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A sensitive monoclonal antibody-based enzyme-linked immunosorbent assay to quantify *Parietaria judaica* major allergens, Par j 1 and Par j 2

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Clinical and Experimental Allergy

Summary

Background *Parietaria* pollen is one of the most important causes of pollinosis in Mediterranean countries. *Parietaria judaica* pollen extract presents two major allergens, Par j 1 and Par j 2, that belong to the lipid transfer protein family.

Objective To develop an ELISA for quantification of both major allergens of *P. judaica* pollen extracts, and to assert correlation of these allergens content with the allergenic activity of extracts.

Methods Natural Par j 1–Par j 2 allergens were purified by gel filtration, ion exchange, and affinity chromatography and identified by mass spectrometry. Rabbit antisera were obtained using this protein preparation as antigen and used for immunoaffinity purification of nPar j 1–Par j 2. BALB/c mice were immunized with the immunopurified nPar j 1–Par j 2 and after fusion and screening by direct ELISA, 5D4 monoclonal antibody was selected as capture antibody to develop a quantitative two-site ELISA. Bound proteins were detected by a biotinylated Par j 1–Par j 2-specific polyclonal antibody.

Results The optimized ELISA was developed from 25 to 8000 pg/mL of purified Par j 1–Par j 2, and a linear portion of 200–1000 pg/mL. The intraassay and interassay coefficients of variation were lower than 7% and 14% respectively. The assay was very sensitive and specific as it had a detection limit of 25 pg/mL and did not detect reactivity with the same family plants, as *Urtica*. Par j 1–Par j 2 allergens content was measured in 14 *P. judaica* and two *P. officinalis* pollen extracts showing a significant correlation with their allergenic activity measured by enzyme allergosorbent test inhibition.

Conclusions The results proved the usefulness of the two-sandwich ELISA for the standardization of *Parietaria* pollen extracts intended for clinical use, because of its good correlation with allergenic potency.

Keywords allergen, biological standardization, immunoassay, monoclonal antibody, Par j 1, Par j 2, *Parietaria judaica*, quantification

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Introduction

Parietaria judaica is a wind-pollinated dicotyledonous weed belonging to the Urticaceae family and is commonly found in the countryside and urban areas, growing on walls and soils with a high nitrogen content [1]. *P. judaica* pollen is the main cause of allergy in the Mediterranean area, with a prevalence of 60–80% in Italy and Greece and 25–40% in Spain and southern France [2]. Nowadays, it is

essential to have available standardized extracts for diagnosis and immunotherapy. The current standardization method is based on the enzyme allergosorbent test (EAST) inhibition, which compares an in-house reference (IHR) extract with new produced batches. This protocol faces two main problems: first, the technique involves the use of human sera, which are difficult to standardize, and secondly, extracts with similar potency can have differences in major allergen ratios [3]. It tends to quantify

major allergen levels in mass units by using monoclonal antibodies (mAbs)-based immunoassays [4] in order to complement the quality control of the extracts.

P. judaica pollen extracts contain at least nine allergens, Par j 1 and Par j 2 being the two major ones as reacted with IgE of 95% and 82% *P. judaica*-allergic patients, respectively [5–7]. These allergens show a high isoform heterogeneity as more than seven different but closely related N-terminal sequences from them have been reported [1, 8, 9]. Par j 1 (14.7 kDa) and Par j 2 (11.3 kDa) belong to the non-specific lipid transfer protein (LTP) family and present a very conserved structural motif composed of four α -helices [10]. This family of proteins has been recently described as a new pan-allergen with a very high degree of cross-reactivity among vegetable foods [11]. Both allergens have three similar allergenic epitopes with high homology and identical conformation [12].

In this study, a solid phase ELISA based on specific antibodies that reacted with Par j 1 and Par j 2 shared epitopes was developed for the first time in order to provide a specific and sensitive estimation of the content of the two *P. judaica* major allergens at the same time.

Materials and methods

Pollen extracts and recombinant allergens

Deffated pollen from *P. judaica* was extracted by magnetic stirring (4 h at 4 °C) at 6.6% (w/v) in 50 mM phosphate buffer plus 65 mM NaCl pH 6.5. The obtained extract was clarified by centrifugation at $13\,000 \times g$ for 30 min, and the supernatant was filtered through AP glass fiber prefilters (Millipore Corp., Bedford, Madison, WI, USA) and dialysed by ultrafiltration with a 5 kDa cut-off in a Pellicon System (Millipore). Recombinant Par j 1 and Par j 2 allergens were produced in *Pichia pastoris* as previously described [13].

Purification of natural and recombinant *Parietaria judaica* major allergens

For chromatographic Par j 1–Par j 2 purification, proteins from 5 g of pollen were extracted with 10 mM phosphate buffer, pH 7.4, plus proteinase inhibitors (1 mM phenylmethanesulphonyl fluoride, 0.1 mM *N*-*p*-Tosyl-L-phenylalanine chloromethyl ketone, 2 µg/mL aprotinin) at 4 °C for 2 h. After centrifugation, the supernatant was concentrated by ultrafiltration using an Amicon Ultra-4 device with a 5 kDa cut-off (Millipore Corp.) and applied to a Superdex S-200 column equilibrated with phosphate-buffered saline (PBS) in an ÄKTA-prime system (Amersham Biosciences, Buckinghamshire, UK). Fractions containing the 14–16 kDa IgE reacting proteins were pooled and, after dialysis with 50 mM sodium acetate, pH 5.2, applied onto a HiTrap SP ion exchange column (Amer-

sham Biosciences). The purified allergens were eluted with a gradient 0–300 mM NaCl. Par j 1 and Par j 2 were isolated in the same fraction and identified by peptide mass fingerprinting as described below.

Polyclonal antiserum against chromatographic purified natural Par j 1–Par j 2 was produced in a New Zealand rabbit injected intramuscularly with 0.2 mg of nPar j 1–Par j 2 emulsified in Freund complete adjuvant. Injections were repeated fortnightly for 75 days and sera were collected 10 days after the last injection. IgG were purified on a HiTrap Protein G (Amersham Biosciences). The fraction used in the two-site sandwich ELISA was labelled with biotin using the ECL protein biotinylation module (Amersham Biosciences) as recommended by the manufacturer.

An immunosorbent column prepared by coupling rabbit anti-Par j 1–Par j 2 polyclonal antibodies to CNBr-activated Sepharose 4B (8 mg of antibodies/mL of gel) as recommended by the manufacturer (Amersham Biosciences) was used to purify natural Par j 1–Par j 2 or recombinants Par j 1 and Par j 2. Therefore, *P. judaica* pollen extract or *P. pastoris* culture supernatants were passed through the column, and after extensive washing with PBS, bound protein was eluted with 100 mM citrate buffer, pH 2.7. Fractions were collected in tubes containing neutralizing buffer (1 M Tris-HCl, pH 8.0), and then pooled, concentrated by ultrafiltration using an Amicon Ultra-4 device with a 5 kDa cut-off (Millipore Corp.), and stored at –40 °C in aliquots. The protein content was determined by the Bradford method with the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA, USA).

Peptide mass fingerprinting

Chromatography-purified Par j 1–Par j 2 were submitted to electrophoresis and stained with Coomassie blue. After electrophoresis, the two 14–16 kDa bands were manually excised from the gel and digested *in situ* with trypsin [14]. An aliquot (0.5 µL) of digested proteins was analysed in an Autoflex II MALDI-TOF (Bruker Daltonics, Billerica, MA, USA) equipped with a reflector using HCCA (α -cyano-4-hydroxycinnamic acid) as matrix in saturated conditions. Mass spectra obtained were used for protein identification in databases. The digestion was further analysed by liquid reverse-phase chromatography coupled with electrospray mass spectrometry with ionic trap Deca-XP (Thermo-Finnigan, San Jose, CA, USA). The fragmentation spectra obtained (ms/ms) were analysed with Turboquest software (Thermo-Finnigan), and homologies were found with partial sequences using the FASTA program (University of Wisconsin, USA).

Production and purification of monoclonal antibodies

mAbs were obtained by immunizing BALB/c mice with the fraction containing natural Par j 1 and Par j 2 allergens

purified by immunoaffinity chromatography. Detailed procedures for the fusion of spleen cells with Sp2/0 myeloma cells and hybridoma maintenance have been previously described [15]. Supernatants were screened by ELISA against nPar j 1–Par j 2 and their recombinant counterparts and hybridomas secreting antibodies that reacted to these antigens were cloned by limiting dilution. Isotype detection was done by a commercial kit (Boehringer Mannheim, Mannheim, Germany). mAbs were purified from hybridoma culture supernatants with a HiTrap Protein G (Amersham Biosciences).

Electrophoresis and Immunoblotting

P. judaica extracts or purified allergens were separated by SDS-PAGE in 12.5% polyacrylamide gels under reducing conditions [16] and stained with Coomassie brilliant blue. Separated polypeptides were electrotransferred onto polyvinylene difluoride membranes (Amersham Biosciences), which were blocked for 1 h at room temperature with 5% skimmed milk and 0.1% Tween 20 in PBS. Then, the blots were incubated with the previously selected mAb 5D4 (0.3 µg/mL) or anti-Par j 1/2 rabbit serum (1/100 000) in PBS containing 0.1% Tween 20 (PBS-T). After washing, the membranes were incubated for 1 h at room temperature with peroxidase-conjugated anti-mouse IgG or anti-rabbit Igs (1/10 000 in PBS-T) and bound antibodies were detected by the chemiluminescence method as recommended by the manufacturer (ECL-Plus; Amersham Biosciences).

Enzyme-linked immunosorbent assay for Par j 1–Par j 2 quantification

Multiwell plates (Greiner, Frickenhausen, Germany) were coated overnight at room temperature with 100 µL/well of mAb 5D4 at 5 µg/mL in PBS. After blocking during 1 h at 37 °C with 200 µL/well of PBS containing 1% bovine serum albumin (BSA) and 0.05% Tween 20, a standard curve was generated by adding 100 µL of serially diluted affinity-purified nPar j 1–Par j 2 (8000–25 pg/mL) to each well and incubating for 1 h at 37 °C. After three washes with 200 µL/well of PBS containing 0.05% Tween 20, 100 µL/well of biotin-labelled Par j 1–Par j 2 polyclonal antibody (625 ng/mL) was added and incubated in the same conditions. The washes were repeated and the plates were incubated with 100 µL/well of streptavidin-horseradish peroxidase conjugate (250 ng/mL) (Sigma, St. Louis, MO, USA). After further washes the development was carried out by incubating at room temperature and in darkness with 200 µL/well of *o*-phenylenediamine solution (Sigma-Fast *o*-phenylenediamine dihydrochloride Tablet Sets, Sigma). Finally the reaction was stopped after 30 min by adding 50 µL/well of 3 M H₂SO₄, and the absorbance was measured at 492 nm. *P. judaica* pollen extracts were

assayed in triplicate and the concentrations were interpolated in the linear portion of the standard curve.

For evaluation of intraassay variations, Par j 1–Par j 2 allergen concentrations (1, 0.9, 0.8, 0.6, 0.5, 0.4, and 0.2 ng/mL) were measured 20 times in the same assay, and for interassay variations 10 curves were done on different days. Coefficient of variation (CV) was defined as the standard deviation divided by the mean and multiplied by 100. Detection limit was calculated as the amount of Par j major allergens, which corresponded to the mean plus 3.33 times the standard deviation obtained after 30 measurements of the zero standard. Using Student's *t*-test (*P* < 0.01) the regression lines of dose–response curves from purified proteins and pollen extracts were checked for parallelism.

Enzyme allergosorbent test-inhibition

The IgE-binding activity of *P. judaica* extracts was measured by EAST inhibition by comparison with the inhibition level obtained in relation to an IHR *P. judaica* extract, previously standardized by the skin prick test (SPT) in accordance with the Nordic Guidelines and the recommendations from the European Academy of Allergy and Clinical Immunology [17, 18]. Briefly, aliquots of a serum pool from *Parietaria* pollen-sensitive patients were pre-incubated with serial dilutions of each *P. judaica* extract. Next, these aliquots were incubated with paper disks coupled to an IHR *P. judaica* pollen extract according to Ceska and Lundkvist [19]. After washing, the disks were incubated with alkaline phosphatase-labelled mouse anti-human IgE (Hycor Biomedical, Kassel, Germany) and developed following the instructions of Hytec-specific IgE enzyme immunoassay (Hycor Biomedical).

Results

Purification and analysis of Par j 1–Par j 2

The purified fraction containing both nPar j 1 and Par j 2 used for immunization showed a double band on Coomassie blue-stained SDS-PAGE with an apparent molecular mass of 15–16 kDa (Fig. 1). The identity of purified Par j 1–Par j 2 was confirmed by mass spectrometry, as the sequences obtained for MS–MS-derived peptides matched registered sequences of databases. Upper and lower bands corresponded to Par j 1 and Par j 2 sequences, respectively (Table 1), although masses corresponding to Par j 2 were found as contaminants of Par j 1 band. SDS-PAGE analysis of immunoaffinity-purified recombinant proteins displayed a band of 15.4 kDa for rPar j 2 and two bands of 18.6 and 16.7 kDa for rPar j 1 (Fig. 1). The latter bands corresponded to glycosylated and non-glycosylated forms of rPar j 1 as demonstrated by periodic acid-Schiff staining (data not shown).

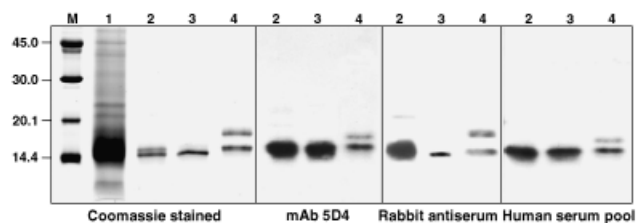


Fig. 1. Coomassie-stained SDS-PAGE and Western blotting of *Parietaria judaica* extract (lane 1), nPar j 1-Par j 2 (lane 2), rPar j 2 (lane 3), and rPar j 1 (lane 4) immunostained with mAb 5D4, anti-Par j 1-Par j 2 polyclonal antiserum, and pool of sera from *P. judaica*-allergic patients. Extract was loaded at 20 µg/lane and purified allergens at 2 or 0.1 µg/lane for Coomassie staining or Western blotting, respectively.

Table 1. Peptide sequences obtained by mass spectrometry analysis for the purified natural Par j 1-Par j 2 and published isoforms

Band (kDa)	Sequence (location in the sequence)	Corresponding isoform*
16	GCYSGAKRLDGETKTGPQR (65-83)	Par j 1.0101, Par j 1.0201
	TYSDIDGK (96-103)	Par j 1.0101, Par j 1.0102
15	KLSEEVK (66-72)	Par j 2.0101, Par j 2.0102
	GISGIKNELVAEVPK (90-114)	Par j 2.0101, Par j 2.0102
	GISGIKNELVAEVPPK (90-115)	Par j 2.0101, Par j 2.0102
	NELVAEVPK (96-114)	Par j 2.0101, Par j 2.0102
	NELVAEVPK (96-115)	Par j 2.0101, Par j 2.0102
	TTLPPITADFDCSK (120-133)	Par j 2.0101, Par j 2.0102

*Swiss-Prot accession numbers: Par j 1.0101, P43217; Par j 1.0102, O04404; Par j 1.0201, Q40905; Par j 2.0101, P55958; Par j 2.0102, O04403.

Production and characterization of antibodies

After fusion of spleen cells of a mouse immunized with immunoaffinity-purified natural Par j 1-Par j 2 and Sp2/0 myeloma cells, 36 hybridomas secreting mAbs anti-Par j 1-Par j 2 were detected by ELISA and their relative affinities to rPar j 1 and rPar j 2 were studied. Among the mAbs, the named 5D4 (IgG1 subclass with *k* light chains) was chosen because it showed the highest absorbance value by ELISA. Moreover, the 5D4 mAb was able to capture natural Par j 1-Par j 2 allergens in solution when biotin-labelled polyclonal antibodies were used for detection. Antibody reactivity was checked by immunoblotting experiments with nPar j 1-Par j 2, rPar j 1 and rPar j 2 (Fig. 1). The 5D4 mAb and the rabbit anti-serum reacted properly to natural and recombinant allergens in reducing and not reducing conditions.

Enzyme-linked immunosorbent assay for Par j 1-Par j 2 quantification

A two-site sandwich ELISA to quantify *P. judaica* major allergens was developed by using the 5D4 mAb as capture,

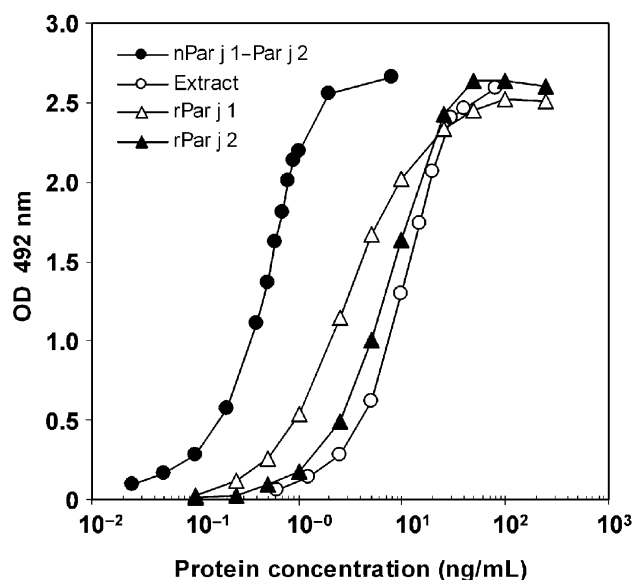


Fig. 2. Dose-response curves in two-site sandwich ELISA of natural Par j 1-Par j 2, rPar j 1, rPar j 2, and *Parietaria judaica* pollen extract. Results represent the average of three (10 for nPar j 1-Par j 2) independent experiments where coefficient of variation was lower than 20%.

the polyclonal antibody as tracer and natural purified Par j 1-Par j 2 as standard curve. The assay was dose dependent, and the standard dose-response curve revealed a sigmoid shape when plotted on a semilogarithmic scale (Fig. 2). The linear portion of the standard curve was found in the range from 200 to 1000 pg/mL, with a coefficient of regression of 0.99. The assay was very sensitive as the antigen concentration that produced a significant positive signal was determined to be of 25 pg/mL. The interassay variation coefficient ranged between 7% and 14% in the linear portion of the standard curve, being less than 20% in the practical working range (100-8000 pg/mL). The intra-assay variation coefficient was 6.6%, 4.5%, 2.1%, 2.7%, 3.4%, 3.2%, and 4.0% at the concentrations of 1, 0.9, 0.8, 0.6, 0.5, 0.4, and 0.2 ng/mL, respectively. These values demonstrated the reproducibility of the technique.

The specificity of the assay was tested in extracts of different origin: tree pollen extracts (*Olea europaea*, *Platanus acerifolia*, *Betula verrucosa*, and *Cupressus arizonica*), grass pollen (*Dactylis glomerata*), other important allergenic weeds included in different taxonomic families (*Artemisia vulgaris*, *Salsola kali*, *Chenopodium album*, *Brassica campestris*, *Plantago lanceolata*, and *Rumex acetosella*), and non-plant extracts (*Alternaria alternata* and *Dermatophagoides pteronyssinus*). None of them gave a significant response in the assay when they were tested at a protein concentration of 1 µg/mL. No reaction was detected with extracts of the same family plants as *Urtica dioica*. However, there was a weak reaction (10 000-fold less) with peach LTP, a protein

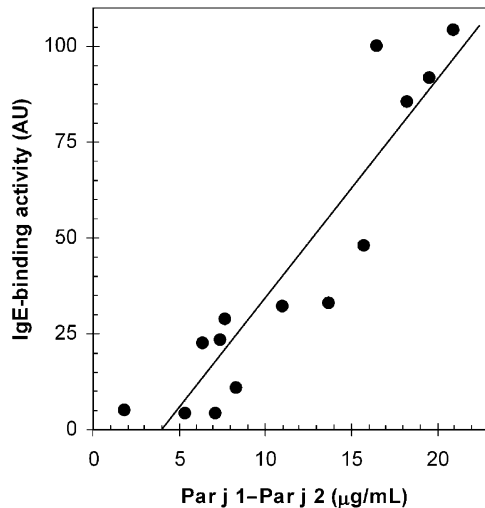


Fig. 3. Correlation between IgE-binding activity values determined by enzyme allergosorbent test inhibition and Par j 1-Par j 2 content measured by the two-site ELISA in 14 *Parietaria judaica* pollen extracts. Results represent the average of three independent experiments where CV was lower than 20%.

structurally related with Par j 1 and Par j 2 [10, 12]. Student's *t*-test indicated that dose-response curves obtained for nPar j 1-Par j 2, rPar j 2, and *P. judaica* extracts were parallel, but that for rPar j 1 was not parallel to the corresponding curve for natural allergens (Fig. 2). No dose-response curves for recombinant proteins were coincident with those of natural allergens and therefore they cannot be used as reference material for the assay.

Quantification of Par j 1-Par j 2 in pollen extracts and correlation with immunoglobulin-binding activity

The described ELISA was applied to assess the major allergens concentration in 14 *P. judaica* and in two *P. officinalis* extracts by interpolation in the linear portion of the standard curve. The concentration of Par j 1-Par j 2 in the extracts analysed ranged from 1.5% to 11.8% of the total protein in *P. judaica* extracts and from 1.4% to 1.8% of that in the two *P. officinalis* extracts (not shown). Data from *P. judaica* extracts were compared by linear regression with the total allergenic activity of the extracts determined by EAST inhibition (Fig. 3). A good correlation ($r=0.923$, $P < 0.001$) was obtained, suggesting that Par j 1-Par j 2 content is highly representative of the total IgE binding activity of the whole pollen extract. Using the described assay, the average of Par j 1-Par j 2 content in maintenance doses of specific immunotherapy with *P. judaica* pollen extracts was 0.12 μg. The IgE-binding activity of nPar j 1-Par j 2 in the *P. judaica* pollen extract was estimated by EAST inhibition. Purified nPar j 1-Par j 2 could inhibit the binding of specific IgE to the whole extract, up to 92% (Fig. 4). The inhibition curve of purified

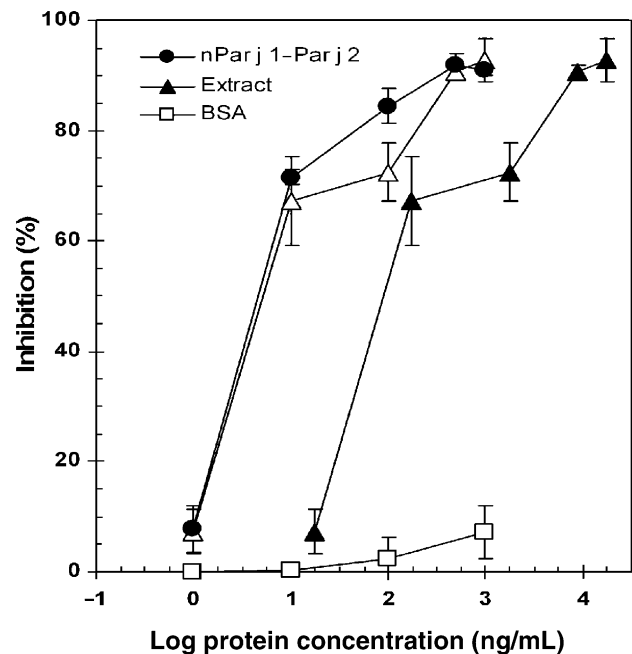


Fig. 4. Enzyme allergosorbent test-inhibition curves. The binding of specific human IgE to *Parietaria judaica* pollen extract was inhibited using nPar j 1-Par j 2, *P. judaica* pollen extract, or bovine serum albumin as control. The estimated Par j 1-Par j 2 content in *P. judaica* pollen extract was represented as empty triangles.

nPar j 1-Par j 2 was coincident to the curve of estimated Par j 1-Par j 2 content in extracts determined according to the present ELISA. These data demonstrated that Par j 1-Par j 2 together are responsible for most of the IgE-binding activity in *P. judaica* pollen extracts.

Discussion

P. judaica pollen is allergenically the most active among pollens distributed around the Mediterranean area. Several studies on the identification and characterization of *P. judaica* allergens have been reported, suggesting that the major allergens of both species show molecular weights ranging between 10 and 14 kDa [8]. Screening of a cDNA expression library allowed the isolation of the two major allergens, Par j 1 (14.86 kDa) and Par j 2 (11.34 kDa), with several isoforms [1]. These two proteins are products of independent genes that belong to a multigene family [7].

Purification of allergens is an important goal in the study of the structure-activity relationship of the immunogen in human atopic allergy. However, this is often impaired by the complex nature of the sources they come from. Because of the molecular weight heterogeneity of Par j 1 and Par j 2 [20] and taking into account that both proteins show an identity of 45% in the amino acid sequence [6] with a very similar three-dimensional structure [10, 12], the purification of only one allergen without losing some isoforms was not feasible. Moreover, as the

two allergens have a similar prevalence among *P. judaica* allergenic patients, we focused on the quantification of both Par j 1 and Par j 2 allergens in a unique assay. Therefore, rabbit antiserum was obtained using a purified fraction containing nPar j 1 and nPar j 2 (confirmed by mass spectrometry) as antigen and subsequently used for immunoaffinity purification of nPar j 1–Par j 2. Mice were immunized with the immunopurified nPar j 1–Par j 2 and the obtained antibodies were tested against rPar j 1 and rPar j 2 to detect those reacting with both proteins. The selected mAbs were applied in a two-site sandwich ELISA to quantify *Parietaria* major allergens. The assay was very sensitive with a detection limit of 25 pg/mL, and, therefore, it was possible to measure Par j 1–Par j 2 content in samples of airborne pollen collected by a Cyclone sampler [21]. The sensitivity was similar to that described for the quantification of Par j 1 by Ayuso et al. [22], where the mAb employed as capture reacted with two proteins named Par j 1A and Par j 1B in *P. judaica* extracts. The N-terminal of these proteins shared significant homology with rPar j 2.0101 [8], so it is not clear if Par j 1 and/or Par j 2 were quantified in that assay. The antibodies used in the present assay reacted with nPar j 1–Par j 2 and their corresponding recombinant counterparts. It has been demonstrated that human IgE-binding sites on Par j 1 do not contain carbohydrates, although these seem to be important for the glycoprotein conformation and thereby influencing the IgE affinity [23]. The different glycosylation pattern of *P. pastoris* or a partial misfolding of the recombinant protein could influence antibodies affinity to recombinant proteins used in the present assay, that show weak antibody binding, and explain the fact that rPar j 1 and rPar j 2 dose–response curves were not coincident to the natural Par j 1–Par j 2 curve. Nevertheless, as no data about the behaviour of purified nPar j 1 or nPar j 2 as isolated proteins in the present assay are available, the results with recombinant proteins cannot completely exclude a possible partial discrimination in the detection of both allergens.

The described assay was specific to *P. judaica* and also to *P. officinalis* pollen extracts as the major *P. officinalis* allergen (Par o 1) [24, 25] shows high homology at the N-terminal amino acid sequence with Par j 1 described isoforms [5, 7, 8], and, moreover, both proteins are extensively cross-reactive [26]. These findings confirm the idea that these allergens are shared in *Parietaria* spp. but not in *U. dioica*, a non-allergenic plant, which also belongs to the Urticaceae family [27].

A significant correlation was found between Par j 1 and Par j 2 content determined by ELISA and IgE-binding activity determined by EAST inhibition. This result and the fact that Par j 1–Par j 2 represented the overall IgE-binding activity in *P. judaica*, as they were able to inhibit 92% of the IgE-binding capacity of serum pool to *P. judaica* extract, may convert the present two-site

sandwich ELISA in a complementary assay to EAST inhibition for extracts quality control. Our data confirm a previous report where it was described, using immunoblot inhibition, that both rPar j 2 and rPar j 1 fully abolished the IgE recognition of the 10–14 kDa native allergen region, suggesting that they were capable of inhibiting the majority of the IgE specific for *P. judaica* allergens [6]. In allergen-specific immunotherapy there is evidence that the optimal maintenance dose should contain 5–20 µg of major allergens [28], but this range should not be necessarily followed as a general rule, specially for highly reactive allergens such as Alt a 1, Cup a 1 or Par j 1, where the major allergen content in maintenance doses of specific immunotherapy is lower than 1 µg.

In conclusion, the described assay is a specific and sensitive method that can be used for the quantification of the major allergens of *P. judaica* extracts. The Par j 1–Par j 2 content provides a reliable indicator of the IgE-binding activity of the whole extract.

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