ORIGINAL ARTICLE

Allergen Profile of London Plane Tree Pollen: Clinical and Molecular Pattern in Central Spain

Nuñez-Borque E[1], Betancor D[2], Fernández-Bravo S[1], Gómez-Cardeñosa A[2], Esteban V[1,3], Garrido-Arandia M[3,4], de las Heras M[2], Pastor-Vargas C[1,3,5]*, Cuesta-Herranz J[2,3]*

[1]Department of Immunology, Instituto de Investigación Sanitaria Hospital Universitario Fundación Jiménez Díaz (IIS-FJD, UAM), Madrid, Spain
[2]Department of Allergy, Hospital Universitario Fundación Jiménez Díaz, Instituto de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD, UAM), Madrid, Spain
[3]RETIC ARADyAL, Instituto de Salud Carlos III, Spain
[4]Centro de Biotecnología y Genómica de Plantas (UPM-INIA), Madrid, Spain
[5]Department of Biochemistry and Molecular Biology, Universidad Complutense de Madrid, Madrid, Spain
*Both authors contributed equally

J Investig Allergol Clin Immunol 2022; Vol. 32(5): 367-374
doi: 10.18176/jiaci.0702

Abstract

Background: Platanus acerifolia (London plane tree) is a deciduous tree of the Platanaceae family. Sensitization to this plant varies with geography. Madrid, located in central Spain, has one of the highest London plane tree pollen concentration levels on the Iberian Peninsula.
Objectives: We evaluated both the clinical characteristics and the molecular sensitization pattern of patients with allergy to London plane tree pollen in the region of Madrid.
Patients and Methods: Thirty-eight patients allergic to London plane tree pollen were selected according to their clinical symptoms and positive results in skin prick testing and/or specific IgE determination. Serum was collected, and allergen components were evaluated using immunodetection techniques as well as ImmunoCAP. The IgE-binding proteins detected were identified and characterized using mass spectrometry.
Results: Analysis of serum samples from allergic patients revealed 9 IgE-binding bands in London plane tree pollen extract. Among these, the 45-kDa protein, which corresponded to Pla a 2, was detected in 76.3% of patients. However, the 18-kDa (Pla a 1) and 9-kDa (Pla a 3) bands were detected in 44.7% and 23.7% of sera, respectively. These results were confirmed using purified proteins. Characterization of the allergen revealed the 27-kDa protein to be glutathione-S-transferase.
Conclusions: The molecular profile of patients sensitized to London plane tree pollen differs from that reported in studies from other locations. In the population we studied, the prevalence of Pla a 2 was higher than that of Pla a 1 and Pla a 3. In addition, the minor allergen previously referred to as Pla a 4 was characterized as glutathione-S-transferase.

Key words: Plane tree. *Platanus acerifolia*. Pollen allergy. Allergen. Pla a 2.

Resumen

Antecedentes: Platanus acerifolia es un árbol de hoja caduca de la familia *Platanaceae*. La sensibilización frente a esta planta varía en función de la zona geográfica. Madrid, ubicada en el centro de España, tiene uno de los mayores niveles de concentración de polen de este árbol en la Península Ibérica.
Objetivo: Evaluar las características clínicas y los patrones moleculares de sensibilización en pacientes con alergia al plátano de sombra en la región de Madrid.
Pacientes y Métodos: Treinta y ocho pacientes alérgicos al polen del plátano de sombra fueron seleccionados de acuerdo con los síntomas clínicos, pruebas cutáneas positivas y/o IgE específica. El suero se recogió y se evaluaron los componentes alérgicos mediante técnicas de inmunodetección, así como ImmunoCAP. Las proteínas que unían IgE fueron identificadas y caracterizadas por espectrometría de masas.
Resultados: El análisis de los sueros de los pacientes alérgicos reveló 9 bandas que captaban IgE en los extractos de polen de plátano de sombra. Entre estas, la proteína de 45 kDa, correspondiente a Pla a 2, se detectó en el 76,3% de los pacientes. Sin embargo, las bandas de 18 kDa (Pla a 1) y 9 kDa (Pla a 3) fueron reconocidas en el 44,7% y 27,3%, respectivamente. Estos resultados se confirmaron usando proteínas purificadas. La caracterización de los alérgenos identificó la proteína de 27 kDa como una glutatión S-transferasa.
Conclusiones: El perfil molecular de los pacientes sensibilizados al polen del plátano de sombra varía respecto al descrito en estudios de otras localizaciones. Nuestra población muestra una mayor prevalencia de Pla a 2 comparado con Pla a 1 y Pla a 3. Además, el alérgeno minoritario previamente denominado Pla a 4 fue caracterizado como una glutatión-S-transferasa.

Palabras clave: Plátano de sombra. *Platanus acerifolia*. Alergia al polen. Alérgeno. Pla a 2.

Introduction

The prevalence of allergy has increased sharply in recent decades, making the disease one of the most widespread worldwide, affecting up to 40% of the general population [1,2]. Allergy is both a health care and a socioeconomic problem, as evidenced by the case of asthma, which has an estimated annual cost of $82 billion in the USA alone [3]. Pollen is one of the most significant sensitizing aeroallergens, and the number of pollen allergen–sensitized individuals has increased in recent decades, especially in industrial areas [4].

Platanus acerifolia (London plane tree), a deciduous tree belonging to the Platanaceae family, is a hybrid of *Platanus orientalis* and *Platanus occidentalis* [5]. Owing to its rapid growth and resistance to atmospheric pollution, it is a widely planted ornamental tree in urban areas of Western Europe, North America, Australia, New Zealand, Iran, and China. The pollination period of *P acerifolia* is short and intense. Daily average concentrations can reach very high levels, often exceeding 1000 pollen grains/m3 of air. Pollination occurs abruptly at the beginning of spring (March-April), reaching peaks of 3263 grains/m³ in Barcelona (March 14, 2014) and 5297 grains/m³ in Madrid (March 31, 2015), according to data from the SEAIC Aerobiology Committee (www.polenes.com). Similarly, the highest annual concentration of *P acerifolia* pollen in Barcelona was in 2013, with 48,626 grains/m³; in Madrid the highest concentration was recorded in 2015, with 23,879 grains/m³. The sensitization rate varies markedly between regions of the world and within each country. On the Iberian Peninsula, prevalence ranges from 52% to 56% in central areas [6-8], 8% to 9% in the northwestern region [9], and 17% in southwestern Spain [10-12].

In 1997, the pollen of the London plane tree was first considered a major source of allergy in the region of Madrid [6]. Since then, *Platanus* pollen has been shown to be one of the most significant causes of pollinosis, and *Platanus* pollen concentrations have increased significantly in recent years [10].

Several reports have evaluated allergens from *P acerifolia* pollen [13-22]. These include 2 major allergens, Pla a 1 and Pla a 2. Pla a 1, a specific nonglycosylated protein with a molecular mass of 18 kDa, is a member of the invertase inhibitor family and is recognized in 80% to 90% of *Platanus*-allergic patients [14,15]. Pla a 2, a 43-kDa glycoprotein, displays polygalacturonase activity and is detected in 84% of patients [15,19]. Another minor allergen, Pla a 3, has also been characterized. Pla a 3 is a 9- to 10-kDa protein grouped under nonspecific lipid transfer proteins (nsLTPs), thus making it cross-reactive with some plant-derived foods [18]. Sensitization to London plane tree pollen was significantly higher in food-allergic patients [18], and nsLTP and profilin may play a role in this close link [17,18].

The aim of the present study was to assess the clinical and molecular profile of patients with allergy to London plane tree pollen in the region of Madrid, located in central Spain.

Materials and Methods

Patient Population

The study population comprised 38 consecutive patients with allergy to *Platanus* pollen seen at the Fundación Jiménez Díaz Hospital, Madrid, Spain. A serum sample was obtained from each patient and stored at –80ºC.

Diagnosis of London plane tree pollen allergy was based on the presence of common symptoms (rhinitis or asthma) during the pollination period and a positive skin prick test and/or specific IgE result for *Platanus* species.

The data recorded were demographic characteristics, presence of previous atopy based on positive skin tests to common aeroallergens, symptoms of *Platanus* allergy (rhinitis and/or asthma), and association with food allergy and/or other types of aeroallergen sensitization based on positive skin prick test or positive specific IgE results.

The Fundación Jiménez Díaz Ethics Committee approved the study, and written informed consent was obtained from all patients.

Skin Prick Test

Skin prick tests were performed with a commercial series (ALK-Abelló SA) of pollen extracts (*Lolium perenne*, *Betula verrucosa*, *Cupressus sempervirens*, *P acerifolia*, *Artemisia vulgaris*, *Parietaria judaica*, *Salsola kali*, *Plantago lanceolata*, and *Olea europaea*), dust mite extracts (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), *Alternaria alternata*, and pet dander (dog and cat). The ALK-Lancet needle (ALK-Lancet, ALK-Abelló) was used for the skin tests, which were performed according to EAACI guidelines [23]. Histamine phosphate at 10 mg/mL and normal saline solution were used as positive and negative controls, respectively. Wheal diameters that were at least 3 mm larger than those of negative controls indicated a positive reaction.

Specific IgE

Allergen-specific IgE (*P acerifolia*, Phl p 7, Phl p 12, and bromelain) was measured using the ImmunoCAP System FEIA (Thermo Fisher Scientific AB) following the manufacturer's recommendations. The clinical characteristics of patients were also analyzed depending on the molecular profile established.

Preparation of Platanus Extracts

P acerifolia pollen was purchased from Iberpolen SL. *Platanus* pollen extract was homogenized in phosphate-buffered saline (PBS), with magnetic stirring overnight at 4ºC. Then, the extract was clarified by centrifugation at 10 000*g* for 30 minutes at 4ºC. The pellet was discarded, leaving the supernatant containing the proteins of interest. Quantification was carried out using the Coomassie Plus protein assay (Pierce) according to the method of Bradford [24].

ELISA

The ELISA inhibition assay was performed using 96-well flat-bottom plates (Immulon 4HBX, Thermo Fisher) and following a procedure described elsewhere [25]. Purified

Pla a 1 and Pla a 2 were obtained from the Allergy Laboratory of the Plant Biotechnology and Genomics Center (CBGP, UPM-INIA). Patients' serum samples were diluted 1:3 when IgE levels were <3 kU_A/L, 1:5 when they were 3-10 kU_A/L, and 1:10 when they were >10 kU_A/L.

SDS-PAGE, Immunoblot Analysis, and Inhibition Assays

Proteins were separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred by semidry blotting as described by Muñoz-García et al [26]. For determination of Pla a 1, incubation was performed with specific anti-Pla a 1 antibody provided by Roxall Medicina España SA and diluted 1:100 000 in PBS-T 0.05 and 2% bovine serum albumin (BSA).

The immunoblot inhibition assays were performed by preincubating serum samples from allergic patients for 4 hours at room temperature under constant stirring with 100 µg/mL of the different inhibitors, ie, total extract, specific allergens (Pla a 1 and Pla a 2), bromelain, and bovine serum albumin. Then, inhibited serum was diluted 1:10 as described above.

Protein Identification and Characterization by Mass Spectrometry

Proteins were identified and characterized using SDS-PAGE (14%) with *Platanus* extract, and proteins bands of interest were stained with PageBlue Protein Staining Solution (Fermentas International, Inc). Proteins were identified using mass spectrometry (MS) based on liquid chromatography–MS in tandem (LC-MS/MS), as described elsewhere [27], in the Proteomics Unit of the Universidad Complutense of Madrid, a member of the ProteoRed Network. Proteins were characterized by searching a nonredundant protein sequence database (NCBI) using the Mascot program (http://www.matrixscience.com).

Statistical Analysis

The statistical analysis was performed using SPSS, Version 21.0 (SPSS Inc). Qualitative variables were expressed as percentages (missing cases not imputed). Quantitative variables were expressed as mean (SD); specific IgE results were expressed as median (IQR). A χ^2 test was used to compare frequencies. Values were considered significant at a *P* value of less than .05.

Results

Patient Characteristics

Thirty-eight consecutive patients with allergy to London plane tree pollen were recruited from Hospital Fundación Jiménez Díaz, Madrid, Spain and evaluated. The mean age was 34.6 years (range 12-81 years); 18 patients (47.3%) were male and 20 (52.6%) female. During the plane tree pollen season, all patients manifested symptoms of rhinoconjunctivitis, and 19 patients (48.7%) also developed asthma symptoms.

Only 4 patients were monosensitized to plane tree pollen, and the rest were polysensitized (89.5%). Among these, the most frequent sensitizing pollens were grasses (76.5%), olive tree (58.8%), and cypress (38.2%). Seven patients (20.6%) were sensitized to dust mites and 4 (11.8%) to animal dander. Nine patients (23.7%) were also allergic to plant-derived foods, 5 (13.2%) to fruits, and 4 (11.8%) to nuts; oral allergy syndrome was the most frequent symptom among these individuals (77.8%). A detailed description of the clinical characteristics of allergic patients according to their molecular pattern is shown in Table 1.

Table 1. Clinical Characteristics of Allergic Patients by Molecular Pattern

	Pla a 1 + Pla a 2 +	Pla a 1 + Pla a 2 –	Pla a 1 – Pla a 2 +	Pla a 1 – Pla a 2 –
Patients, No.	15	2	14	7
Age, y	39.6 (14.6)	46.5 (7.78)	27.28 (18.9)	32.7 (16.2)
Sex, Male	8 (53.3%)	0 (0%)	7 (50%)	3 (42.8%)
Female	7 (46.7%)	2 (100%)	7 (50%)	4 (47.1%)
Previous atopy	4 (26.6%)	1 (50%)	8 (57.14%)	5 (71.4%)
Asthma symptoms	7 (46.67%)	1 (50%)	9 (64.28%)	2 (28.6%)
Food allergy	3 (20%)	0 (0%)	6 (42.85%)	0 (0%)
Monosensitization to Platanus	2 (13.3%)	0 (0%)	1 (7.14%)	1 (14.28%)
Sensitization to:				
Grass	10 (66.67%)	1 (50%)	10 (71.42%)	5 (71.42%)
Olive tree	5 (33.33%)	1 (50%)	9 (64.28%)	3 (42.85%)
Cypress	8 (53.3%)	0 (0%)	6 (42.86%)	4 (57.14%)
Dust mites	5 (33.3%)	0 (0%)	2 (14.26%)	0 (0%)
Molds	1 (6.67%)	0 (0%)	0 (0%)	1 (14.28%)
Pet dander	1 (6.67%)	0 (0%)	1 (7.14%)	2 (28.57%)

Table 2. Frequency of IgE-Binding Band Detection in Platanus Pollen Extract by Immunoblotting With Patients' Serum

	9 kDa	18 kDa	27 kDa	32 kDa	42 kDa	45 kDa	60 kDa	64 kDa	90 kDa
Total	9/38	17/38	10/38	4/38	19/38	29/38	16/38	17/38	16/38
Average	23.7%	44.7%	26.31%	10.3%	50.0%	76.31%	42.1%	44.74%	42.10%

The mean specific IgE concentration for *P acerifolia* was 4.43 kU_A/L (range 0.75-100 kU/L). Nine patients (23.6%) were sensitized to profilin (Phl p 12), 5 (13.1%) to Phl p 7 (polcalcin), and 3 (7.9%) to bromelain.

SDS-PAGE and Immunodetection

SDS-PAGE of *Platanus* extracts revealed multiple protein bands with an apparent molecular weight ranging from 6 kDa to 200 kDa (Figure 1). IgE immunoblotting carried out with the sera of 38 *Platanus*-allergic patients revealed a spectrum of IgE-binding bands between 9 kDa and 90 kDa. Control immunoblot assays with pooled serum from nonatopic patients did not show IgE-binding bands (Figure 2). The molecular mass of the IgE-binding bands was around 9, 18, 27, 32, 42, 45, 60, 64, and 90 kDa; the frequency of recognition is shown in Table 2. The most frequent IgE-binding band was a 45-kDa protein, which was detected in 76.3% of patients, while the 18-kDa and 9-kDa bands were recognized by 44.7% and 23.68% of sera, respectively. Based on their molecular weight, and according to an allergen database (www.allergen.org), these bands corresponded to Pla a 2 (45 kDa), Pla a 1 (18 kDa), and Pla a 3 (9 kDa).

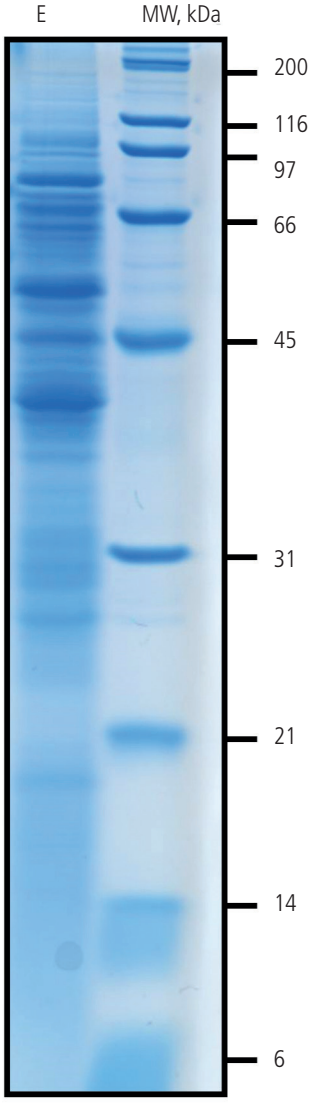

Figure 1. SDS-PAGE of *Platanus* pollen extract under reducing conditions. MW indicates molecular weight in kDa; E, *Platanus* pollen extract.

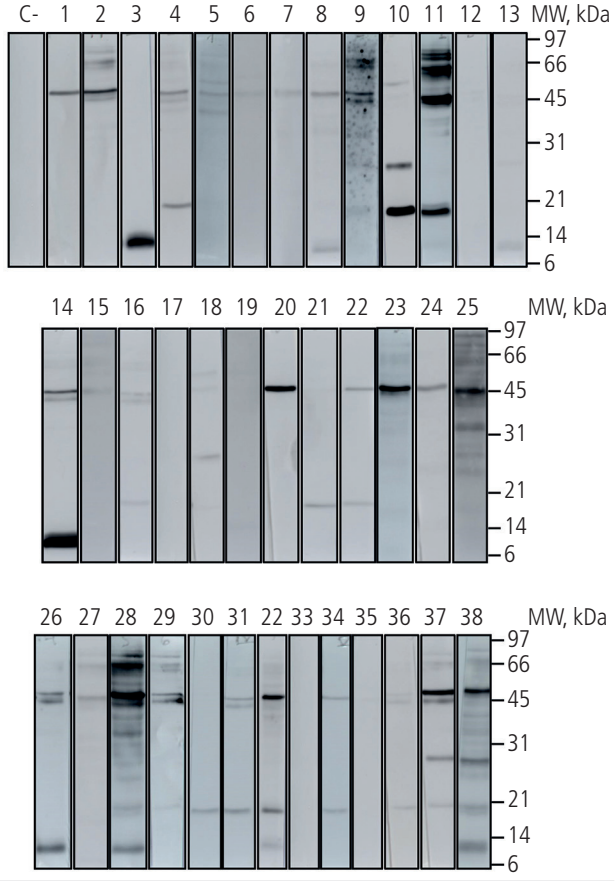

Figure 2. IgE-binding bands in *Platanus* pollen extract by immunoblotting. Lanes 1 through 38 represent serum from patients. C– indicates negative control of serum pool from nonatopic patients. MW indicates molecular weight in kDa.

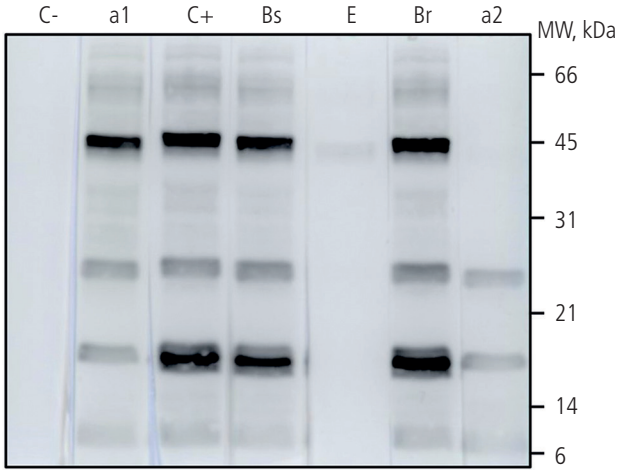

Figure 3. Immunoblot inhibition analysis of Platanus pollen extract with a serum pool from *Platanus*-allergic patients. C– indicates serum pool from nonatopic patients; A1, serum pool from *Platanus*-allergic patients inhibited with Pla a 1 purified protein; C+, serum pool from *Platanus*-allergic patients (noninhibited); Bs, serum pool from *Platanus*-allergic patients inhibited with BSA; E, serum pool from *Platanus*-allergic patients inhibited with *Platanus* pollen extract; Br, serum pool from *Platanus*-allergic patients inhibited with bromelain; A2, serum pool from *Platanus*-allergic patients inhibited with purified Pla a 2 protein. MW indicates molecular weight in kDa.

Specific IgE (ELISA) and IgE Immunoblot Inhibition

ELISA with purified proteins and immunoblot inhibition were performed to confirm that Pla a 1 and Pla a 2 were IgE-binding bands. ELISA was performed with solid-phase purified Pla a 2 and Pla a 1 using individual sera. Twenty patients with 45-kDa protein-specific IgE and 10 patients with 18-kDa protein-specific IgE (specific IgE greater than 1 kU_A/mL) were selected. All individual sera were positive (data not shown).

In addition, to confirm that Pla a 1 and Pla a 2 were IgE-binding bands, an immunoblot inhibition assay was performed by preincubating pooled sera from 2 London plane tree–sensitized patients with purified Pla a 1 and Pla a 2 (Figure 3). The serum pool was incubated with total extract, bromelain, BSA, and purified proteins of both Pla a 1 and Pla a 2. IgE binding to the London plane tree allergen was blocked using total extract, while no inhibitory effect was detected in the negative inhibition control experiment with BSA. A negative control immunoblot assay with serum from nonatopic patients did not display stained bands in the *Platanus* extract, while positive controls with the serum pool revealed the IgE-binding band spectrum. Furthermore, incubation with Pla a 2 blocked binding to that protein and to all those weighing more than 42 kDa. Furthermore, no inhibitory effects were displayed when samples were incubated with bromelain, confirming that protein recognition was not mediated by carbohydrate. However, a partial inhibitory effect was observed when incubation was performed with Pla a 1.

Identification of Proteins and Characterization by MS

To identify the 18-, 27-, 32-, and 45-kDa IgE-binding proteins, selected bands were cut out from the gels and analyzed using MS/MS. However, it was not possible to isolate the 9-kDa protein from the gel; nonetheless, based on its weight, it corresponded to the *Platanus* allergen Pla a 3, which was previously identified as LTP [16,18]. Research conducted with protein databases revealed the 27-kDa protein to be a glutathione-S-transferase and the 32-kDa band to be a protein from the 14-3-3 protein family. Furthermore, a 45-kDa peptide sequence corresponded to the protein polygalacturonase and was identified as Pla a 2. However, no significant homologies were found for the 18-kDa protein.

As the 18-kDa band was not identified by MS and immunoblotting inhibition with purified allergen Pla a 1 did not completely block its recognition, it was characterized by immunodetection. For this, an SDS-PAGE analysis carried out with both purified proteins, ie, Pla a 1 and Pla a 2 (Figure 4A), confirmed that they corresponded by weight to the 18- and 45-kDa bands, respectively.

On the other hand, immunoblot analysis in which the London plane tree extract was incubated with a specific antibody against Pla a 1 revealed that the band corresponded to that recognized by patients (Figure 4B). Furthermore, a negative control immunoblot assay with the pooled serum from nonatopic patients did not display stained bands in *Platanus* extract.

Discussion

London plane tree pollen is the most abundant pollen during the pollen season in Madrid. Its short and explosive pollination contrasts with that of other species pollinating during the same period (eg, *Plantago*), which is less variable and longer lasting. Interestingly, these high concentrations of

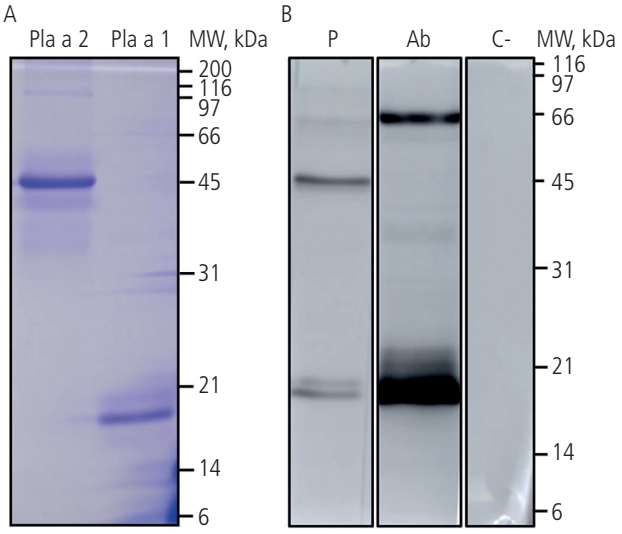

Figure 4. A, SDS-PAGE of purified Pla a 1 and Pla a 2 proteins under reducing conditions. A1 indicates Pla a 1 purified protein; A2, Pla a 2 purified protein. B, Pla a 1 determination in *Platanus* pollen extract by immunoblotting with specific antibody. C– indicates negative control of a serum pool from nonatopic patients; Ab, anti-Pla a 1 specific antibody; P, serum from *Platanus*-allergic patients; MW, molecular weight in kDa.

London plane tree pollen contrast with the low frequency of sensitization produced when compared to other pollens with lower environmental levels, eg, grass or olive.

In this report, we evaluate both the clinical characteristics and molecular sensitization pattern of London plane tree pollen–allergic patients in the center of Spain. We found important differences in the molecular pattern of allergens and believe this finding merits special attention owing to its potential implications for the standardization of London plane tree pollen extract, as well as for component-revolved diagnosis and the choice of the allergen extract for immunotherapy. Most of the studies on London plane tree pollen allergens that evaluate sensitization to purified allergens have been conducted with patients from the Barcelona area of Spain [13-15,17,19-21,24]. Madrid and Barcelona are relatively close to one another (600 km) and have similar annual indexes and maximum daily concentrations of London plane tree pollen [12,28,29]. However, the specific environmental conditions of each city are extremely different and should be taken into account. Barcelona is a coastal city with a Mediterranean climate, while Madrid, in the central area of Spain, has a continental climate. As a result, the primary cause of allergy in Barcelona is dust mites and, less frequently, pollen, with London plane tree pollen being the most frequent cause of pollen allergy in the area [30]. In contrast, the most common cause of allergy in Madrid is pollen (grasses, olive trees, and cypress, in that order), as befits a continental climate, with dust mites being the cause of allergy in a small percentage of patients. We believe that the different environmental and climatic conditions (humidity, temperature, contamination, pruning, winds) [7,29] might account for some of the differences in our findings on the frequency of sensitization to purified allergens. Similar reports have been published in different areas for other allergen sources, such as olive pollen [31], peanut [32], and nuts [33].

Consistent with Varela et al [6], we found that very few patients (10.5%) were monosensitized, that is, most London plane tree pollen–allergic patients (89.5%) were also sensitized to other pollens. Taking this into account, together with the 2 previously mentioned factors (high concentration, low frequency of allergy, and polysensitization), we hypothesize that the allergenic potency of this pollen is not likely to be very high, with only the most atopic patients sensitized and, therefore, polysensitized. These data suggest that sensitization to London plane tree pollen increases the probability of allergy to another pollen and could be considered a marker of polysensitization, although this hypothesis must be confirmed in studies from other areas.

The frequency of asthma in London plane tree pollen–allergic patients (48.7%) was within the range of values previously reported for pollen-allergic patients in Spain, taking into account the frequency of asthma in patients allergic to pollen with food allergy (59%) or without food allergy (47%) [31].

We assessed allergens from London plane tree pollen extracts by SDS-PAGE and immunoblotting studies under reducing conditions. The highest IgE-binding capacity (76.3%) was associated with the 45-kDa IgE-binding band, which corresponds to the Pla a 2 allergen [15,23]. The percentage was lower for the 18-kDa band, identified as Pla a 1 by immunoblotting (44.7%) and was not sufficiently frequent to be considered a major allergen. These results clearly contrast with those previously reported by Asturias et al [15,20], who found that Pla a 1 and Pla a 2 were present in 92% and 83% of monosensitized patients and, together, were responsible for 79% of total specific IgE-binding activity.

A review of the data published by other researchers revealed important differences in the frequency of sensitization to Pla a 1 and Pla a 2 described initially. For example, the results reported by Wangorsch et al [21] differed widely, with sensitization to Pla a 2 demonstrated in only 20%-27% of patients, depending on whether they had peach allergy or not.

As for the frequency of sensitization to Pla a 1, the first studies [15] reported a frequency of 84%, which was later confirmed by the same researchers. Subsequently, using the sera of patients from the same city (Barcelona), Wangorsch et al [21] found that the frequency of sensitization to Pla a 1 was 60% in patients allergic only to London plane tree pollen and 33% if they also had peach allergy. The results of our study show that the frequency of sensitization to Pla a 1 differed considerably from that reported in studies conducted in the Barcelona area; however, it was within the range of sensitization frequencies (depending on whether patients were allergic to food) reported by Wangorsch et al, namely, 31.6% to 44.7%.

Although the differences found in our study may be due to methodological variations, we highlight that 2 different techniques (ELISA and immunoblotting) showed Pla a 1 to be a minor allergen in *Platanus* tree pollen–allergic patients from Madrid.

In addition, given that Pla a 2 is a glycoprotein with polygalacturonase activity, the frequency of sensitization to Pla a 2 could be affected by the presence of cross-reactive carbohydrate determinants (CCDs). Our results, like those reported by Wangorsch et al [21], showed that only 3 patients had specific IgE to CCDs, suggesting that CCDs do not interfere with calculations of the frequency of sensitization to Pla a 2. The higher frequency of sensitization to Pla a 2 and the low interference of CCDs in the determination of Pla a 2 indicate that Pla a 2 would be a better marker of primary sensitization than Pla a 1 in this area.

Pla a 3 is an allergen belonging to the nsLTP family. It is homologous to Pru p 3 from peach fruit, which has been associated with allergy to plant-derived foods. In the series we report, 23.7% of the patients were sensitized to Pla a 3. It is therefore relevant that 23.7% of the patients were allergic to plant-derived food. A review of data from the literature shows that the frequency of sensitization to Pru p 3 depends primarily on the percentage of patients who had food allergy in the population studied [16].

For the remaining 6 IgE-binding bands detected by immunoblotting, 4 were inhibited with Pla a 2 (42, 60, 64, and 90 kDa), thus potentially explaining the presence of aggregates. The other 2 bands (27 and 32 kDa) were not inhibited by incubation with Pla a 1, bromelain, or Pla a 2 and were maintained in patients. The 32-kDa protein (protein 14-3-3) has never been reported to be an allergen. However, in 2008, Pazouki et al [22] reported the 27-kDa band to be a possible London plane tree pollen allergen (Pla a 4).

However, the authors did not characterize which protein it was. The results we obtained with MS identified this band as the glutathione-S transferase protein, which has already been reported to be a pollen allergen (Bet v 8) in *Betula pendula* [29].

In summary, this study shows that the molecular profile of sensitized patients in Madrid differs from that described in previous studies, where Pla a 1 and Pla a 2 were identified as major allergens. Our results demonstrate that *Platanus*-allergic patients from Madrid were sensitized mainly to Pla a 2. This report will improve both diagnosis and immunotherapy in central Spain. Furthermore, the allergen previously described as Pla a 4 was identified and characterized for the first time as a glutathione-S transferase protein.

Acknowledgments

We would like to thank Borja Bartolome from Roxall Medicina España SA for providing the anti-Pla a 1 antibody.

We would like to thank Oliver Shaw (Fundación IIS-Fundación Jiménez Díaz) for reviewing the manuscript for language-related issues.

Funding

This work was supported by grants from Instituto de Salud Carlos III (ISCIII) cofunded by Fondo Europeo de Desarrollo Regional – FEDER for the Thematic Networks and Co-operative Research Centres: ARADyAL (RD16/0006/003 and RD16/0006/0014).

Conflicts of Interest

The authors declare that they have no conflict of interests.

References

1. Doğruel D, Bingöl G, Altıntaş DU, Seydaoğlu G, Erkan A, Yılmaz M. The Trend of Change of Allergic Diseases over the Years: Three Repeated Surveys from 1994 to 2014. Int Arch Allergy Immunol. 2017;173:178-82.
2. Chereches-Panta PCS, Dumitrescu D, Marshall M, Mirestean I, Muresan M, Iacob D, et al. Epidemiological survey 6 years apart: increased prevalence of asthma and other allergic diseases in schoolchildren aged 13-14 years in Cluj-Napoca, Romania (based on ISAAC questionnaire). Maedica. 2011;6:10-6.
3. Nurmagambetov T, Kuwahara R, Garbe P. The Economic Burden of Asthma in the United States, 2008-2013. Ann Am Thorac Soc. 2018;15:348-56.
4. D'Amato G, Liccardi G. Pollen-related allergy in the European Mediterranean area. Clin Exp Allergy. 1994;24:210-9.
5. Besnard G, Tagmount A, Baradat P, Vigouroux A, Berville A. Molecular approach of genetic affinities between wild and ornamental Platanus. Euphytica. 2002;126:401-12.
6. Varela S, Subiza, J, Subiza JL, Rodríguez R, García B, Jerez M, et al. Platanus pollen as an important cause of pollinosis. J Allergy Clin Immunol. 1997;100:748-54.
7. Alcazar P, Garcia-Mozo H, Trigo MM, Ruiz L, Gonzalez-Minero FJ, Hidalgo P, et al. Platanus pollen season in Andalusia (southern Spain): trends and modeling. J Environ Monit. 2011;13:2502-10.
8. Subiza J, Jerez M, Gavilan MJ, Varela S, Rodrıguez R, Nargenes MJ, et al. Which are the pollens that produce pollinosis in the urban environment of Madrid. Rev Esp Alergol Inmunol Clin. 1998;13:107-19.
9. Iglesias I, Rodríguez-Rajo FJ, Méndez J. Behavior of Platanus hispanica Pollen, an Important Spring Aeroallergen in Northwestern Spain. J Invest Allergol Clin Immunol. 2007;17:145-56,
10. Alcázar P, Cariñanos P, Castro C, Guerra F, Moreno C, Domínguez-Vilches E. Airborne plane-tree (Platanus hispanica) pollen distribution in the city of Córdoba, South-western Spain, and possible implications on pollen allergy. J Invest Allergol Clin Immunol. 2004;14:238-43.
11. Recio M, Trigo MM, Toro FJ, Cabezudo B. Caracterización del contenido polínico invernal de la atmósfera de Málaga. Acta Bot Malacitana. 2008;21:65-70.
12. Díaz de la Guardia C, Sabariego S, Alba F, Ruiz L, García Mozo H, Toro Gil FJ, et al. Aeropalynological study of the genus Platanus L. in the Iberian Peninsula. Polen. 1999;10:93-101.
13. Valero AL, Rosell E, Amat P, Sancho J, Roig J, Piulats J, et al. Hipersensibilidad al polen de Platanus acerofilia: detección de las fracciones alergénicas. Alergol Inmunol Clin. 1999;14:220-6.
14. Asturias JA, Ibarrola I, Eraso E, Arilla MC, Martínez A. The major Platanus acerifolia pollen allergen Pla a 1 has sequence homology to invertase inhibitors. Clin Exp Allergy. 2003;33:978-85.
15. Asturias JA, Ibarrola I, Bartolomé B, Ojeda I, Malet A, Martínez A. Purification and characterization of Pla a 1, a major allergen from Platanus acerifolia pollen. Allergy. 2002;57:221-7.
16. Scala E, Cecchi L, Abeni D, Guerra EC, Pirrotta L, Locanto M, et al. Pla a 2 and Pla a 3 reactivities identify plane tree-allergic patients with respiratory symptoms or food allergy. Allergy. 2017;72:671-4.
17. Enrique E, Alonso R, Bartolomé B, San Miguel-Moncín M, Bartra J, Fernández-Parra B, et al. IgE reactivity to profilin in Platanus acerifolia pollen-sensitized subjects with plant-derived food allergy. J Investig Allergol Clin Immunol. 2004;14:335-42.
18. Lauer I, Miguel-Moncin MS, Abel T, Foetisch K, Hartz C, Fortunato D, 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. 2007;37:261-9.
19. Ibarrola I, Arilla MC, Martínez A, Asturias JA. Identification of a polygalacturonase as a major allergen (Pla a 2) from Platanus acerifolia pollen. J Allergy Clin Immunol. 2004;113:1185-91.
20. Asturias JA, Ibarrola I, Bartolomé B, Ojeda I, Malet A, Martínez A. Purification and characterization of Pla a 1, a major allergen from Platanus acerifolia pollen. Allergy. 2002;57:221-7.
21. Wangorsch A, Larsson H, Messmer M, García-Moral A, Lauer I, Wolfheimer S, et al. Molecular cloning of plane pollen allergen Pla a 3 and its utility as diagnostic marker for peach associated plane pollen allergy. Cin Exp Allergy. 2016;46:764-74.

22. Pazouki N, Sankian M, Nejadsattari T, Khavari-Nejad RA, Varasteh, AR. Oriental plane pollen allergy: identification of allergens and cross-reactivity between relevant species. Allergy Asthma Proc. 2008;29:622-8.
23. Skin tests used in type I allergy testing Position paper. Sub-Committee on Skin Tests of the European Academy of Allergology and Clinical Immunology. Allergy. 1989;44(S10):11-59.
24. 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.
25. Cases B, Pastor-Vargas C, Dones FG, Perez-Gordo M, Maroto AS, de las Heras M, et al. Watermelon profilin: characterization of a major allergen as a model for plant-derived food profilins. Int Arch Allergy Immunol. 2010;153:215-22.
26. Muñoz-García E, Luengo-Sánchez O, Haroun-Díaz E, Maroto AS, Palacín A, Díaz-Perales A, et al. Identification of thaumatin-like protein and aspartyl protease as new major allergens in lettuce (Lactuca sativa). Mol Nutr Food Res. 2013;57:2245-52.
27. Pastor C, Cuesta-Herranz J, Cases B, Pérez-Gordo M, Figueredo E, de las Heras M, et al. Identification of major allergens in watermelon. Int Arch Allergy Immunol. 2009;149:291-8.
28. Subiza J, Cabrera M, Valdivieso R, Subiza JL, Jerez M, Jiménez JA, et al. Seasonal asthma caused by airborne Platanus pollen. Clin Exp Allergy. 1994;24:1123-9.
29. Gabarra E, Belmonte J, Canela M. Aerobiological behaviour of Platanus L. pollen in Catalonia (North-East Spain). Aerobiologia. 2002;18:185-93.
30. Puiggròs A, Muñoz-Cano R, Roger Reig A, Raga E, Belmonte J, Valero A. Prevalence of sensitization to pollen from trees planted in Barcelona city. J Investig Allergol Clin Immunol. 2015;25:150-1.
31. Cuesta-Herranz J, Barber D, Blanco C, Cistero-Bahíma A, Crespo JF, Fernández-Rivas M, et al. Differences among pollen-allergic patients with and without plant food allergy. Int Arch Allergy Immunol. 2010;153:182-92.
32. Vereda V, van Hage M, Ahlstedt S, Ibañez MD, Cuesta-Herranz J, van Odijk J, et al. Peanut allergy: Clinical and immunologic differences among patients from 3 different geographic regions. J Allergy Clin Immunol. 2011;127:603-7.
33. Haroun-Díaz E, Azofra J, González-Mancebo E, de Las Heras M, Pastor-Vargas C, Esteban V, et al. Nut Allergy in Two Different Areas of Spain: Differences in Clinical and Molecular Pattern. Nutrients. 2017;9:909.
34. Deifl S, Zwicker C, Vejvar E, Kitzmüller C, Gadermaier G, Nagl B, et al. Glutathione-S-transferase: a minor allergen in birch pollen due to limited release from hydrated pollen. PloS One. 2014;9:e109075.

Manuscript received March 22, 2021; accepted for publication May 4, 2021.

Carlos Pastor-Vargas

Departamento de Bioquímica y Biología Molecular
Facultad de C.C. Químicas
Universidad Complutense de Madrid
28040 Madrid, Spain.
E-mail: cpasto01@ucm.es

Javier Cuesta-Herranz

Servicio de Alergología
Hospital Universitario Fundación Jiménez Díaz
Av. Reyes Católicos 2
28040 Madrid, Spain.
E-mail: j.cuestaherranz@gmail.com