

Oriental plane pollen allergy: Identification of allergens and cross-reactivity between relevant species

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ABSTRACT

Pollen from different tree and grass species represent the greatest inducers of type I allergy worldwide. Oriental plane trees, as *Platanus orientalis*, are an important source of airborne allergens in cities of the southwest Asia and southeast Europe. This study was aimed to identify relevant allergens of *P. orientalis* pollen and to ascertain whether *P. orientalis* allergens have cross-reactivity with related plane trees, such as *Platanus acerifolia* and *Platanus occidentalis* pollen components. Nineteen patients with a clinical history of reaction to *P. orientalis* pollen and a positive skin-prick test (SPT) to *P. orientalis* pollen extract were included in this study. Identification of IgE-binding proteins in *Platanus* pollen extracts was elucidated by immunoblotting using sera from *P. orientalis* pollen-sensitive patients. Cross-reactivity studies among *P. orientalis* allergens and relevant species was evaluated by immunoblot-inhibition and ELISA inhibition assays. All the patients were polysensitive and exhibited positive SPT to grass and tree pollen allergens. The IgE-binding pattern of *P. orientalis* pollen extract was well defined. The inhibition experiments showed that the capacity of *P. occidentalis* was greater than *P. acerifolia* pollen extract in preventing the reactivity of immobilized *P. orientalis* pollen extract with the IgE antibodies of patients. The 28-kDa molecule was the most frequent allergen among nine IgE-binding proteins of *P. orientalis* pollen. The IgE-reactive components of *P. orientalis* pollen showed a higher level of cross-reactivity with *P. occidentalis* pollen in comparison with *P. acerifolia* pollen.

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Key words: Allergen characterization, cross-reactivity, ELISA inhibition, immunoblot inhibition, *Platanus acerifolia*, *Platanus occidentalis*, *Platanus orientalis*, pollen allergy, SDS-PAGE immunoblotting

During the past 10 years, the researches of many groups have been focused on the identification and characterization of proteins responsible for IgE-mediated allergies, because type I allergy is becoming a major world health problem.¹ Currently, allergy diagnosis and specific immunotherapy are performed with allergenic extracts that contain a variety of allergenic and nonallergenic components that make them too complex to be standardized.² *Platanus orientalis* (Oriental plane) is the dominant tree species in parks and streets in most cities of Iran.³ The other important plane trees are American sycamore (*P. occidentalis*) and London plane tree (*P. acerifolia* or *P. hybrida*).⁴ Research has shown that *Platanus* pollen is a major contributor to pollinosis symptoms during March and April.^{5–7} A

high prevalence of positive skin-prick tests (SPTs; 43.6%) has been recorded for *P. orientalis* in Mashhad, Iran. The highest concentrations of its pollen have been detected during the flowering season, reaching up to 15% of the total pollen in Mashhad (A.-R. Varasteh, unpublished results, 2008). Despite the abundance of airborne *P. orientalis* pollens and its proven implication in pollinosis, few studies have actually addressed the molecular characteristics of *P. orientalis* pollen-derived allergens. The purpose of this study was to identify the most important allergens of *P. orientalis* pollen and to ascertain whether *P. orientalis* allergens have cross-reactivity with related plane trees, such as *P. acerifolia* and *P. occidentalis* pollen components.

MATERIALS AND METHODS

Patients and SPTs

Nineteen subjects (10 male and 9 female patients; aged 10–42 years; mean age, 25 ± 14.5 years) were included in the study. The patients fulfilled the following criteria: (1) seasonal rhinitis and/or symptoms of asthma throughout the flowering season of plane tree and (2) a positive SPT to *P. orientalis* total pollen extract. All patients gave their consent to participate in the study. All subjects underwent the SPTs using the crude extract of *P. orientalis* pollen (0.5 mg/mL) and commercial pollen extracts of the most prevalent trees

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This study was approved by the Institutional Review Board (IRB) and informed consent was obtained from each patient

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Table 1 Clinical data, total and specific IgE, and SPT results of the selected *Platanus orientalis* pollen-allergic patients

Patient No.	Age (yr)	Sex	Total IgE (kU/L)	Specific IgE (OD)	SPT (mm ²)	Outdoor Allergens (Pollens)	
						Tree	Grass
1	42	F	900	0.84	60	ai, ac, cu	am, cy, ch, ar, sa, po
2	18	M	185	1.28	55	ai, ac, cu, fr	am, cy, ch, ar, sa, po
3	22	F	198	1.26	40	ai, ac, fr	am, cy, ch, ar, sa, po
4	27	F	800	0.64	25	ac, fr	cy, ch, ar, sa, po
5	38	M	580	0.68	12	ai, ac, cu, fr	—
6	18	M	760	1.7	90	ai, ac, cu, fr	am, cy, sa, ar, po
7	24	F	>1000	0.94	24	ai, ac, cu, fr	am, cy, ch, ar, sa, po
8	19	M	>1000	0.78	30	ai, ac, cu, fr	am, cy, ch, ar, sa, po
9	23	F	270	0.2	25	ai, ac, fr	am, cy, ch, ar, sa, po
10	28	F	320	0.22	15	—	am, ch, sa
11	30	F	450	0.48	49	ai, ac, cu, fr	am, cy, ch, ar, sa, po
12	27	M	870	0.64	25	—	am, cy, ch, ar, sa, po
13	36	F	220	0.54	25	ai	am, cy, ch, sa, po
14	10	M	>1000	0.8	30	ai	am, cy, ch, ar, sa, po
15	30	M	250	0.42	25	ai, ac, fr	am, cy, ch, ar, sa, po
16	22	M	300	0.48	20	ai, cu, fr	am, cy, ch, ar, sa, po
17	18	M	>1000	0.2	12	ai, ac, cu, fr	am, cy, ch, ar, sa, po
18	22	F	470	0.16	16	ai, ac, cu, fr	am, cy, ch, ar, sa, po
19	30	M	640	0.64	12	ai, ac, cu, fr	am, cy, ch, ar, sa, po

Allergens: ai = *Ailanthus altissima*; ac = *Acacia longifolia*; cu = *Cupressus arizonica*; fr = *Fraxinus americana*; am = *Amaranthus retroflexus*; cy = *Cynodon dactylon*; ch = *Chenopodium album*; ar = *Artemisia vulgaris*; sa = *Salsola kali*; po = *Poa pratensis*. Sex: F, female; M, male. OD = optical density; SPT = skin-prick test.

and grasses including *Ailanthus altissima*, *Acacia longifolia*, *Cupressus arizonica*, *Fraxinus americana*, *Amaranthus retroflexus*, *Cynodon dactylon*, *Chenopodium album*, *Artemisia vulgaris*, *Salsola kali*, and *Poa pratensis* (Hollister-Stier, Spokane, WA). SPTs were performed on the volar side of the forearm using sterile 1-mm-tip lancets. Readings were taken after 15 minutes. A wheal diameter of ≥ 3 mm was considered positive.⁸ Histamine, 10 mg/mL, and saline were used as positive and negative controls, respectively. The clinical data of the selected patients are summarized in Table 1. A control group ($n = 9$) with no history of allergic diseases and a negative SPT to *P. orientalis* pollen was selected.

Preparation of Pollen Allergenic Extracts

Fresh pollen samples were collected from *P. orientalis* trees during peak pollination months. The batch contained <1% nonpollen impurities. After microscopic examination to check purity and to exclude contaminations, *P. orientalis* pollen was defatted with acetone before extraction. One gram of *P. orientalis* pollen was added to 100 mL of acetone and shaken overnight at 4°C. The mixture was centrifuged ($15,000 \times g$ for 30 minutes at 4°C). The supernatant was discarded, and the pellet containing *P. orientalis* pollen was dried at room temperature. The dried pollen was extracted

overnight at 4°C in 10 mL of phosphate-buffered saline (PBS). After centrifugation ($15,000 \times g$ for 30 minutes at 4°C), the supernatant was dialyzed (cutoff point of 3.5 kDa) against phosphate buffer 5 mM (pH 8) and filtered through a 0.22- μ m membrane (Millipore Corp., Bedford, MA). The filtrate was then lyophilized and stored -20°C in sterile vials.⁹ Determination of the protein concentration was performed to the method of Bradford with bovine serum albumin (BSA) as standard.¹⁰ To use this extract for SPT, glycerol was added to a final concentration of 50% (V/V). The same protocol was followed to isolate allergens from *P. occidentalis* and *P. acerifolia* pollens (Pharmacia Allergon AB, Engelholm, Sweden).

Total and Specific IgE

Total IgE. Measurement of total IgE was performed with the IgE total kit (Radim, Roma, Italy) according to the to the manufacture's protocol.

Measurement of *P. orientalis* Pollen Allergen-Specific IgE in Sera. The wells of the Nunc-Immuno plate (Nalgen Nunc International, Roskilde, Denmark) were coated with 100 μ L of *P. orientalis* pollen allergenic extraction at a concentration of 0.1 mg/mL in coating

buffer (15 mM of Na₂CO₃ and 35 mM of NaHCO₃, pH 9.6) at 4°C for 16 hours. After blocking with 150 µL of 2% BSA in PBS at 37°C for 30 minutes, the plates were incubated with 100 µL of patients' sera (diluted 1/5 in PBS) for 3 hours at room temperature followed by incubating with a goat biotinylated anti-human IgE (Kirkegaard and Perry Laboratories, Gaithersburg, MD) diluted 1/1000 in PBS containing 1% BSA for 2 hours. The wells were then incubated for 1 hour with horseradish peroxidase-labeled streptavidin (Sigma Chemical Co., St Louis, MO) diluted 1/2000 in PBS containing 1% BSA. Each incubation step was followed by washing five times with PBS-T (PBS containing 0.05% Tween 20). Enzyme reaction was performed using tetramethyl benzidine/H₂O₂ as the substrate. The reaction was stopped by 3 M of HCl after 30 minutes at room temperature in the dark. Absorbance was determined at 450 nm with an ELISA reader. Results were expressed as optical density (OD) units. Based on the mean value of nine normal sera (<0.105 OD units), OD values of >0.21 were considered positive.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and IgE Immunoblot Analysis

SDS-PAGE was performed according to Laemmli¹¹ in 12.5% polyacrylamide gel using a BioRad Mini Protean II system (BioRad, Hercules, CA). Protein bands were visualized by Coomassie brilliant blue staining. Separated protein bands were electrotransferred onto polyvinylidene difluoride (PVDF) membranes (Immobilon-P; Millipore Corp.), essentially by the method of Towbin.¹² Immunodetection was performed on PVDF after treatment with methanol for 15 seconds and blocking with Superblock (Pierce, Rockford, IL) at 4°C for 16 hours. Membranes were probed with individual sera from *P. orientalis*-allergic patients (diluted 1/5 in PBS containing 1:10 v/v blocking buffer) or with a pool of sera from nonallergic subjects, diluted as mentioned previously, for 4 hours at room temperature. PBS buffer with 1% BSA was used as a negative control. Membranes were then washed four times for 5 minutes with 0.05% Tween-20 in PBS and incubated for 2 hours with a goat biotinylated anti-human IgE (Kirkegaard and Perry Laboratories) diluted 1/1000 in PBS containing blocking buffer (1:10 v/v). After washing, blots were incubated for 1 hour with horseradish peroxidase-labeled streptavidin diluted 1/40,000 (Sigma Chemical Co.). The peroxidase reaction was developed with SuperSignal West Pico Chemiluminescent substrate (Pierce) for 5 minutes, and IgE-binding proteins were detected by ECL Hyperfilm (Amersham Pharmacia Biotech, Uppsala, Sweden) after exposure for 5 minutes. The molecular mass of protein bands was estimated with Kodak digital science 1 DayImage Analysis

Software (Eastman Kodak, Rochester, NY) by comparison with protein markers of known molecular weight (LMW Electrophoresis Calibration kit; Amersham Pharmacia Biotech).¹³

IgE Reactivity of Sera from Patients to *P. acerifolia* and *P. occidentalis* pollens

The *P. acerifolia* and *P. occidentalis* total pollen extracts were examined for reactivity with the eight sera from allergic patients to *P. orientalis* pollen after separation of proteins by SDS-PAGE and electroblotting onto a PVDF membrane.

ELISA Inhibition Experiments

To assess cross-reactivity of *P. orientalis* pollen allergens to *P. acerifolia* and *P. occidentalis* pollen allergens, ELISA inhibition was performed as follows: 100 µL of a pooled serum comprising five sera (patients 2, 3, 5, 6, and 12; diluted 1/5 in PBS) were preincubated with 100 µL of different concentrations of *P. orientalis*, *P. acerifolia*, and *P. occidentalis* pollen extracts and BSA for 2 hours at room temperature. All of the selected sera had high levels of *P. orientalis* pollen-specific IgE and also revealed IgE reactivity to most of the *P. orientalis* pollen allergens. Preincubated sera were then added to a flat-bottom microtiter plate that had been coated with *P. orientalis* pollen extract (10 µg/well). The ELISA procedure thereafter was the same as described for measurement of *P. orientalis* pollen allergen-specific IgE. Inhibition rates were calculated as percentage of the noninhibitor values of the sera under consideration of the nonspecific binding control.

IgE Immunoblot Inhibition Assay

To confirm the specificity of the detection and to obtain further information of species-specific allergen, an IgE immunoblot inhibition test was performed. For the IgE immunoblot inhibition experiment, a serum pool from patients (patient No. 1, 4, 15, and 19) who were monosensitized to the major allergen (28 kDa) of *P. orientalis* (diluted 1/5 in PBS) was preincubated overnight at 4°C with 30 µg of *P. orientalis*, *P. occidentalis*, and *P. acerifolia* whole pollen extracts and BSA (negative control) as inhibitors. Preincubated sera were used to probe the PVDF-blotted membrane strips containing total *P. orientalis* pollen extract. The pools of sera were also used to probe the membrane strips containing *P. orientalis*, *P. occidentalis*, and *P. acerifolia* total pollen extracts. Subsequent steps were the same as described for the immunoblot analysis.

RESULTS

Patients

Table 1 summarizes the data of the *P. orientalis* pollen-allergic subjects. Adverse reactions were reported

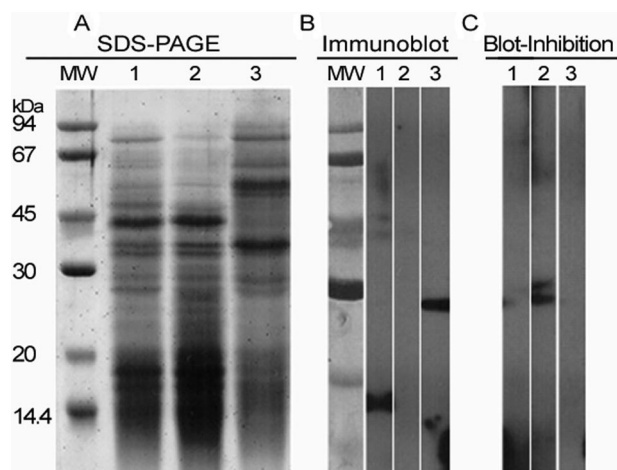


Figure 1. (A) Protein components of *Platanus occidentalis* (lane 1), *Platanus acerifolia* (lane 2), and *Platanus orientalis* (lane 3) pollen extracts in reducing sodium dodecyl sulfate polyacrylamide gel electrophoreses (SDS-PAGE; 12.5%). Protein patterns were stained by Coomassie blue. (B) Immunoblot analysis of pollen extract of *Platanus* species (lane order is the same as SDS-PAGE) with a 28-kDa-specific pool sera. (C) IgE-immunoblot inhibition analysis of *P. occidentalis* (lane 1), *P. acerifolia* (lane 2), and *P. orientalis* (lane 3) pollen extracts with a 28-kDa-specific pool sera. *P. orientalis* pollen extract was immobilized on polyvinylidene difluoride in immunoblot inhibition assays. MW, molecular weight marker.

by 100% of patients to *P. orientalis* pollen in spring. All patients had positive skin challenge responses to *P. orientalis* total pollen extract. The mean total IgE in the *P. orientalis* pollen-allergic and nonallergic individuals was 580 ± 315 kU/L and 27 ± 15.7 kU/L, respectively. All *P. orientalis* pollen-allergic subjects exhibited positive SPT to grass and tree pollens allergens. The frequency of immunologic reactivity to these pollen allergens were *A. altissima* (16/19), *A. longifolia* (13/19), *C. arizonica* (11/19), *F. Americana* (14/19), *A. retroflexus* (17/19), *C. dactylon* (16/19), *C. album* (18/19), *A. vulgaris* (16/19), *S. kali* (18/19), and *P. pratensis* (17/19). Sixteen of 19 patients showed significantly high levels of IgE to *P. orientalis* extract in ELISA (Table 1).

Pollen Crude Extraction and SDS-PAGE

Crude extract of *P. orientalis*, *P. acerifolia*, and *P. occidentalis* pollens were obtained by PBS extraction. Proteins were analyzed by SDS-PAGE under reducing conditions and visualized by Coomassie blue staining. Protein staining revealed a complex pattern of components from ~10 to 100 kDa in all allergenic extracts (Fig. 1 A). Approximately the same pattern of SDS-PAGE was observed for *P. acerifolia* and *P. occidentalis* extracts, but *P. orientalis* extract showed a different proteins profile.

Identification of *P. orientalis* Allergens

Allergens of *P. orientalis* pollen were identified using immunoblotting with individual patient's sera (Fig. 2 A). Patients showed different IgE-binding patterns to *P. orientalis* pollen extract. After immunoblotting, nine proteins with molecular weights ranging from 12 to 68 kDa have been identified. Apparent molecular masses of the main allergens of *P. orientalis* pollen extract were 12, 18, 24, 28, 37, 42, 48, 50, and 68 kDa with prevalences of 27, 21, 11, 64, 16, 27, 16, 16, and 21%, respectively. The most prevalent IgE reactive component of *P. orientalis* was a 28-kDa protein band. Four patients (patients 1, 4, 15, and 19) indicated IgE reactivity with only the 28-kDa component of *P. orientalis* extract.

IgE Reactivity of Sera with *P. acerifolia* and *P. occidentalis* Pollens

The *P. acerifolia* and *P. occidentalis* total pollen extracts were examined for reactivity with the serum from individual patients with allergy to *P. orientalis* pollen after separation of proteins by SDS-PAGE and electroblotting onto a PVDF membrane. The pattern of IgE binding to *P. orientalis* (Fig. 2 A, patients 2, 3, and 4) was more similar to *P. occidentalis* (Fig. 2 C) than to *P. acerifolia* (Fig. 2 B) using the sera of *P. orientalis* pollen-sensitive sera, although some of the sera revealed different reactivity for each pollen extract (Fig. 2, patients 6 and 5).

The inhibition ELISA experiments confirmed immunoblot results and showed that the capacity of *P. occidentalis* was greater than *P. acerifolia* pollen extract to prevent the reactivity of immobilized *P. orientalis* pollen extract with IgE antibodies of patients who suffer from *P. orientalis* pollen allergy (Fig. 3).

IgE Immunoblot Inhibition Experiment

Immunoblot inhibition assay was also performed with *P. acerifolia*, *P. occidentalis*, and *P. orientalis* total pollen extracts as inhibitors. The serum pool from patients whose IgE antibodies recognized a 28-kDa allergen from *P. orientalis* pollen (Fig. 1 B, lane 3), showed strong reactivity with the 18-kDa protein in *P. occidentalis* pollen extract (Fig. 1 B, lane 1). Preincubation sera with *P. orientalis* pollen extract completely inhibited 28-kDa-specific IgE (Fig. 1 C, lane 3). *P. occidentalis* pollen extract also completely inhibited the 28-kDa-specific IgE binding to this allergen when used as inhibitor (Fig. 1 C, lane 1). No inhibition was observed when the serum pool was preincubated with *P. acerifolia* extract (Fig. 1 C, lanes 2). Sensitive sera recognized no 28-kDa protein in *P. acerifolia* total pollen extract (Fig. 1 B, lane 2).

DISCUSSION

Most of the allergenic pollens contain an enormous number of IgE binding proteins that usually display

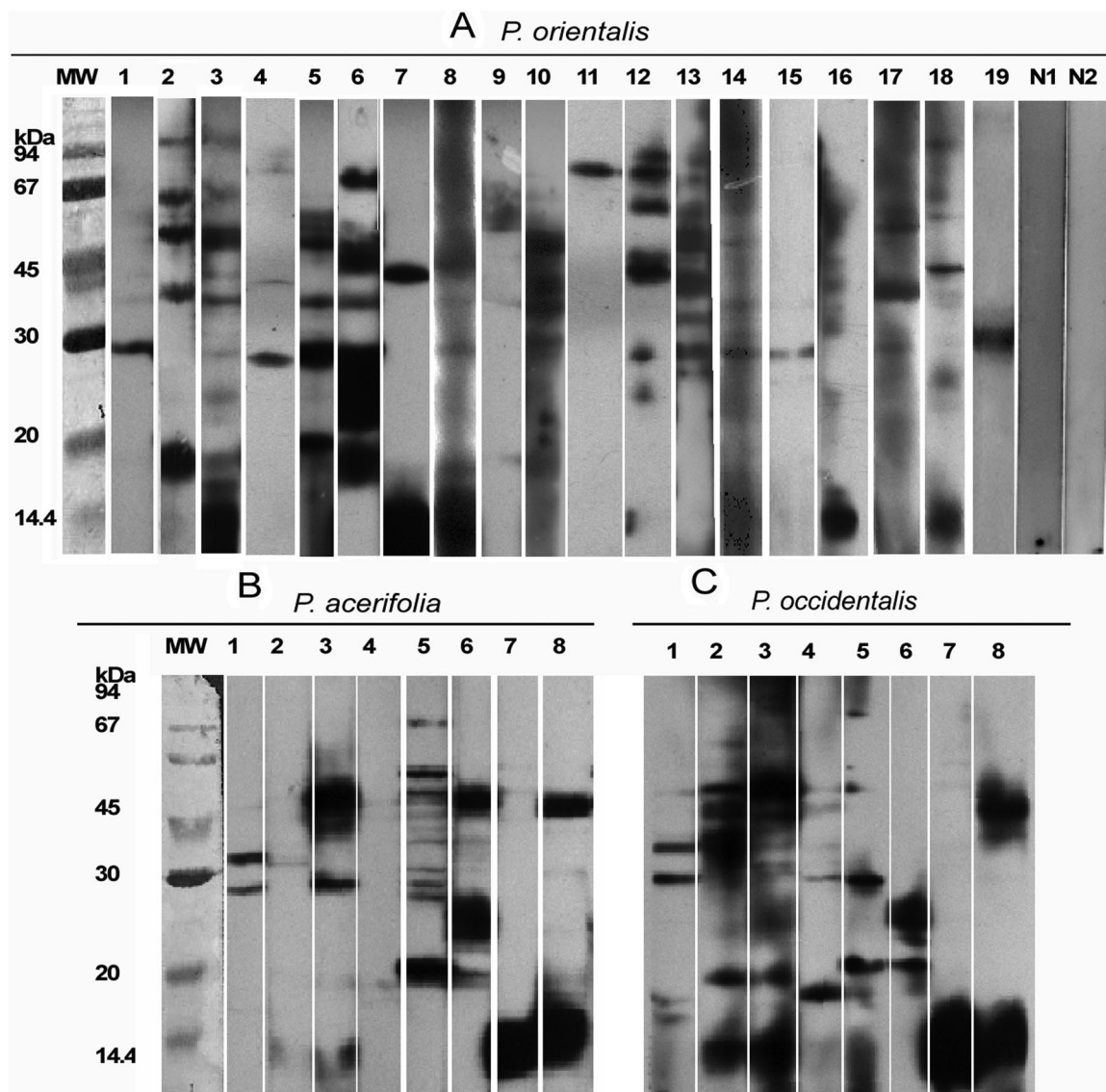


Figure 2. (A) The allergenic profile of the *Platanus orientalis* pollen extract and the IgE-reactivity of 19 *P. orientalis*-allergic patients and negative controls. The pattern of IgE binding to (B) *Platanus acerifolia* and (C) *Platanus occidentalis* pollen extracts using the sera of *P. orientalis* pollen-sensitive patients. The patient numbers are indicated above the lines. MW, molecular weight marker.

molecular masses lower than 50 kDa.¹⁴ *P. orientalis* pollen is a highly represented pollen in the atmosphere of many Iranian cities. Air concentration of this allergenic pollen has its main peak in early spring.¹⁵ To characterize antigens recognized by allergen-sensitive sera and to identify their cross-reactivity between divergent species, IgE immunoblotting and immune inhibition assays were performed on the crude extract of *P. orientalis* pollen. SPTs showed that subjects are usually sensitive to a number of different pollens.

Total extracts from *P. orientalis*, *P. acerifolia*, and *P. occidentalis* pollens were separated in reducing SDS-PAGE. Approximately the same pattern was observed for *P. acerifolia* and *P. occidentalis* extracts, but *P. orientalis* extract showed a different protein profile. Despite these varieties in electrophoresis pattern, the pattern of

IgE-reactivity to *P. orientalis* pollen extract was very similar to the *P. acerifolia* and *P. occidentalis*. Although there were minor differences in the IgE reactivity of some sera with certain protein components of these tree pollens, these differences could be caused by amino acid sequence variations of proteins among relevant tree species.

Immunoblot studies using sera from 19 subjects allergic to oriental plane tree pollen identified nine IgE antibody-binding components in the crude extract of pollen of *P. orientalis*. The IgE-binding patterns of the tested sera were heterogeneous, indicating heterogeneity of immune response. The highest IgE-binding capacity was associated with the 28-kDa allergen that is designated as Pla or 4 (accession no. P85814). The 18-kDa protein from the *P. orientalis* pollen, with 21% prevalence among plane tree

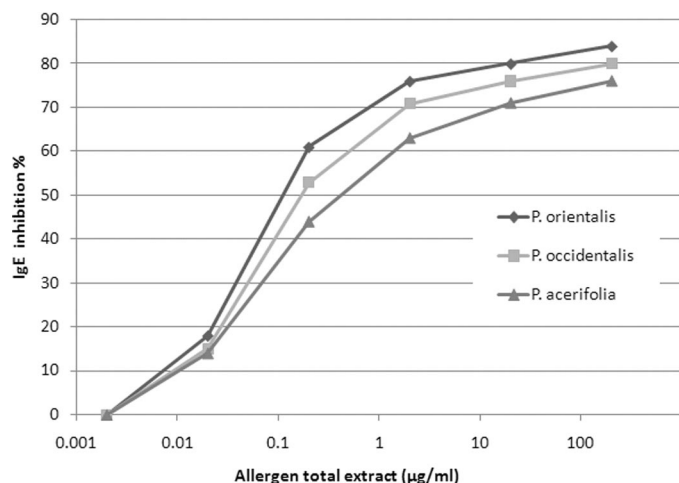


Figure 3. Inhibition of the IgE binding to immobilized *Platanus orientalis* pollen extract by using a pool serum that was incubated with *Platanus* pollen extracts. Inhibition was assayed by a competitive ELISA method.

pollen-allergic patients, was named Pla or 1 (accession no. EU296476). The 42-kDa component with 27% prevalence was named Pla or 2 (accession no. EU296477). The 12-kDa allergen with 27% prevalence was defines Pla or 3 (accession no. EU296478).

Until now, several allergens with the same molecular weight have also been described in *P. acerifolia* pollen extract, including Pla a 1, a nonglycosylated protein of 18 kDa that belongs to a invertase inhibitors family,^{16,17} and Pla a 2, an allergen with a molecular weight of 43 kDa. Pla a 2 allergen has been isolated and characterized as a polygalacturonase protein.¹⁸ *P. acerifolia* lipid transfer protein, Pla a 3, with a size of 12 kDa, has also been identified as an allergen in plane tree pollen-allergic patients.¹⁹ Panallergen profilin has been identified as another IgE-binding protein in pollen of *P. acerifolia*.²⁰ IgE-reacting proteins of 17 and 45 kDa have previously been reported as allergens of *P. acerifolia* pollen, although no data on their biochemical or allergenic features have been described.^{5,21} Unfortunately, there are no reports about molecular characteristics of *P. occidentalis* pollen allergens.

In this study, we investigated the presence of a 28-kDa allergen in divergent species of *Platanus* by immunoblot inhibition experiment. We have found that *P. occidentalis* pollen extract inhibits the IgE binding of sera to the 28-kDa protein in *P. orientalis* pollen extract; it also appeared that the same sera recognized strongly a protein of ~18 kDa in *P. occidentalis* pollen extract, suggesting that the *P. occidentalis* pollen extract may contain an isoform or a degraded form (homologous protein) of the 28-kDa allergen. *P. acerifolia* pollen extract was not able to inhibit the binding of 28 kDa-specific IgE to this allergen. In addition, a pool of sera of 28-kDa-monosensitize patients recognized no 28-

kDa component in *P. acerifolia* pollen extract. Until now, there are no reports of an allergen with a molecular weight of 28 kDa in the total extract of *P. acerifolia* pollen.

In conclusion, our results showed that the IgE-reactive components of *P. orientalis* pollen showed a higher level of cross-reactivity with *P. occidentalis* pollen in comparison with *P. acerifolia* pollen. Analysis of IgE binding proteins of *P. occidentalis* revealed nine different IgE-binding proteins in *P. orientalis* pollen that some of them had counterparts in the other species of *Platanus*, *P. acerifolia*, which were designated as Pla or 1 (18 kDa), Pla or 2 (42 kDa), and Pla or 3 (12 kDa) according to the World Health Organization/International Union of Immunological Societies Allergen Nomenclature Subcommittee recommendation. Pla or 4 allergen, which showed highest IgE-binding frequency, could be a species-specific allergen of *P. orientalis* pollens.

REFERENCES

1. Johansson SGO, and Haahtela T. World Allergy Organization Guidelines for Prevention of Allergy and Allergic Asthma. *Allergy Clin Immunol Int* 16:176–185, 2004.
2. Vanree R. Analytic aspects of the standardization of allergenic extracts. *Allergy* 52:795–805, 1997.
3. Poorkhabbaz A. The influence of air pollution on Plane (*Platanus orientalis* L.). In Faculty of Forest and Forest Ecology. Göttingen: George-August University of Göttingen 2007. 11–14.
4. Hsiao J. A numerical taxonomic study of the genus *Platanus* based on morphological and phenolic characters. *Am J Bot* 60:678–684, 1973.
5. Varela S, Subiza J, Subiza JL, et al. *Platanus* pollen as an important cause of pollinosis. *J Allergy Clin Immunol* 100:748–754, 1997.
6. Subiza J, Cabrera M, Valdivieso R, et al. Seasonal asthma caused by airborne *Platanus* pollen. *Clin Exp Allergy* 24:1123–1129, 1994.
7. Gonianakis MI, Baritaki MA, Neonakis IK, et al. A 10-year aerobiological study (1994–2003) in the Mediterranean island of Crete, Greece: Trees, aerobiologic data, and botanical and clinical correlations. *Allergy Asthma Proc* 27:371–377, 2006.
8. Dreborg S, and Frew A. Allergen standardization and skin tests. EAACI position paper. *Allergy* 48:49–75, 1993.
9. Chakraborty P, Ghosh D, Chowdhury I, et al. Aerobiological and immunochemical studies on *Carica papaya* L. pollen: An aeroallergen from India. *Allergy* 60:920–926, 2005.
10. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254, 1976.
11. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685, 1970.
12. Towbin H, Staehelin T, and Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc Natl Acad Sci U S A* 76:4350–4354, 1979.
13. Sankian M, Yousefi M, Pazouki N, et al. One-step purification of histidine-tagged profilin with high purity and yield by using metal precipitation. *Biotechnol Appl Biochem* 47:185–189, 2007.
14. Huecas S, Villalba M, and Rodríguez R. Ole e 9, a major olive pollen allergen is a β -1,3-glucanase. Isolation, characterization,

- amino acid sequence and tissue specificity. *J Biol Chem* 276: 27959–27966, 2001.
15. Shafiee A. Studies of atmospheric pollen in Tehran, Iran, 1974–75. *Ann Allergy* 37:133–137, 1976.
 16. Asturias JA, Ibarrola I, Bartolome B, et al. Purification and characterization of Pla a 1, a major allergen from *Platanus acerifolia* pollen. *Allergy* 57:221–227, 2002.
 17. Asturias JA, Ibarrola I, Eraso E, et al. The major *Platanus acerifolia* pollen allergen Pla a 1 has sequence homology to invertase inhibitors. *Clin Exp Allergy* 33:978–985, 2003.
 18. Ibarrola I, Arilla MC, Martínez A, et al. Identification of a polygalacturonase as a major allergen (Pla a 2) from *Platanus acerifolia* pollen. *J Allergy Clin Immunol* 113:1185–1191, 2004.
 19. Lauer I, Miguel-Moncin MS, Abel T, et al. Identification of a plane pollen lipid transfer protein (Pla a 3) and its immunological relation to the peach lipid-transfer protein, Pru p 3. *Clin Exp Allergy* 37:261–269, 2007.
 20. Enrique E, Alonso R, Bartolome B, et al. IgE reactivity to profilin in *Platanus acerifolia* pollen-sensitized subjects with plant-derived food allergy. *J Investig Allergol Clin Immunol* 14:335–342, 2004.
 21. Bartolome B, Olive A, Vives R, et al. Estudio y caracterización de los alérgenos del polen de *Platanus acerifolia*. *Rev Esp Alergol Inmunol Clin* 11:172, 1996. □

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