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Molecular Cloning and Expression of Pro J 1: A New Allergen of *Prosopis Juliflora* Pollen

Fatemeh Dousti[1], Mohammad-Ali Assarehzadegan[1,2], Payam Morakabati[1], Gholam Reza Khosravi[1], and Bahareh Akbari[1]

[1] Department of Immunology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2] Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Pollen from mesquite (*Prosopis juliflora*) is one of the important causes of immediate hypersensitivity reactions in the arid and semi-arid regions of the world. The aim of present study is to produce and purify the recombinant form of allergenic Ole e 1-like protein from the pollen of this allergenic tree. Immunological and cross-inhibition assays were performed for the evaluation of IgE-binding capacity of purified recombinant protein.

For molecular cloning, the coding sequence of the mesquite Ole e 1-like protein was inserted into pTZ57R/T vector and expressed in *Escherichia coli* using the vector pET-21b(+). After purification of the recombinant protein, its immunoreactivity was analysed by in vitro assays using sera from twenty one patients with an allergy to mesquite pollen.

The purified recombinant allergen was a member of Ole e 1-like protein family and consisted of 150 amino acid residues, with a predicted molecular mass of 16.5 kDa and a calculated isoelectric point (pI) of 4.75. Twelve patients (57.14%) had significant specific IgE levels for this recombinant allergen. Immunodetection and inhibition assays indicated that the purified recombinant allergen might be the same as that in the crude extract.

Herein, we introduce an important new allergen from *P. juliflora* pollen (Pro j 1), which is a member of the Ole e 1-like protein family and exhibits significant identity and similarity to other allergenic members of this family.

Keywords: Allergen; Cloning; Expression; Prosopis

INTRODUCTION

Mesquite (*Prosopis juliflora*) is a tree that belongs to the Fabaceae family. It is native to the tropical and subtropical areas of the United States, Asia, Africa and Australia.[1-7] Mesquite is commonly planted in thearidand semi-arid regions of Iran and other Middle East countries, as a roadside and an ornamental shade tree in parks and gardens. Flowering occurs twice a year, primarily in spring and early summer.[1, 8]

Corresponding Author: Mohammad-Ali Assarehzadegan, MD; Department of Immunology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, and Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran. Tel: (+98 61) 3336 7543-50, Fax: (+98 61) 3333 2036, E-mail: assarehma@gmail.com

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To date, several researches from various countries have reported that mesquite pollen is one of the most important sources for triggering respiratory allergies such as seasonal allergic rhinitis and asthma. [1-7, 9, 10] It has been documented that the frequency of sensitization to mesquite pollen among allergic patients in Iran and neighbouring countries along the Persian Gulf ranges from 24% to 66%.[2-4,11,12]

Previous studies on the analysis of allergenic proteins of mesquite pollen have shown several components with molecular weights ranging from 10 to 99 kDa with IgE-binding properties.[5,8-10] In addition, mesquite tree pollen proteins exhibit significant IgE cross-reaction with those of other plants such as *Acacia farnesiana* (*Needle bush*), *Ailanthus excelsa* (Tree of heaven), *Cassia siamea* (Kassod tree) and *Salvadora persica* (Mustard tree).[8-10,13] Till date, Pro j 2, the first reported allergen from *P. juliflora* pollen, was identified as belonging to the family of profilins. However, despite the high rates of sensitization to mesquite pollen, only a few studies exist on the molecular characterisation of *P. juliflora* pollen allergens. In this research, we identified a novel allergen from mesquite pollen, which is a member of the Ole e 1-like protein family. The prototypic member of this family is the major olive pollen allergen, Ole e 1.[14] Until now, similar allergens from this protein family have also been identified from other plants such as *Fraxinus excelsior* (Fra e 1)[15], *Ligustrum vulgare* (Lig v 1)[16], *Syringa vulgaris* (Syr v 1)[17], *Salsola kali* (Sal k 5)[18], and *Chenopodium album* (Che a 1).[19]

We expressed Pro j 1 in *Escherichia coli* and evaluated its protein sequence homology with the most common allergenic Ole e 1-like proteins from plants in tropical regions. Identification and production of the recombinant forms of the common allergens of these pollens may lead to the development of new procedures for diagnostic, therapeutic and protective purposes.

MATERIALS AND METHODS

Mesquite Pollen and Crude Extract

Pollens of mesquite tree were collected from well-identified plants during the pollination season (February-March and August-September) in Ahvaz city, southwest Iran. Pollen collection and processing were carefully performed by trained pollen collectors. Floral parts other than pollen grains were separated using the sieves with different sizes (0.1, 0.07 and 0.05 millimeter meshes) successively. The purity of the final powder was determined by examining the pollen content under an optical microscope. Pollen materials with more than 96% pollen and less than 4% floral parts of the same plant were taken for protein extraction. Pollen grains were degreased by frequent changes in diethyl ether. The pollen was extracted by following a method described previously[20] and the extract was freeze-dried. The protein content of the extract was measured using Bradford's method.[21] The final extract stored at -20°C until further use.

Human Sera

Serum samples were obtained from 21 mesquite-allergic patients referred to Immunology and allergy department from outpatient clinics in random. They showed positive skin prick test (SPT) to the crude extract of mesquite pollen. These patients also had a history of respiratory allergy and were asked to complete a detailed allergy questionnaire. Six healthy subjects who showed negative SPT responses and no specific IgE to *P. juliflora* pollen extract were used as negative controls. The human ethics committee of the institute approved the study protocol with written informed consent from each patient. Serum samples of all the patients were stored at -20°C until further use.

Determination of Total and Specific IgE

Total serum IgE levels were determined by a commercially enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (Euroimmun, Lübeck, Germany). For measurement levels of specific IgE to mesquite pollen proteins in allergic patients, an indirect ELISA was developed. Briefly, 2 µg/well of mesquite pollen extract diluted in 100 µL of carbonate buffer (15 mMNa_2CO_3 and 35 mM $NaHCO_3$, pH 9.6) was added into each of the ELISA microplate (Nunc A/S, Roskilde, Denmark) and incubated overnight at 4°C. Then, following blocking with 150 µl of phosphate buffered saline (PBS) and 2% bovine serum albumin (BSA) solution for 1 hour at 37°C, the plates were incubated with 100 µl patients' sera for 3 hours at room temperature with shaking. Afterwards, biotinylated goat anti-human IgE antibody (Nordic- MUbio, Susteren, Netherlands) at1:500 dilution in 1% BSA was added and incubated for 2 hours at room temperature. This was followed by addition of 100 µl of 1:8000 dilution of horseradish peroxidase (HRP)-conjugated

streptavidin (Bio-Rad Laboratories, Hercules, CA, USA) (diluted in PBS containing 1% BSA) and incubation for 1 hour at room temperature. Then, 100 µl of tetramethylbenzidine (TMB-H_2O_2; Sigma-Aldrich, St. Louis, MO, USA) solution was added as the enzyme substrate and the plate was incubated at room temperature for 20 min before which the reaction was stopped by addition of 100 µl of 3 M HCl. Finally, the optical density (OD) of each well was assessed at 450 nm by an ELISA reader. An OD four times greater than the mean values of three determinations of pooled sera from negative controls (i.e. >0.10 OD units) was considered to be positive.[22]

Amplification of Pro j 1 cDNA and Determination of Nucleotide Sequence

For cloning the sequence encoding Pro j 1, we isolated total RNA using 65 mg of *P. juliflora* pollen following the method of Chomczynski and Sacchi[23] and then cDNA was synthesised using RevertAid TM First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Sense degenerate and antisense primers were designed according to consensus nucleotide sequence for the reported allergens from the Ole e 1-like protein family with a high degree of amino acid sequence identity using Gene Runner ver. 4.0 software.[15,18,19,24-26] The sense primer was 5′-ACKATKTTYCCMAACCTCCA-3′ and the antisense primer was 5′-TTAATTAGCTTTAACATCATAAAGATCC-3′.

Following insertion of the polymerase chain reaction (PCR) product into the cloning vector pTZ57R/T (InsTAcloneTM PCR Cloning Kit, Thermo Scientific), *E. coli* TOP10 cells (Invitrogen, Carlsbad, CA, USA) were transformed with the ligation products using the manufacturer's protocol. The recombinant plasmid was then purified from the gel using a Plasmid Extraction Kit (GeNet Bio, Chungnam, Korea), sequenced by the dideoxy method and analysed at the SeqLab Sequence laboratories (Gottingen, Germany). The sequence was submitted to the GenBank database of NCBI (http://www.ncbi.nlm.nih.gov/) with the accession number KR870436.

Expression of the Plasmid Carrying Pro j 1 cDNA and Purification of Recombinant Pro j 1 (rPro j 1)

For direct cloning of the coding sequence of Pro j 1 into the expression plasmid pET-21b (+) (Novagen, Gibbstown, NJ, USA), cDNA encoding Pro j 1 was first amplified using two specific primers carrying two restriction sites for *Not* I and *Xho* I. These primers contained the following overhangs: the sense primer (5′-TCC**GCGGCCGC**ACKATKTTYCCMAACCTCCA -3′, *Not* I restriction site is bolded) and the antisense primer (5′-CC**CTCGAG**TTAATTAGCTTTAACATCATAAAGAT-3′, *Xho* I restriction site is bolded). Following PCR amplification, the resulting product was digested with *Not* I and *Xho* I restriction enzymes according to the manufacturer's protocol (Thermo Scientific), and ligated into the digested pET-21b(+) plasmid using the same enzymes. The constructs were transformed into competent *E. coli* BL21 (DE3) cells (Novagen).

For production of rPro j 1, the recombinant plasmid pET-21b(+)/Pro j 1 was inoculated into 3 ml of lysogeny broth (LB) medium containing 100 µg/ml of ampicillin and incubated at 37°C. Expression was induced by isopropyl β-D thiogalactopyranoside (IPTG) (0.6 mM) and then the cells were harvested by centrifugation (3,500 ×*g*, 15 min, 4°C) and resuspended in lysis buffer (50 mM Tris–HCl pH 6.8, 15 mM imidazole, 100 mM NaCl, 10% glycerol, and 0.5% Triton X-100). Finally, the cells were disrupted by subjecting them to three freeze-thaw cycles in liquid nitrogen. Purification of rPro j 1 was performed with Ni-NTA affinity chromatography (Invitrogen) from the soluble phase of lysate, following the manufacturer's instructions.

Indirect ELISA and Immunoblotting Assays

To measure the levels of specific IgE to rPro j 1 in patients' sera, an indirect ELISA was developed as described previously[27] except that each wells of microplate was coated with 2 µg/ml of the purified rPro j 1 protein.

The proteins of the mesquite pollen extract were separated by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 12.5% acrylamide separation gels under reducing conditions.[22, 28] The molecular masses of protein bands were estimated with Image Lab Analysis Software (Bio-Rad Laboratories) by comparison with protein markers of known molecular weights (Amersham Low molecular weight Calibration Kit for SDS electrophoresis, GE Healthcare, UK). Following SDS-PAGE of the mesquite pollen proteins or the purified rPro j 1, they were electro-transferred to polyvinylidene difluoride (PVDF) membranes (GE Healthcare) as described

earlier.[20] Briefly, after blocking and washing, the membranes were incubated with a serum pool or individual sera from patients sensitized to mesquite pollen or with control sera (1:5 dilutions) for 3 hours. The blotted membranes were incubated with biotinylated goat anti-human IgE (Nordic-MUbio) (1:1000 v/v in PBS) and then with HRP- conjugated streptavidin (Sigma-Aldrich, St. Louis, Mo, USA) in TPBS (PBS containing 0.05% Tween 20) at a dilution of 1:10,000 v/v in PBS. After every step, the membranes were washed several times with TPBS. The reactive blotted proteins were visualized using Super Signal West Pico Chemiluminescent Substrate Kit (Thermo Scientific, Waltham, MA, USA) and ChemiDoc XRS+ system (Bio-Rad Laboratories).

ELISA and Immunoblotting Inhibition Assays

ELISA inhibition assays were performed according to previous studies. [8, 29] The pooled sera (1:2 v/v) from mesquite-allergic patients (Nos.1, 2, 8, 9, 11 and 12) were pre-incubated overnight at 4°C with either 1000,100, 10, 1, 0.1 or 0.01 µg of rPro j 1 as an inhibitors or with BSA as a negative control and were subsequently used in the ELISA inhibition assays. The percentage of inhibition was calculated using the following formula: (OD of sample without inhibitor- OD of sample with inhibitor / OD of sample without inhibitor) ×100.

To study cross-inhibition between natural and recombinant Pro j 1, a mixture of 100 µl of pooled sera (1:5 v/v) was incubated with natural mesquite pollen extract (20 µg/ml, as an inhibitor), rPro j 1 (10 µg/ml, as an inhibitor), or BSA (as a negative control) overnight at 4°C with shaking. Preincubated sera were used to assess the reactivity of a PVDF membrane blotted with natural mesquite pollen extract and rPro j 1.

RESULTS

Patients and Skin Prick Tests

The 21 patients in the present study included 10 males and 11 females (mean age: 28.61±5.65 years; age range 20-38 years). All the patients suffered from respiratory allergies and seasonal rhinitis and all were positive to SPT performed using *P. juliflora* pollen extracts (Table 1). A serum pool of 7 non-allergic subjects who showed negative SPT responses and no specific IgE against mesquite pollen extract were considered as negative control.

Serum Total and Specific IgE Levels

The mean total serum IgE in the subjects was determined to be 220.63 IU/ml. In patients reactive to Pro j 1, the mean of total IgE was 188.45 IU/ml (Table 1). Serum from 21 allergic patients were assessed for

Table 1. Clinical characteristics, SPT responses and specific IgE values of patients reactive to recombinant Pro j 1.

Allergic Patients	Age (years)/gender[1]	Clinical history[2]	Total IgE (IU/ml)	*P. juliflora* pollen extract		rPro j 1
				Skin test[3]	Specific IgE[4]	Specific IgE
1.	34/M	A,R,L	152	7	1.75	0.98
2.	26/M	A, R	232	11	2.10	1.94
3.	27/F	A, R	145	8	1.86	0.95
4.	38/F	A, L	172	7	0.96	0.81
5.	20/M	A, R, L	125	9	0.86	0.73
6.	24/F	A, R, L	352	12	2.00	0.93
7.	32/M	A, R	201	9	0.83	0.75
8.	34/F	A, R	152	9	1.80	1.13
9.	21/F	A, R	164	8	1.32	1.10
10.	36/F	A, L	133	6	0.76	0.64
11.	26/F	A, R, L	245	10	1.95	0.96
12.	29/M	A,R,L	322	10	1.93	1.11

[1] M, male ; F, female. [2]A, Allergic rhinitis; L, lung symptoms (breathlessness, tight chest, cough, wheeze); R, rhinoconjunctivitis. [3] The mean wheal areas are displayed in mm^2. Histamine diphosphate (10 mg/ml)-positive control; Glycerin-negative control. [4] Determined in specific ELISA as OD (optical density) at 450 nm.

specific IgE binding to proteins from the mesquite pollen extract. All of these patients showed significantly elevated specific IgE levels to the extract of mesquite pollen (mean $OD_{450,}$ 1.32± 0.54; range: 0.76-2.2). The mean OD_{450} for specific IgE in rPro j 1 reactive patients was 1.00 ± 0.33, ranging from 0.76 to 2.1 (Table 1).

Nucleotide and Deduced Amino Acid Sequences of Pro j 1

Sequence analysis of Pro j 1 allergen revealed an open reading frame of 453 bp coding for 150 amino acid residues with a predicted molecular mass of 16.527 kDa and a calculated isoelectric point (pI) of 4.75. The obtained nucleotide sequence was submitted to NCBI GenBank.

The deduced amino acid sequence of Pro j 1 was compared with those of other known allergenic plant-derived Ole e 1-like proteins available in the protein database (Figure 1). A significant degree of sequence identity (89%) was detected between Pro j 1, Che a 1 and Cro s 1 (Table 2).

SDS-PAGE and IgE-binding Components of Mesquite Pollen Extract

The SDS-PAGE separation of the mesquite pollen extract revealed several protein bands in the crude extract with molecular weights ranging from approximately 15 to 90 kDa (Figure 2). IgE-binding reactivity of the separated protein bands from the electrophoresis of the Acacia pollen extract was assessed by conducting immunoblotting experiments.

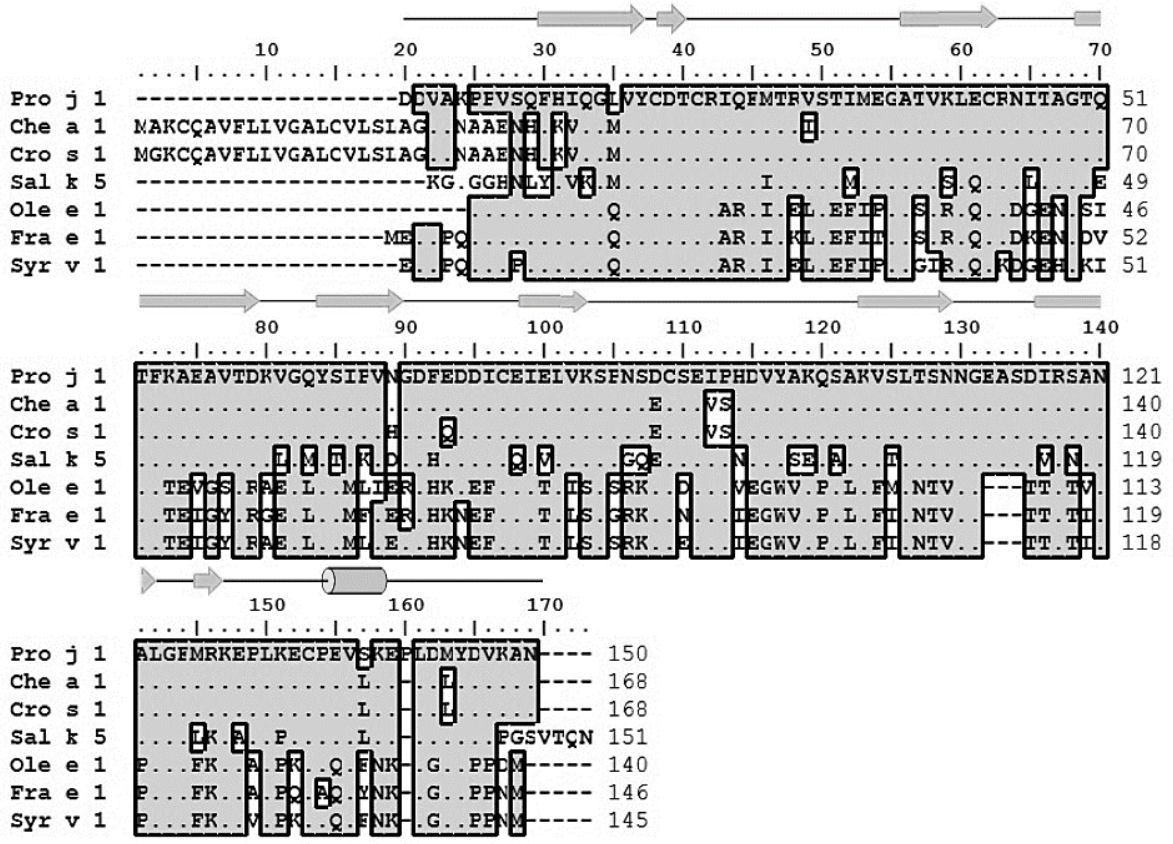

Figure 1: Comparison of the *P. juliflora* Ole e 1-like protein (Pro j 1) amino acid sequence with allergenic Ole e 1-like protein from other plants. *Chenopodium album* (Che a 1, G8LGR0.1), *Crocus sativus* (Cro s 1, XP004143635.1), *Salsola kali* (Sal k 5, ADK22842.1), *Olea europaea* (Ole e 1, ABP58635.1), *Fraxinus excelsior* (Fra e 1, AAQ83588.1) and *Syringa vulgaris* (Syr v 1, S43243). The amino acid sequence identity and the similarity of Pro j 1 (KR870436) to other members of the Ole e 1-like family are indicated in table 2. The top line indicates the location of secondary structures that created by PSIPRED protein sequence analysis (http://bioinf.cs.ucl.ac.uk/psipred/). Cylinder, arrows and black line correspond to alpha helices, beta strands and coil structure, respectively.

Table 2. Percentage of similarity and identity between Pro j 1 and selected allergenic Ole e 1-like proteins

Allergens*	GenBank	Pro j 1	
	Accession No.	% Similarity	% Identity
Che a 1	G8LGR0.1	93	89
Cro s 1	AAX93750.1	93	89
Sal k 5	ADK22842.1	91	75
Ole e 1	ABP58635.1	61	46
Fra e 1	AAQ83588.1	62	46
Syr v 1	S43243	62	45

* Che a 1 (*C. album*); Cro s 1 (*C. sativus*); Sal k 5 (*S. kali*); Ole e 1 (*O. europaea*); Fra e 1 (*F. excelsior*); Syr v 1 (*S. vulgaris*).

The results indicated that several IgE-reactive bands ranging from around 15 to 85 kDa.

Production and Purification of Pro j 1

The *E. coli* strain BL21 (DE3) pLysS was transformed with the recombinant pET-21b(+)/*Pro j 1*, and the rPro j 1 as a fusion protein with His_6-tag in the C-terminus was expressed. The rPro j 1 was secreted into the cell culture supernatant in a soluble form, from which it was further purified using Ni^{2+} affinity chromatography and quantified by Bradford's protein assay. A yield of approximately 14 mg/L of the rPro j 1 protein was obtained from the bacterial expression medium. SDS-PAGE revealed that the apparent molecular weight of the fusion protein was about 17.5 kDa (Figure 3A). The allergenic Ole e 1-like protein from mesquite pollen, as a new allergen, was designated Pro j 1 by the WHO/IUIS Allergen Nomenclature Subcommittee.

IgE-binding Analysis of rPro j 1

The specific IgE to the purified rPro j 1 were determined using 21 individual patients' sera. Out of the 21 patients, 12 (57.14%) had significant specific IgE levels to rPro j 1 (Table 1). Serum samples from the patients allergic to mesquite pollen were further tested for IgE reactivity to rPro j 1 using immunoblotting assays. The results revealed that the recombinant form of Pro j 1 was reactive with 12 individuals' sera (Figure 3B). These results were consistent with those obtained from specific IgE ELISA (Table 1).

In vitro Inhibition Assays

ELISA inhibition assays were used to evaluate the IgE-binding reactivity of the purified rPro j 1 compared with its natural counterpart in *P. juliflora* pollen extract.

The ELISA inhibition results showed a dose-dependent inhibition of the IgE directed towards rPro j 1 in patients' sera positive to mesquite. Pre-incubation of pooled sera with 1000 µg/ml of rPro j 1 and *P. juliflora* pollen extract revealed significant inhibition (93% and 87%, respectively) of IgE binding to rPro j 1 in microplate wells (Figure 4).

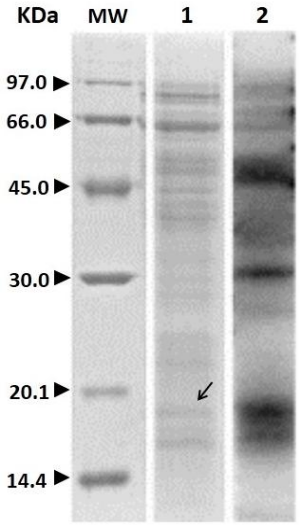

Figure 2. SDS–PAGE and immunoreactivity of *P. juliflora* pollen extract. Lane MW: molecular weight marker (GE Healthcare, Little Chalfont, UK); lane 1: Coomassie Brilliant Blue stained SDS–PAGE of the crude extract of mesquite pollen (12.5% acrylamide gel); lane 2: Immunoblotting of mesquite pooled extract. The strip was first blotted with mesquite pollen extract and then incubated with pooled sera of mesquite allergic patients ((Nos.1, 2, 8, 9, 11 and 12) and detected for IgE reactive protein bands. Natural Pro j 1 is shown by arrow.

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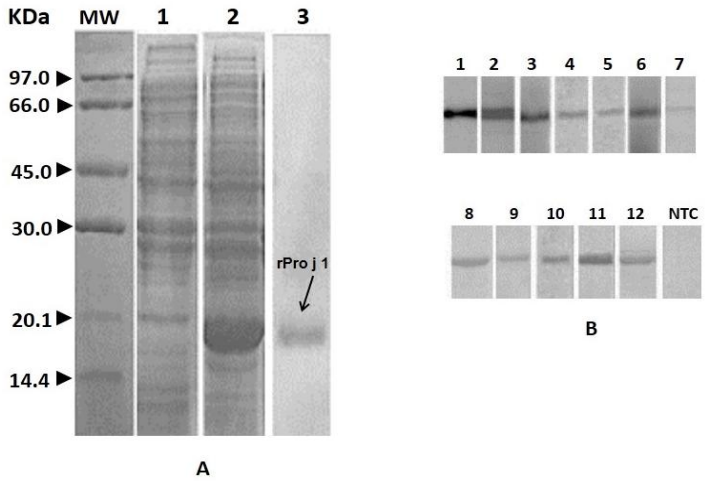

Figure 3. SDS–PAGE and immunoreactivity of recombinant Pro j 1 (rPro j 1). A. lane MW: Molecular Weight marker (GE Healthcare, Little Chalfont, UK); lane 1: Coomassie Brilliant Blue stained SDS–PAGE of soluble fraction of cell culture (IPTG-induced pET-21b(+) without insert); lane 2: rPro j 1 (IPTG-induced pET-21b(+)/Pro j 1) in soluble fraction; lane 3: purified rPro j 1 (as an approximately 18-kDa recombinant protein) with Ni-NTA affinity chromatography on 12.5% acrylamide gel. B. IgE immunoblot of purified rPro j 1 using allergic patients' sera. lanes 1–12, probed with sera from patients with positive for rPro j 1; lane NTC: negative control.

Immunoblot inhibition assays showed that pre-incubation of serum samples with rPro j 1 nearly completely inhibited the IgE binding to a protein band with an apparent molecular weight of 17 kDa (Figure 5, line 3). Altogether, in vitro inhibition assays revealed a similar IgE reactivity for rPro j 1 and its natural counterpart in mesquite pollen extract. In addition, the results showed that pre-incubation of serum samples with native crude extract of mesquite pollen inhibited the IgE binding to natural Pro j 1 in mesquite pollen extract and other reactive proteins (Figure 5, line 2). However, pre-incubation of the pooled sera with BSA did not affect the IgE-reactivity to rPro j 1 (Figure 5, line 1).

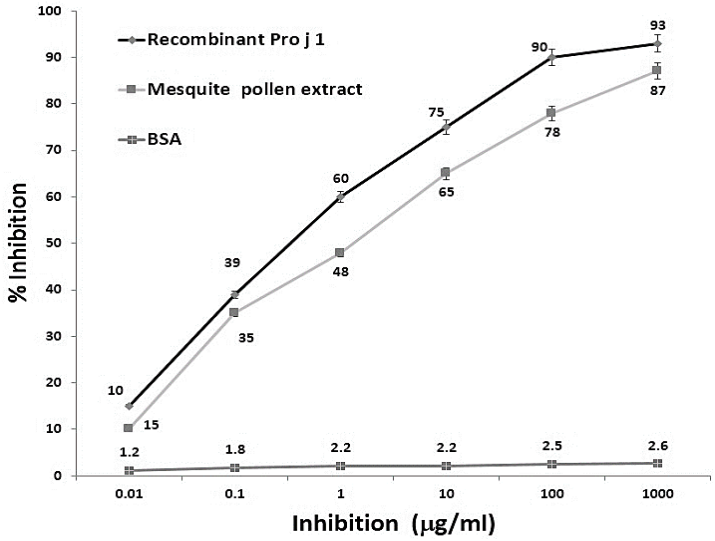

Figure 4. ELISA inhibition with mesquite pollen extract and rPro j 1. Inhibition of IgE-binding to rPro j 1 by ELISA using mesquite pollen extract and rPro j 1. Control experiments were performed with BSA.

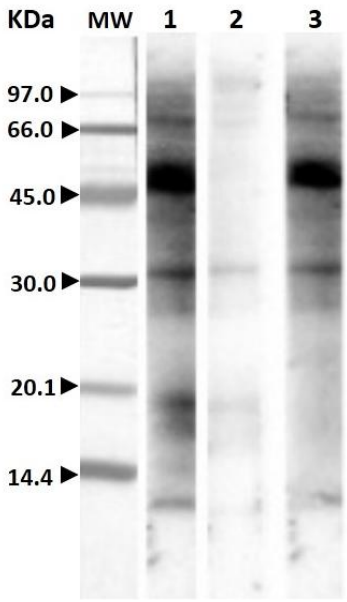

Figure 5. Immunoblotting inhibition assays. lane MW: molecular weight marker (GE Healthcare, UK); lane 1: Mesquite protein strip incubated with pooled sera without inhibitor (negative control); lane 2: Mesquite protein strip incubated with pooled sera containing 70 µg of mesquite pollen extract as an inhibitor (positive control); lane 3: mesquite protein strip incubated with pooled sera containing 25 µg purified rPro j 1, as an inhibitor.

DISCUSSION

P. juliflora has been recognised as a cause of respiratory allergy in various parts of tropical and subtropical regions of the United States, Asia, Africa and Australia. [1-7] In the present study, the cDNA encoding Pro j 1, a new allergen of mesquite pollen, was cloned and expressed in a prokaryote expression system. Pro j 1 has been identified as a member of the Ole e 1-like protein family, and the immunoassay experiments results showed an IgE-reactivity in 57% (12/21) of the allergic patients. Earlier studies have also identified several allergens from this family, such as Sal k 5, Che a 1 , Cro s 1, Pla l 1, Syr v 1, Lig v 1 and Fra e 1. [15, 18, 19, 24-26]

The cDNA encoding Pro j 1 contained 453 bases that express a polypeptide of 150 amino acids with a molecular weight of approximately 16.5 kDa. Previous studies have also reported proteins with different molecular weights from members of the Ole e 1-like proteins allergens from various pollen sources, such as 17.08- 17.62 kDa in two members of the Amaranthaceae family (Che a 1, Sal k 5), 20 kDa in *C. sativus* pollen (Cro s 1), and 17-20 kDa (glycosylated and non-glycosylated) in *P. lanceolata* (Pla l 1). [18, 24, 30, 31] These variations in molecular weight may be attributed to the diversities in few amino acid residues, the levels of glycosylation, or the methods used to measure the molecular weights. Moreover, Pro j 1 has six cysteine residues in its sequence and like other member of the Ole e 1-like protein family, such as Che a 1, Cro s 1 and Sal k 5, it also has a conserved sequence for potential N-glycosylation in the same position of the polypeptide chain (Asn-Ile/Leu-Thr-Ala), which is actually occupied by a glycan in these proteins.

Immunoblotting assay of mesquite pollen crude extract using pooled sera from the patients was indicated an IgE-binding protein band with an estimated molecular weight of 17.5 kDa (Figure 1). The IgE-binding potential of the purified rPro j 1 to the sera obtained from mesquite-allergic patients was evaluated using specific ELISA and immunoblotting assays to confirm that rPro j 1 was correctly folded and bound to IgE, similar to its natural counterpart in mesquite extract. The immunoblot analysis using individual patient serum demonstrated various IgE reactivity with several proteins with 15, 17, 20, 28, 35, 45, 66, 85 and 85 kDa molecular weights as the main IgE-binding components. The immunoblotting assays results using natural Pro j 1 with an apparent molecular weight of 17 kDa were consistent with those obtained for the rPro j 1. A nearly complete inhibition of IgE-binding to natural Pro j 1 was also observed after pre-incubation of pooled sera with purified rPro j 1. Thus, rPro j 1 probably consists of similar IgE-epitopes to those of its natural counterpart.

Cross-reactivity of the mesquite pollen components with those of other allergenic pollens has been described previously. [8-10, 13] Mesquite pollens and other allergenic pollens from the the Fabaceae (*A. farnesiana*) and Amaranthaceae (*S. kali*, *C. album*) families are a well-known source of respiratory allergies.[1-3, 32]

Amino acid sequence analysis revealed that Pro j 1 has a high level of identity and similarity with the selected allergenic Ole e 1-like proteins from most of the common allergenic regional plants, particularly *C. album* (Che a 1), *C. sativus* (Cro s 1) and *S. kali* (Sal k 5) (89%, 89% and 75%, respectively). This fact increases the probability of cross-reactivity among

these allergens, although further studies are needed to demonstrate this assumption.

In conclusion, we identified a new allergen from the mesquite pollen, Pro j 1, with a detectably specific IgE in 57% of mesquite allergic-patients. Pro j 1 is another member of the Ole e 1-like protein family. In addition, *E. coli* can be used as a heterologous expression system for the production of rPro j 1 with immunoreactivity similar to the natural form of the allergen. Furthermore, a high level of homology between the amino acid sequence of Pro j 1 and that of several allergenic members of the Ole e 1-like protein family from other plants also predicted the potential cross-reactivity among these allergenic plants.

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REFERENCES

1. Al-Frayh A, Hasnain SM, Gad-El-Rab MO, Al-Turki T, Al-Mobeireek K, Al-Sedairy ST. Human sensitization to Prosopis juliflora antigen in Saudi Arabia. Ann Saudi Med 1999; 19(4):331-6.
2. Assarehzadegan MA, Shakurnia A, Amini A. The most common aeroallergens in a tropical region in Southwestern Iran. World Allergy Organ J 2013; 6(1):7.
3. Assarehzadegan MA, Shakurnia AH, and Amini A. Sensitization to common aeroallergens among asthmatic patients in a tropical region affected by dust storm. J Med Sci 2013; 13(7):592–7.
4. Bener A, Safa W, Abdulhalik S, Lestringant GG. An analysis of skin prick test reactions in asthmatics in a hot climate and desert environment. Allerg Immunol (Paris) 2002; 34(8):281-6.
5. Killian S, McMichael J. The human allergens of mesquite (Prosopis juliflora). Clin Mol Allergy 2004; 2(1):8.
6. Thakur IS. Purification and characterization of the glycoprotein allergen from Prosopis juliflora pollen. Biochem Int 1991; 23(3):449-59.
7. Buys PJC. Aeroallergens in Namibia: review article. Curr Allergy Clin Immunol 2013; 26(4):210-2.
8. Assarehzadegan MA, Khodadadi A, Amini A, Shakurnia AH, Marashi SS, Ali-Sadeghi H, et al. Immunochemical characterization of Prosopis juliflora pollen allergens and evaluation of cross-reactivity pattern with the most allergenic pollens in tropical areas. Iran J Allergy Asthma Immunol 2015; 14(1):74-82.
9. Dhyani A, Arora N, Gaur SN, Jain VK, Sridhara S, Singh BP. Analysis of IgE binding proteins of mesquite (Prosopis juliflora) pollen and cross-reactivity with predominant tree pollens. Immunobiology 2006; 211(9):733-40.
10. Dhyani A, Singh BP, Arora N, Jain VK, Sridhara S. A clinically relevant major cross-reactive allergen from mesquite tree pollen. Eur J Clin Invest 2008; 38(10):774-81.
11. Hasnain SM, Al-Frayh AR, Subiza JL, Fernández-Caldas E, Casanovas M, Geith T, et al. Sensitization to indigenous pollen and molds and other outdoor and indoor allergens in allergic patients from saudi arabia, United arab emirates, and Sudan. World Allergy Organ J 2012; 5(6):59-65.
12. Suliaman FA, Holmes WF, Kwick S, Khouri F, Ratard R. Pattern of immediate type hypersensitivity reactions in the Eastern Province, Saudi Arabia. Ann Allergy Asthma Immunol 1997; 78(4):415-8.
13. Shamsbiranvand MH, Khodadadi A, Assarehzadegan MA, Borsi SH, Amini A. Immunochemical characterization of acacia pollen allergens and evaluation of cross-reactivity pattern with the common allergenic pollens. J Allergy (Cairo) 2014; 2014:409056.
14. de Dios Alché J, M'rani-Alaoui M, Castro AJ, Rodríguez-García MI. Ole e 1, the major allergen from olive (Olea europaea L.) pollen, increases its expression and is released to the culture medium during in vitro germination. Plant cell physiol 2004; 45(9): 1149-57.
15. de Dios Alché J, M'rani-Alaoui M, Castro AJ, Rodríguez-García MI. Isolation of the main allergen Fra e 1 from ash (Fraxinus excelsior) pollen: comparison of the natural and recombinant forms. Ann Allergy Asthma Immunol 2006; 96(4):557-63.
16. Batanero E, Gonzalez De La Peña MA, Villalba M, Monsalve RI, Martin-Esteban M, Rodríguez R. Isolation, cDNA cloning and expression of Lig v 1, the major allergen from privet pollen. Clin Exp Allergy. 1996; 26(12):1401-10.
17. González E, Villalba M, Rodríguez R. Immunological and molecular characterization of the major allergens from lilac and privet pollens overproduced in Pichia pastoris. Clin Exp Allergy 2001; 31(2):313-21.

18. Castro L, Mas S, Barderas R, Colás C, García-Sellés J, Barber D, et al. Sal k 5, a member of the widespread Ole e 1-like protein family, is a new allergen of Russian thistle (Salsola kali) pollen. Int Arch Allergy Immunol 2014; 163(2):142-53.
19. Barderas R, Villalba M, Lombardero M, Rodríguez R. Identification and characterization of Che a 1 allergen from Chenopodium album pollen. Int Arch Allergy Immunol 2002; 127(1):47-54.
20. Assarehzadegan MA, Sankian M, Jabbari F, Noorbakhsh R, Varasteh A. Allergy to Salsola Kali in a Salsola incanescens-rich area: role of extensive cross allergenicity. Allergol Int 2009; 58(2):261-6.
21. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72:248-54.
22. Ali-Sadeghi H, Khodadadi A, Amini A, Assarehzadegan MA, Sepahi N, Zarinhadideh F. Pro j 2 is mesquite profilin: molecular characteristics and specific IgE binding activity. Asian Pac J Allergy Immunol 2015; 33(2):90-8.
23. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162(1):156-9.
24. Calabozo B, Díaz-Perales A, Salcedo G, Barber D, Polo F. Cloning and expression of biologically active Plantago lanceolata pollen allergen Pla l 1 in the yeast Pichia pastoris. Biochem J 2003; 372(Pt 3):889-96.
25. Asturias JA, Arilla MC, Gómez-Bayón N, Martínez J, Martínez A, Palacios R. Cloning and expression of the panallergen profilin and the major allergen (Ole e 1) from olive tree pollen. J Allergy Clin Immunol 1997; 100(3):365-72.
26. Varasteh A-R, Sankian M, Midoro-Horiuti T, Moghadam M, Shakeri MT, Brooks EG, et al. Molecular cloning and expression of Cro s 1: an occupational allergen from saffron pollen (Crocus sativus). Rep Biochem Mol Biol 2012; 1(1):1-8.
27. Assarehzadegan MA, Sankian M, Jabbari F, Tehrani M, Varasteh A. Expression of the recombinant major allergen of Salsola kali pollen (Sal k 1) and comparison with its low-immunoglobulin E-binding mutant. Allergol Int 2010; 59(2):213-22.
28. Zarinhadideh F, Amini A, Assarehzadegan MA, Borsi SH, Sepahi N and Ali-Sadeghi H. Immunochemical and molecular characterization of allergenic profilin (Koc s 2) from Kochia scoparia pollen. J Korean Soc Appl Biol Chem 2015; 58(3):443-51.
29. Assarehzadegan MA, Sankian M, Jabbari F, Noorbakhsh R, Varasteh A. Allergy to Salsola Kali in a Salsola incanescens-rich area: role of extensive cross allergenicity. Allergol Int 2009; 58(2):261-6.
30. Vahedi F, Sankian M, Moghadam M, Mohaddesfar M, Ghobadi S, Varasteh AR. Cloning and expression of Che a 1, the major allergen of Chenopodium album in Escherichia coli. Appl Biochem Biotech 2011; 163(7):895-905.
31. Varasteh AR, Moghadam M, Vahedi F, Kermani T, Sankian M. expression of the allergen Cro s 2 profilin from saffron (Crocus sativus). Allergol Int 2009; 58(3):429-35.
32. Fereidouni M, Hossini RF, Azad FJ, Assarehzadegan MA, Varasteh A. Skin prick test reactivity to common aeroallergens among allergic rhinitis patients in Iran. Allergol Immunopathol (Madr) 2009; 37(2):73-9.