ORIGINAL ARTICLE

Identification of *Plantago lanceolata* Pollen Allergens Using an Immunoproteomic Approach

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Abstract

Background: Airborne *Plantago* pollen triggers respiratory allergies in Mediterranean countries.
Objectives: We aimed to study sensitization in patients with seasonal respiratory allergy and identify proteins of *Plantago lanceolata* pollen that could be responsible for hypersensitivity reactions in sensitized patients. We also determined the airborne pollen concentration of *Plantago* species from 2004 to 2011.
Methods: IgE-binding proteins were analyzed and characterized using 1D and 2D gel electrophoresis and immunoblotting with sera from individuals sensitized to *P lanceolata* pollen extracts, mass spectrometry analysis, and protein data mining. We used aerobiological methods to study airborne pollen.
Results: P lanceolata pollen accounts for 3% of the annual pollen spectrum in the air of Porto. Of a total of 372 patients, 115 (31%) showed specific IgE levels to *P lanceolata* pollen extracts. All sera from *P lanceolata*–allergic patients recognized 8 prominent groups of IgE-reactive allergens. Separation of proteins using 2D gel electrophoresis followed by identification with mass spectrometry revealed the presence of other IgE-reactive components that could be involved in sensitization.
Conclusions: We detected proteins in *P lanceolata* pollen extracts that, to our knowledge, have not yet been studied and could worsen sensitization to this weed pollen species. The proteins identified were involved in a variety of cellular functions. By applying 2D electrophoresis and immunoblotting with a pool of 2 sera from different *P lanceolata*–allergic patients, we obtained a more detailed characterization of the *P lanceolata* allergen profile.

Key words: *Plantago lanceolata*. IgE-binding protein. Allergens. Airborne pollen. Mass spectrometry. 2D gel electrophoresis.

Resumen

Antecedentes: El polen de *Plantago* provoca alergia respiratoria en los países mediterráneos.
Objetivos: El objetivo de este estudio fue analizar las sensibilizaciones de pacientes con alergia estacional e identificar las proteínas de polen de *Plantago lanceolata* que puedan ser responsables de las reacciones de hipersensibilidad en pacientes sensibles. Adicionalmente determinamos la concentración de polen de *Plantago* spp en el aire, en los años 2004-2011.
Métodos: Las proteínas que se unen a la IgE fueron analizadas y caracterizadas a través de electroforesis en gel 1-D y 2-D e inmunobloting con suero de pacientes sensibilizados al polen de *P. lanceolata*. Se analizó mediante espectrometría de masas el contenido en las proteínas y se aplicaron métodos aerobiológicos para estudiar el espectro de polen en el ambiente.
Resultados: En cuanto a los resultados obtenidos, el polen de *P. lanceolata* representa el 3% del espectro de polen anual en la atmósfera de Oporto. De los 372 pacientes, el 31% presentaban IgE específica frente al polen de *P. lanceolata*. Todos los sueros de los pacientes alérgicos a *P. Lanceolata* reconocían los ocho grupos prominentes de alérgenos reactivos a IgE. La separación de proteínas mediante electroforesis en gel 2-D, seguida de la espectrofotometría de masas permitieron identificar en el polen la presencia de otros componentes IgE reactivos que podrían estar implicados en la sensibilización de estos pacientes.
Conclusiones: En conclusión, este estudio muestra la presencia de proteínas en el polen de *P. Lanceolata* que hasta ahora no habían sido estudiadas y que pueden intervenir en la sensibilización a éste polen. Se detectaron proteínas involucradas en una gran variedad de funciones celulares. Mediante las técnicas aplicadas en este estudio, entre ellas el inmunobloting, nos permite realizar una detallada caracterización del perfil alergénico del polen de *P. lanceolata*.

Palabras clave: *P. lanceolata*. Proteína de unión a IgE. Alérgenos. Polen aerotransportado. Espectrofotometría de masas. Electroforesis bidimensional.

Introduction

Pollen-induced respiratory allergies are seasonal diseases that involve an immunoreactive response of susceptible individuals to allergens present in the pollen of various trees, grasses, and weeds that are released into the atmosphere during the flowering season.

Sensitization to pollen allergens varies between different regions of the world and is closely related to climatic conditions, degree of urbanization, and plant distribution [1,2]. In Mediterranean countries or countries influenced by the Mediterranean climate, airborne *Plantago* pollen is, together with Poaceae, *Olea* species, and *Cupressus* species, one of the 10 most common triggers of respiratory allergy [3-7].

Monosensitization to the pollen of *Plantago lanceolata* (plantain) is unusual. However, cross-reactivity with grass and olive pollen has been reported [3,7]. Consequently, it is difficult to ascertain the exact contribution of plantain to the etiology of pollinosis. Nonetheless, *P lanceolata* pollen extracts are included in the pan-European standardized allergen batteries for use in clinical practice and research proposed by the Global Asthma and Allergy European Network [8].

The genus *Plantago* (Plantaginaceae) comprises approximately 250 species. It usually grows in neglected gardens and humid meadows and at roadsides. It invades lawns, spreads steadily, and is considered an undesirable weed, with no economic or aesthetic value [9].

Few studies have tried to identify the allergens in *Plantago* pollen [10-12], and to date, only 1 allergen, Pla l 1, has been purified and characterized. cDNA of 3 isoforms has been cloned, and the recombinant proteins produced are classified into 3 types: nonglycosylated, 17 kDa; glycosylated, 23 kDa; and dimeric, 32-39 kDa [13]. Other allergens identified as specific to *P lanceolata* include a cytochrome c protein [14] and a calcium-binding protein [15].

In this study, we determined the airborne pollen concentration of *Plantago* species in the city of Porto, Portugal, from 2004 to 2011 and studied the prevalence of sensitization among individuals with seasonal respiratory allergy. Furthermore, using a proteomic approach, we identified novel proteins of *P lanceolata* pollen that could be responsible for hypersensitivity reactions in sensitized patients.

Material and Methods

Study Area

This study was conducted in the Porto region of northwest Portugal. According to the Köppen climate classification, the climate of this area is Mediterranean with an Atlantic influence. The temperature is mild; January and July are the coldest and the hottest months, respectively. The mean (SD) minimum temperature is 10ºC (0.7ºC) and the mean (SD) maximum temperature is 19ºC (0.6ºC). Annual mean relative humidity ranges between 75% and 80%, and rainfall is mainly concentrated in winter and spring [16].

Aerobiological Monitoring

Airborne pollen levels were monitored from 2004 to 2011, using a 7-day Hirst-type volumetric spore sampler (Burkard) set on the roof of the Faculty of Sciences of the University of Porto (41º11´ N, 8º39´ W), which is approximately 20 meters above ground level, and calibrated to sample air at 10 L per minute. Pollen was trapped on a Melinex tape coated with silicone oil, which was then cut into daily segments and mounted on slides with a glycerol gel. The daily mean concentration of *Plantago* pollen was estimated using an optical microscope (4 full lengthwise traverses at a magnification of x400). Pollen counts were expressed as the sum of the number of pollen grains per cubic meter of air for a 24-hour period.

Sensitization Data

Data from the sera of patients presenting specific IgE reactivity to pollen extracts (ImmunoCAP FEIA, Phadia AB) were randomly obtained from a central hospital in the Porto region between 2006 and 2011. Patients presenting IgE only to *P lanceolata* (w9) or to *P lanceolata* (w9) and a weed mixture (wx1: *Ambrosia elatior, Ambrosia artemisiifolia, Artemisia vulgaris, P lanceolata, Chenopodium album,* and *Salsola kali*) were evaluated, and a brief descriptive statistical analysis was performed according to different levels of specific IgE recognition: 0.35-0.7 kU_A/L; 0.7-3.5 kU_A/L; 3.5-17.5 kU_A/L; 17.5-50 kU_A/L; and 50-100 kU_A/L. We considered that ethical approval was unnecessary, since our study was based only on anonymous files of sensitization test results.

Pollen Samples

During the *Plantago* flowering season, anthers were collected, dried at 27°C, and gently crushed. The pollen released was sieved and stored at –20°C.

Patient Sera

Thirty random atopic patients were assessed using skin prick tests, and the serum from each individual was separated from whole blood and measured using ImmunoCAP FEIA. Positive ImmunoCAP results with *P lanceolata* pollen extracts (w9) were used individually in the immunoblotting experiments. The specific IgE levels ranged from 0.05 kU_A/L (lowest value) to 70.3 kU_A/L (highest value). One serum from a nonatopic individual with IgE of 4.34 IkU/L and no results from ImmunoCAP with *P lanceolata* pollen was chosen as a negative control. Each serum represents only 1 sensitized individual with a regular volume of around 0.5-0.7 mL, which is sufficient for a single experiment.

Protein Extraction and 1D Gel Electrophoresis

P lanceolata pollen was suspended in 1:20 (wt/vol) phosphate-buffered saline at pH 7.4. Soluble proteins were extracted by continuous stirring (4 hours) and the supernatant was centrifuged at 13 200 rpm (30 minutes, 4°C). The resultant suspension was filtered through a 0.45-µm Millipore filter and centrifuged. The total soluble protein content was quantified colorimetrically using the Coomassie Protein Assay Reagent kit (Thermo Scientific) [17]. Proteins were profiled using SDS-PAGE (30 µg per lane) under reducing conditions [18] and stained with Coomassie Blue R-250. The molecular weight of the protein bands was estimated by comparison with a standard protein marker (Fermentas).

2D Gel Electrophoresis and Protein Selection

P lanceolata pollen protein extracts (400 µg) underwent 2D gel electrophoresis as described by Gomes et al [19]. Briefly, pollen proteins were precipitated (ProteoExtract, Calbiochem), resuspended (7 M urea, 2 M thiourea, 4% [vol/vol] CHAPS, and 0.0002% bromophenol blue) with 0.2% of ampholyte, and quantified (2D Quant Kit, GE Healthcare). Passive rehydration of the strips was performed overnight with 250 µg of sample using 11 cm IPG strips pH 3-10 NL (ReadyStrip, Bio-Rad) at room temperature. Isoelectric focusing (IEF) was performed using Protean IEF Cell (Bio-Rad) with an initial voltage of 250 V for 15 minutes and then by applying a voltage gradient up to 8 000 V, with limiting current of 50 µA per strip at 20°C, until 25 000 V was reached. IEF proteins were then reduced and alkylated by incubation with 2% DL-dithiothreitol followed by 2.5% iodoacetamide in an equilibration buffer (6 M urea, 2% SDS, 0.002% bromophenol blue, 75 mM Tris pH 8.8, 29.3% glycerol) for 10 minutes each with gentle shaking. The strips were then packed in a 1% low gel (1% agarose in running buffer: 25 mM Tris, 192 mM glycine, and 0.1% [wt/vol] SDS, pH 8.3; Bio-Rad) on a precast gel (Any kD, Bio-Rad). 2D gel electrophoresis was performed using a Criterion system (Bio-Rad) with 1xTris/glycine/SDS buffer at a constant voltage of 125 V. 2D electrophoresis gels were stained with colloidal Coomassie Brilliant Blue G-250 (PageBlue, Thermo Scientific) or blotted onto a nitrocellulose membrane (Protran, Whatman Schleicher & Schuell) for Western blotting.

Immunoblotting

Protein bands/spots were separated by SDS-PAGE and electrophoretically transferred to a nitrocellulose membrane. Transfers were carried out in a solution of 192 mM glycine, 25 mM Tris, and 20% methanol (transfer buffer) for 2 hours at 200 mA. The membranes were saturated for 1 hour with 5% nonfat dry milk and 0.1% goat serum in TBS-T (20 mM Tris, 150 mM NaCl with 0.1% Tween 20). 1D gel electrophoresis was performed using a panel of 30 sera from individuals sensitized to *P lanceolata* pollen protein extracts to assess IgE binding to *P lanceolata* pollen proteins. For the 2D gel electrophoresis, 1 blot was incubated with a pool of 2 sera from individuals allergic to *P lanceolata* pollen extracts (1:10). After washing, blots were probed with horseradish peroxidase–conjugated mouse antihuman IgE (1:2000). The reaction was revealed using peroxidase ECL substrate and exposed to Amersham Hyperfilm ECL (GE Healthcare).

Protein Selection and Identification by Mass Spectrometry

The immunoreactive protein spots revealed by the sera pool of 2 individuals sensitized to *P lanceolata* pollen extracts were matched with the Coomassie Blue gels and excised with a spotpicker (OneTouch 2D gel spotpicker, 1.5 mm diameter, Gel Company). These protein spots were washed, reduced with dithiothreitol, alkylated with iodoacetamide, and digested in gel with trypsin.

Proteins were identified using matrix-assisted laser desorption/ionization–time of flight (MALDI TOF/TOF) mass spectrometry (4700 Proteomics Analyzer MALDI-TOF/TOF, AB SCIEX) according to a procedure published elsewhere [19]. The processed peptide samples were desalted and concentrated using ZipTips (Millipore) and crystallized onto a MALDI sample plate. The peptide mass spectrum was acquired for each sample in reflector-positive mode with a mass window of 700-4000 m/z. Some of the peptide peaks were selected for MS/MS peptide sequencing. The proteins were identified by combining data from the peptide mass fingerprinting and MS/MS peptide sequencing approaches using the Mascot protein search software (Matrix Science) integrated in the GPS Explorer software (AB SCIEX). Proteins were then searched for against the UniProt Green plants protein sequence database. The maximum error tolerance was 50 ppm and up to 2 missed cleavages were allowed.

Results

Aerobiological Monitoring

Plantago pollen was present in the air of the Porto region from March to September, with most pollen sampled from May to July (Figure 1). This pollen type represents around 3% of the annual pollen load, corresponding to the ninth most abundant airborne pollen.

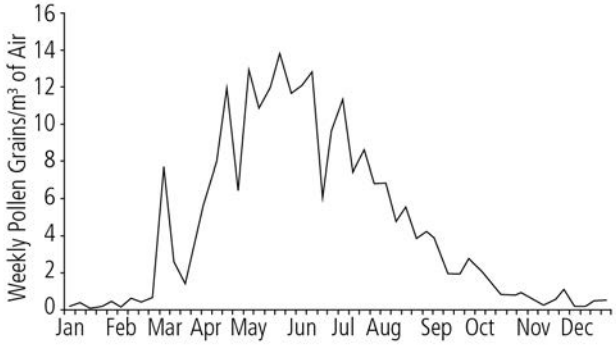

Figure 1. Average weekly pollen concentration of airborne *Plantago* species in the Porto region (2004-2011).

Sensitization Data

Of 372 patients with specific IgE reactivity to at least 1 pollen extract, only 115 patients (31%) presented specific IgE levels to *P lanceolata*. Of these, 70% (n=80) were positive to w9 only and 30% (n=35) to both w9 and wx1. As for specific IgE levels, most patients had 0.7-3.5 kU_A/L followed by 3.5-17.5 kU_A/L. Demographic data were not available for reasons of confidentiality.

IgE-binding Protein Profile by 1D Gel Electrophoresis and Immunoblotting

The polypeptide profile of *P lanceolata* pollen, which was obtained using 12.5% SDS-PAGE, revealed multiple molecular-weight bands ranging from 70 kDa to 13 kDa after staining with Coomassie Blue R-250 (Figure 2). 1D gel electrophoresis revealed 8 prominent groups of IgE-reactive allergens in *P lanceolata*. Out of 30 sera, more than 70%

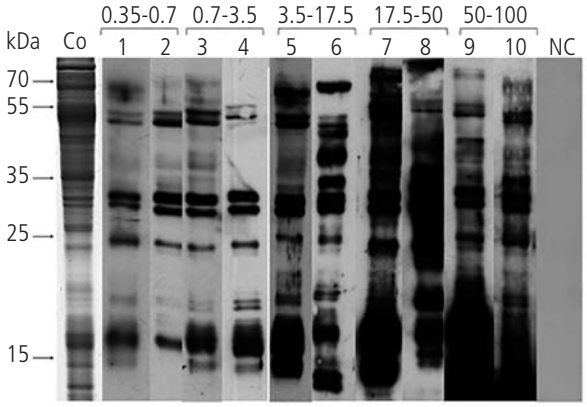

Figure 2. SDS-PAGE and immunodetection of *Plantago lanceolata* pollen extracts assayed with a panel of sera from individuals sensitized to *P lanceolata*. These 10 immunoblots were taken as representative of the 30 processed. Blots from 2 different individuals are shown for each IgE level group. Coomassie Blue staining pattern of total protein (Co). IgE-reactive profiles of patient sera with escalating specific IgE levels (0.35-0.7, 0.7-3.5, 3.5-17.5, 17.5-50, and 50-100 kU_A/L) were used (1-10). A nonatopic patient serum was used as a negative control (NC).

exhibited IgE reactivity to protein bands of 50-53, 29-31, 23-25, 18-19, and 16-17 kDa. Other allergenic polypeptides in the range of 70-73, 40-42, 34-36, and 13-14 kDa were detected in at least 55% of cases of recognition of IgE. Figure 2 shows 10 allergen profiles corresponding to 10 individual sera presenting specific IgE levels ranging from 0.05 kU_A/L to 70.3 kU_A/L. These 10 immunoblots were taken as representative of the overall 30 (blots from 2 different individuals are shown for each IgE level group).

Analysis of Immunoreactive Pollen Proteins Using 2D Gel Electrophoresis

After testing a panel of sera from individuals sensitized to *P lanceolata* pollen protein extracts using 1D electrophoresis, we verified that several IgE-reactive components were uncharacterized protein bands of around 53, 42, and 35 kDa. We attempted to identify these *P lanceolata* pollen-reactive peptides by separating their proteins using 2D gel electrophoresis (Figure 3A) followed by immunoblotting (Figure 3B).

2D gel electrophoresis revealed several spots with a pI ranging from 3 to 10 and a molecular mass of 10 kDa to 100 kDa. Most of the proteins profiled ranged from 35 kDa to 55 kDa and had acidic or neutral pI. In order to visualize the binding of IgE to pollen proteins, immunoblots were developed with a pool of 2 sera from 2 sensitized individuals in order to correlate with the results of the 1D gel electrophoresis and immunoblotting experiments. These sera were not used for 1D gel electrophoresis, as we received them afterwards. This type of study is limited by the fact that each serum represents only 1 sensitized individual and that sera are kept in batches of around 0.5-0.7 mL, which is often enough for only 1 experiment.

Immunoreactive proteins ranged from 15 kDa to 53 kDa in weight and, in contrast to findings with Coomassie Blue staining, most had neutral or basic pI. Several significant IgE

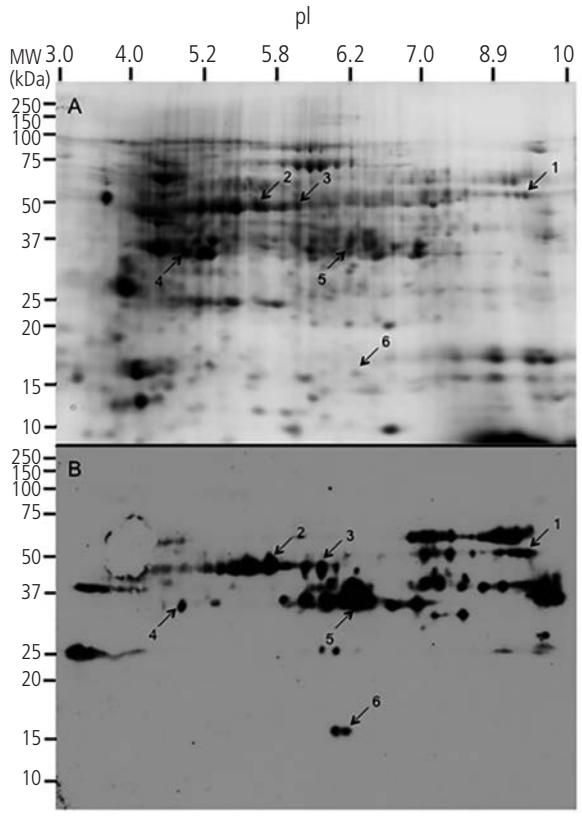

Figure 3. 2D gel electrophoresis of protein extracts of *Plantago lanceolata* pollen. A, Gel stained with Coomassie blue. B, Western blot analysis after transfer to a nitrocellulose membrane. This blot was incubated with a pool of sera from 2 sensitized individuals presenting specific IgE to *P lanceolata* pollen. Proteins were identified by matching the spots indicated by arrows (1-6) to Coomassie Blue gels. These spots were then excised for protein identification by MALDI-TOF/TOF analysis.

immunoreactive spots were excised from the corresponding Coomassie-stained gel (Figure 3) and analyzed using mass spectrometry.

Protein Identification Using MALDI-TOF/TOF Mass Spectrometry

The Table shows IgE-reactive *P lanceolata* pollen proteins identified using MALDI-TOF/TOF mass spectrometry. The peptides identified were polygalacturonase (B9SJM2_RICCO, spot 1), UDP-glucose-6-dehydrogenase (Q9AUV6_ORYSJ, spot 2 and I1LWR5_SOYBN, spot 3), adenosylhomocysteinase (G8GJ68_LINUS, spot 2), inositol-3-phosphate synthase (INO1_SESIN, spot 2), alpha−1,4−glucan−protein synthase [UDP-forming] (UPTG_MAIZE, spot 4), glyceraldehyde-3-phosphate dehydrogenase (D7L218_ARALL, spot 5), and fructose-biphosphate aldolase (Q38HV4_SOLTU, spot 5). Spot 6 (PLAL1_PLALA) seems to correspond to Pla l 1, a major allergen of *P lanceolata* pollen whose low molecular mass (around 15 kDa) is consistent with the Official List of Allergens of the International Union of Immunological Societies.

Table. Immunoreactive Proteins Identified From IgE Sera Pool by MALDI TOF/TOF MS[a]

Spot	Protein Description	Accession Number	MASCOT Protein C.I. %	Peptide Count	% Cov	Peaks Matched	MOWSE Score	MW, kDa	pI
1	Polygalacturonase	B9SJM2_RICCO	100	4	14	5	135	43.4	9.1
2	UDP-glucose 6-dehydrogenase	Q9AUV6_ORYSJ	100	7	18	9	188	53.4	5.8
	Adenosylhomocysteinase	G8GJ68_LINUS	100	12	20	14	126	61.9	5.7
	Inositol-3-phosphate synthase	INO1_SESIN	100	9	20	14	114	56.4	5.6
3	UDP-glucose 6-dehydrogenase	I1LWR5_SOYBN	100	11	28	15	232	53.6	6.1
4	Alpha-1,4-glucan-protein synthase [UDP-forming]	UPTG_MAIZE	100	10	28	18	263	41.7	5.8
5	Glyceraldehyde-3-phosphate dehydrogenase	D7L218_ARALL	100	11	35	12	135	37.0	6.6
	Fructose-bisphosphate aldolase	Q38HV4_SOLTU	100	7	23	8	125	38.8	8.5
6	Major pollen allergen Pla l 1	P82242 (PLAL1_PLALA)	100	10	68	13	213	14.9	7.6

[a]Uniprot database search for green plants taxonomic selection: http://www.uniprot.org/

Discussion

Airborne *Plantago* pollen was detected in Porto during the spring and early summer (2004-2011) at concentrations of <14 grains/m^3 of air, which are considered low-risk for allergenic reactions (Portuguese Aerobiology Network). However, the extended main pollen season with its concomitant presence of high levels of pollen from other allergenic sources, such as grasses, olives, and *Parietaria* [20,21], increases the exposure period and the allergenic pollen load, possibly triggering cross-reactivity phenomena [9,22,23]. Sensitization to this pollen species (evaluated using skin prick tests) has been widely reported, particularly in the Mediterranean area, where rates of sensitization to *P lanceolata* are higher than expected, ranging from 14.6% to 38% [1,3-5,7]. In our study, sensitization to *P lanceolata* was 31%. Consequently, this allergen is a major cause of pollinosis and should be taken into consideration when diagnosing seasonal allergy and included in standard skin prick test arrays in this region [3].

We observed IgE-reactivity to 8 groups of protein bands, which were similar to prominent bands observed in the 1D electrophoresis profile. Asero et al [10] detected 3 specific allergens of 17, 19, and 40 kDa using sera from *P lanceolata*–monosensitized individuals. Calabozo et al [13,24,25] identified, characterized, and produced the recombinant form of the major *P lanceolata* allergen, Pla l 1, and observed that it consisted of a polymorphic allergen that can occur in different forms: unglycosylated (17 kDa), glycosylated (23 kDa), and dimeric (32-39 kDa). Using 1D and 2D electrophoresis and immunoblotting, we found that serum IgE also recognized proteins of similar molecular weights to Pla l 1 (Figures 2 and 3). Therefore, consistent with previous results, the 15-16–kDa band identified could correspond to the unglycosylated form of Pla l 1.

When we used sera with increasing specific IgE levels, low-molecular-weight allergens (13-14 kDa) were recognized, thus making it more difficult to distinguish protein bands. Low-molecular-weight proteins were also recognized using rabbit polyclonal IgG ZmPRO3 antibody as a probe instead of serum IgE (unpublished data); these proteins were from the panallergen family of profilins [26], even though they were not identified by mass spectrometry. In a study based on skin prick test results, 74.4% of profilin-sensitive patients were also sensitized to plantain pollen, suggesting that this panallergen shares determinant epitopes with a 14-13–kDa *P lanceolata* pollen protein [27]. Asero et al [10] observed a 30 kDa band recognized by IgE of *P lanceolata* and grass-sensitive patients. Probing the extracts with Phl p 1 and Phl p 5 only revealed IgE reactivity in the latter. Therefore, the authors suggested that only group 5 grass pollen allergens could be a cause for plantain/grass cross-reaction. We identified 2 immunoreactive proteins of around 39 kDa and 37 kDa (Figure 3, spot 5); these proteins correspond to fructose-bisphosphate aldolase and glyceraldehyde-3-phosphate dehydrogenase, respectively. Shotgun proteomic analysis revealed that both peptides are known to work together in metabolic pathways such as glycolysis and were also recognized as IgE-binding proteins in *Lolium perenne* pollen extracts [29]. Glyceraldehyde-3-phosphate dehydrogenase was also found to be a *Triticum aestivum* food allergen [30].

In our study, a 40-42–kDa protein was observed in more than 50% of sera (lanes 2, 3, 5, 6, 7, 8, 9, and 10 [Figure 2]). In fact, a 40-kDa *P lanceolata* protein described as a cross-reactive protein in *Olea europaea* and *P lanceolata* may correspond to a β-glucanase protein [22], which comprises a family of pathogen-related proteins and has been addressed as a novel type of panallergen [26,27]. However, we identified an α-1,4-glucan-protein synthase (Figure 3, spot 4), which is

involved in the synthesis of cellulose and shows allergenic activity in *Phoenix dactylifera* pollen [28].

Asero et al [10] and Calabozo et al [24] reported protein bands of 53 kDa and 50 kDa, respectively, both of which were weakly recognized by IgE sera in *P lanceolata* pollen extracts. However, our data showed a set of 2 highly prevalent bands of 50-53 kDa detected in more than 96% of the 30 sera (Figure 2). Proteomic analysis revealed several immunoreactive proteins (Figure 3, spots 1, 2, and 3) with a molecular weight that was similar to those described above. The polygalacturonase protein identified (spot 1) was found in *Ricinus communis* and had functional enzyme activity. It was first described as a pollen allergen in *Platanus acerifolia* and was characterized as a major allergen, Pla a 2 [31]. Polygalacturonases are usually distributed in plants, fungi, and bacteria and are involved in the ripening process and in pollen development and germination.

A UDP-glucose-6-dehydrogenase of around 53 kDa was identified in spots 2 (pI 5.8) and 3 (pI 6.1) of the 2D gel electrophoresis blot. This protein was previously reported in the germination of the pollen of *Oryza sativa* and *Arabidopsis thaliana* [32,33]. An inositol-3-phosphate synthase (56 kDa) was also identified in spot 2. Both proteins were able to bind to *P lanceolata* pollen protein extracts, even though they were not reported as having allergen-related activities in other organisms. Furthermore, an adenosylhomocysteinase of around 62 kDa identified in spot 2 had already been reported as an immunoreactive protein of *Aspergillus fumigatus* [34].

Proteomic analysis of pollen extracts is a suitable method for identification of potential novel IgE-binding proteins. Most of the spectra produced correspond to peptides that share a high degree of homology with other sequenced peptides from taxonomically related species. Hence, the production of purified allergens (natural or recombinant) as predictive markers of clinical reactivity could prove useful for distinguishing the pollen allergen responsible for eliciting allergy symptoms and diagnosis of true plantain-allergic patients [1,13,35].

Knowledge of pollen allergens is important for the study of true plantain allergy. Using 2D gel electrophoresis, we identified the presence of potentially novel proteins of *P lanceolata* pollen extracts. However, some of the IgE-binding proteins identified may be the outcome of cross-reaction to homologous allergens from other sources. Our results should be used as the basis for further molecular characterization of allergens in *P lanceolata*, which is a prerequisite for the treatment of allergic patients. It is important to remember that individual patients, even when sensitized to the same pollen type, show different IgE-binding protein profiles and therefore do not necessarily develop clinically relevant allergic symptoms. This possibility should be confirmed using other allergenicity tests.

Acknowledgments

We thank Gabriel Pereira for technical support.

Funding

This work was supported by FEDER funds through the Operational Program Competitiveness Factors (COMPETE) and national funds through FCT – Foundation for Science and Technology in the Project "Effects of atmospheric non-biological pollutants on pollen grains" (Ref. PTDC/AACAMB/102796/2008) and PEst-OE/CTE/UI0039/2011-UI 39. Helena Ribeiro holds a scholarship (SFRH/BDP/43604/2008) funded by QREN-POPH and FCT.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Previous Presentation

This work was presented in part as an abstract at the 2nd Southern European Allergy Societies Congress, Estoril, Portugal (SEAS 2011).

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Manuscript received April 24, 2013; accepted for publication July 19, 2013.

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