is unusual: allergens, as antigens, in general have conformational epitopes [4–8]. Dominant linear epitopes represented by peptides of 4–9 aminoacid

Table 1. Prevalence of 6a epitope specific IgG and IgE antibodies

A							
Patient	a ¹	IgG ab b ¹	c ¹	a ¹	IgE ab b ¹	c ¹	
N.21	982	951 (−3.2%)	259 (−73.6%)	1268	1114 (−12.2%)	387 (−69.5%)	
N.22	4904	4760 (−4.1%)	354 (−73.0%)	1600	1599 (−0.1%)	611 (−61.8%)	
N.23	1999	1967 (−1.7%)	613 (−69.3%)	281	249 (−11%)	78 (−72%)	
N.25	2840	3472 (+1.2%)			777 (−92.9%)		
N.26	3448	2915 (−14.5%)			1338 (−61.2%)		
pool I	1304	1404 (+1.8%)			291 (−77.7%)		
pool T	2661	2562 (−3.8%)			368 (−86.2%)		
B							
Patient	a ²	IgE ab b ²	c ²	Patient	a ²	IgE ab b ²	c ²
N.01	304	351 (+1.15%)	21 (−94.2%)	T.73	2170	2242 (+1.03%)	462 (−80%)
N.02	790	775 (−2.9%)	56 (−92.9%)	T.74	908	855 (−5.8%)	10 (−98.9%)
N.03	1400	1332 (−4.9%)	110 (−92.1%)	T.88	1300	1251 (−3.8%)	231 (−82.2%)
N.04	1100	1182 (+1.07%)	110 (−91%)	T.89	234	193 (−17.6%)	91 (−61.1%)
N.05	120	109 (−9.2%)	13 (−89.2%)	T.90	246	286 (+1.16%)	111 (−66.4%)
N.06	1375	1342 (−2.4%)	111 (−91.9%)	T.91	1444	1426 (−1.2%)	238 (−83.5%)
N.07	142	120 (−15.5%)	7 (−95.1%)				
N.08	1154	1104 (−4.3%)	76 (−93.4%)				
N.09	181	194 (+1.07%)	7 (−96.7%)	I.01	202	194 (−4%)	109 (−46%)
N.10	794	836 (+1.05%)	79 (−90.7%)	I.02	630	698 (+1.11%)	205 (−72.7%)
N.11	602	554 (−8%)	2 (−99.7%)	I.03	300	285 (−5%)	179 (−40.3%)
N.12	220	198 (−1%)	10 (−95.5%)	I.17	880	785 (−4.3%)	366 (−55.4%)
				I.13	600	597 (−0.5%)	331 (−44.8%)
				I.18	1180	1163 (−1.4%)	596 (−59.5%)
T.70	485	442 (−8.9%)	136 (−72%)	I.20	900	953 (+1.6%)	384 (−60.6%)
T.72	916	888 (−3.1%)	112 (−88%)	I.25	444	431 (−3%)	354 (−29.3%)

A. DARIA

¹IgG or IgE bound cpms expressed in arbitrary units (cfr. Methods). In parenthesis % of inhibition.

Column a: sera (100 µL) preabsorbed with 10 µL of BBS. Column b: sera preabsorbed with 2.35 µg of control betagalactosidase in 10 µL of BBS. Column c: sera preabsorbed with 2.35 µg of 6a FP in 10 µL of BBS. Following incubation with 1 ng (11.450 cpm) of radiolabelled *Par o I*, antigen-antibody complexes were precipitated by human IgG or IgE specific goat antiserum.

B. IgG ELISA.

²OD at 405 nm. In parenthesis % of inhibition.

25 µL of human serum mixed with 25 µL of PBS (column a) or of preimmune rabbit serum (column b) or of anti 6a (column c) was added to *P. officinalis* extract coated microtiter wells (5 µg). Bound IgE was measured using radiolabelled goat anti human IgE.

Patients N.1–N.12 and T.70–T.91 were Italian, obtained from Dr. G. Sacerdoti and Dr. L. Tarentino, respectively; patients I.01–I.25 were Israeli, obtained from Dr Geller-Bernstein.

residues have been described in the response to viral and protozoan antigens [16–18].

The data presented here suggest that antibodies with the 6a specificity play an important role in allergy to *Parietaria*. *Parietaria* specific pollinosis is characterized by high proportion of monosensitization: 39% of *Parietaria* allergic patients are not sensitized to other allergenic sources. (M. d'Amato *et al.* manuscript in preparation).

Par o I and *Par j I* are highly immunogenic/allergenic [8–10]. In naturally sensitized patients, the antibody response is often restricted to these crossreacting allergens: an analysis of immunoblot patterns of 40 individual sera has shown that although several potential allergens were present in the pollen extract, in 1/3 of the cases IgE antibody bound only to the the major allergen band [19]. The experiments reported in the present study confirm

these observations by showing that anti-6a-specific antibody response is highly prevalent among naturally immunized patients. In addition the high level of inhibition achieved by absorption of individual sera with the fusion protein suggests that antibody response to *Par o* I and *Par j* I is focused on the 6a epitope. The comparatively lower percentage inhibition obtained for sera from Israeli patients as compared with sera from the Italians may reflect exposure to distinct species or varieties of *Parietaria*.

The high immunogenicity of the 6a epitope may reflect crossreactivity with microbial or autoantigens and/or intrinsic properties of *Par o* I/*Par j* I molecules in the interaction with B cells and/or preferential pairing of 6a-specific B cells with helper T cells of a dominant specificity. A pertinent question is whether in the response to *Par o* I, T cells exhibit a restricted TCR repertoire as reported for other allergens [20,21].

The focusing of antibody response to a specific epitope singles *Par o* I and *Par j* I out among known allergens. The search for epitopes which are antigenic/allergenic for a large proportion of individuals responding to a given antigen and/or dominate restricted individual antibody repertoires has been pursued for several major allergens. Dominant epitopes have been attributed to the pollen allergens *Amb a* I from *Ambrosia* [2], *Lol p* I, and *Poa p* I from grass [3,4], *Sin a* I from *Sinapis alba* [5] and *Bet v* I from birch [6]. These epitopes are defined by mouse MoAbs inhibiting a high proportion of human antibody binding to the respective allergen. A caveat is that high binding inhibition may reflect the occurrence of human autoantibodies crossreacting with variable regions of mouse immunoglobulins [22] or may be unspecific due to allosteric hindrance [23]. The prevalence of epitope-specific response among patients naturally sensitized to specific allergens has also been investigated: an analysis of the response of 38 tree allergic patients to MoAbs defined group I epitopes [24] and a similar study of the response of 14 mite allergic patients to *Der p* I epitopes defined by large fusion peptides [25], yield no evidence of dominance of specific epitopes and showed that different individuals responded to different epitopes.

The observation that the same epitopes are target for IgG and IgE antibodies is concordant with studies on the *Chironomid* allergen *Chir t* I [26] and on the mite allergen *Der p* I [25]. A strict relationship between the development of IgG and IgE antibody response is suggested also by studies indicating that in the response to certain allergens antibodies of these isotypes share idiotypes [27]. In the mouse it has recently been shown that IgG1 and IgE class switching are sequential [28].

The 6a epitope is potentially an interesting reagent for immunotherapy. There are at least three approaches to

therapy based on allergen specific modulation of the immune response which have proven to be at least partially effective: vaccination with allergenic extracts, injection of antigen-antibody complexes and vaccination with modified allergens. Different but not mutually exclusive mechanisms have been postulated, including promotion of the synthesis of blocking antibodies, modulation of TH₁/TH₂ development, production of anti-idiotypic autoantibodies [29–31]. The availability of a well defined reactive which can be modified in a programmed way may help to test these hypotheses.

Species crossreactivity such as that displayed by the 6a epitope characterizes human antibody response to major allergens from several sources. Unlike viral and bacterial parasite antigens, which usually induce strain specific antibodies, allergens elicit antibodies which are cross-reactive among species and often among distinct molecules in the same species [32–34]. It could be speculated that in allergy chronic or repeated exposure to a mixture of molecules from genetically heterogeneous populations targets the immune response to protein domains conserved in evolution or shared between different gene products because of genomic events such as exon shuffling or gene duplication [34]. Perhaps whereas the study of the immune response to parasites has drawn the attention to evolutionary mechanisms creating variability, the immune response in allergy underlines evolutionary conservation of certain structures and genome mobility, important concepts in modern genetics.

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