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Purified allergens vs. complete extract in the diagnosis of plane tree pollen allergy

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Clinical and Experimental Allergy

Summary

Background Plane tree pollen allergy is a clinical disorder affecting human population in cities of Europe, North America, South Africa, and Australia.

Objective To compare IgE-reactivity of the natural and recombinant forms of two major plane allergens, Pla a 1 and Pla a 2, with the reactivity of *Platanus acerifolia* pollen extract.

Methods Forty-seven patients with *P. acerifolia* allergy, 15 of them monosensitized, and 24 control subjects were included in the study. Natural Pla a 1 and Pla a 2 were purified by standard chromatographic methods and recombinant proteins were expressed in *Escherichia coli*. Skin prick test and determination of specific IgE were performed with commercial *P. acerifolia* extract and natural and recombinant purified allergens.

Results Pla a 1 and Pla a 2 were responsible for 79% of the IgE-binding capacity against *P. acerifolia* pollen extract. A high correlation has been found between the IgE response to nPla a 1 ($R=0.80$; $P<0.001$) or nPla a 2 ($R=0.79$; $P<0.001$) vs. *P. acerifolia* extract as well as between natural and recombinant Pla a 1 ($R=0.89$; $P<0.001$). Skin testing showed no significant differences between extract and nPla a 2, whereas a higher reactivity was found with nPla a 1. In contrast, rPla a 1 revealed markedly reduced sensitivity in comparison with extract by skin prick test and specific IgE. The sensitivity of the mix Pla a 1+Pla a 2 was 100% and 87.5% for monosensitized and polysensitized patients, respectively, with no false-positive reactions detected.

Conclusion Pla a 1 and Pla a 2 are sufficient for a reliable diagnosis of *P. acerifolia* in most patients and induce comparable skin test reactivity as a whole extract.

Keywords component-resolved diagnosis, IgE detection, Pla a 1, Pla a 2, plane tree allergy, recombinant allergen, skin prick tests

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Introduction

The diagnosis of pollen allergy is based on clinical history and detection of IgE-specific antibodies with *in vitro* tests or skin tests, but both techniques are influenced by the inherent heterogeneity of allergenic extracts. Nowadays, purified proteins for *in vitro* assays instead of complex allergenic extracts are more widely used as some allergens are available, allowing a component-resolved diagnosis [1–3]. Diagnostic studies using allergenic molecule-based approaches for skin testing have also been performed [4–6]. Purified allergens have even been used for allergen-specific immunotherapy [7, 8] and for monitoring antibodies response after allergen-specific immunotherapy [9].

Platanus acerifolia is one of the most common trees found in European cities because of its resistance to diseases and urban-polluted environment. In Spain, this pollen is the most important allergen during its flowering season (March–April) [10] with prevalences by skin prick test (SPT) of 56% in Madrid, 35% in western Spain and 8.5% in Barcelona [11–13]. The clinical importance of *Platanus* pollen has also been demonstrated in studies from southern France and Switzerland [14, 15]. An association between *Platanus* pollen and some vegetable foods such as hazelnut, peanut, banana, and celery has been found in 84% of patients allergic to plant foods [16] and cross-reactivity between lettuce-LTP and *Platanus*-pollen extract has also been reported [17]. *P. acerifolia*

allergens described ranged from 5 to 66 kDa [18–20], but only two major allergens have been characterized. Pla a 1 is a nonglycosylated protein of 18 kDa related to invertase inhibitors, which reacted with 92% of monosensitized and 83% of polysensitized *P. acerifolia*-allergic patients [21, 22]. Pla a 2 is a glycoprotein of 43 kDa with polygalacturonase activity and a prevalence of 84% [23].

In this study, we evaluate the potential of the major *P. acerifolia* allergens, Pla a 1 and Pla a 2, with regard to their capacity to bind specific IgE and to elicit SPT reactivity in 47 patients with *P. acerifolia* allergy in comparison with standardized *P. acerifolia* extract. In addition, we check the ability of their recombinant counterparts to substitute natural proteins.

Materials and methods

Patients

Seventy-one subjects were included in this study: 15 allergic patients monosensitized to *P. acerifolia* pollen (group MS); 32 allergic patients polysensitized to *P. acerifolia* pollen and other aeroallergenic sources such as dust mites and pollen from grass, olive, cypress, and ragweed (group PS); and 24 control subjects, 13 nonatopics, and 11 with other unrelated allergies. The diagnosis of *P. acerifolia* allergy was based on the typical clinical history of recurrent rhinitis in coincidence with the higher-plane tree pollen counts, a positive SPT response to *P. acerifolia* extract (Bial-Aristegui, Bilbao, Spain), and specific IgE serum level to *P. acerifolia* as determined by using the ImmunoCAP System (Pharmacia Diagnostics, Uppsala, Sweden). Control subjects included healthy people and patients sensitized to different allergenic sources, such as house dust mites, dog dander, and pollens unrelated to *P. acerifolia*. The use of natural and recombinant allergens for SPT was approved by local committees, and all subjects provided oral and written informed consent before enrolment in the study.

P. acerifolia extract and purification of Pla a 1 and Pla a 2

Crude extracts of *P. acerifolia* pollen (IberPolen, Málaga, Spain) were obtained by PBS extraction [24]. Natural Pla a 1, nPla a 2, and rPla a 1 were purified as previously described [21–23]. Full-length cDNA-coding for Pla a 2 was inserted between the *Nde*I and *Hind*III sites of the expression vector pKN172 [25] and used to transform *E. coli* BL21 (DE3) (Novagen, Madison, WI, USA). rPla a 2 was expressed as previously described for Pla a 1 [22] and purified as described for its natural counterpart [23] but including treatment with 6 M guanidinium, refolding in 6 M urea, and step dialysis in PBS containing 4, 2, and 0 M urea. Plane profilin was purified by poly-L-proline affinity chromatography [26].

Electrophoresis and immunoblotting

P. acerifolia extracts and purified proteins were separated by SDS-PAGE (12.5% polyacrilamide gels) under reducing conditions and stained with Coomassie Blue. For immunoblot studies, separated proteins were transferred electrophoretically to PVDF (Hybond-P, GE-Healthcare, Uppsala, Sweden). After blocking, membranes were incubated at 4 °C overnight with diluted human sera, and then washed and bound antibodies were detected with horseradish peroxidase-conjugated anti-human IgE (Dako, Copenhagen, Denmark). The blot was washed and developed by the ECL+Western blotting detection system (GE-Healthcare). For IgE dot-blot, 2 µL of purified allergens (100 µg/mL) was deposited onto a nitrocellulose membrane (Hybond-C+, GE-Healthcare). After drying, the membrane was blocked and incubated as above described.

Determination of specific serum immunoglobulin levels

Specific serum IgE levels to *P. acerifolia* extract and purified allergens were evaluated by enzyme allergosorbent test (EAST). *P. acerifolia* extract (400 µg/mL) and purified proteins (50 µg/mL) were coupled to CNBr-activated paper discs [27]. Sera were added to the discs and incubated at 37 °C for 1 h. After washing, the discs were incubated 30 min at 37 °C with 50 µL of alkaline phosphatase-labelled mouse anti-human IgE and developed following the instructions of Hytec IgE EIA (Hycor Biomedical, Kassel, Germany).

Inhibition assays

Enzyme linked immunosorbent assay (ELISA) inhibition assays were carried out in microtitre plates (Greiner, Frickenhausen, Germany) that were coated with 100 µL of plane pollen extract at 12 µg/mL in 0.1 M bicarbonate buffer, pH 9.6, and blocked with 1% BSA, 0.05% (v/v) Tween 20 in PBS. 100 µL of a pool of sera from 12 monosensitized plane pollen-allergic patients (diluted 1:4) was pre-incubated overnight at 4 °C with tenfold serial dilutions of *P. acerifolia* pollen extract and equimolar ratio of Pla a 1 and Pla a 2 from 10 000 to 1 ng/mL. Pre-incubated sera were added to the plates, incubated for 1 h at 37 °C and developed as previously described [28].

Skin tests with *Platanus. acerifolia* extract and purified allergens

SPT was performed with a commercial *P. acerifolia* extract (Bial-Aristegui), nPla a 1, rPla a 1, and nPla a 2. The individual proteins were diluted in NaCl 0.9% at 0.3, 3, 30, and 100 µg/mL. Histamine 10 mg/mL and NaCl 0.9% served as controls. All skin tests were performed in

duplicate on the volar forearm and applied in two opposite directions with a standardized prick needle (Morrow-Brow, Derby, UK). Weals were carefully outlined with a ballpoint pen and transferred to a paper by means of adhesive tape. The weal areas (in mm²) were measured by computerized planimetry (AutoCAD 11, Autodesk Inc., San Rafael, CA, USA). A weal surface area greater than 7 mm² was considered to be positive.

Data analysis

Sensitivity was calculated as the proportion of true positives divided by *P. acerifolia* allergic patients. Specificity was calculated as the proportion of true negatives divided by control subjects. Statistical data analysis was performed by using the Wilcoxon test and the Spearman's ρ correlation included in the statistical software SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA). A *P* value of less than 0.05 was considered significant.

Results

Patients

A total of forty-seven *P. acerifolia*-allergic patients entered the study. The mean age of subject included in the MS group was 33 ± 4 years (range, 6–60 years) and 31 ± 9 years (range, 12–57 years) in the PS group. All patients from the MS group had rhinitis/rhinoconjunctivitis during flowering season of *P. acerifolia* while patients in the PS group had asthma ($n = 3$), rhinitis/rhinoconjunctivitis ($n = 23$), or rhinitis/rhinoconjunctivitis and asthma ($n = 6$). Five patients of this group also had concomitant allergy to plant foods such as peach, kiwi, nuts, and strawberry. The atopic control group consisted of 11 patients who had asthma ($n = 2$), rhinitis/rhinoconjunctivitis ($n = 7$) or rhinitis/rhinoconjunctivitis and asthma ($n = 2$) against aero-allergens of different *P. acerifolia* (mean age: 34 ± 15 years; range, 15–67 years). The non-atopic control group was composed of 13 subjects with a mean age of 43 ± 17 years (range, 9–70 years).

Pla a 1 and Pla a 2 as markers for *Platanus acerifolia* allergy

Allergograms of *P. acerifolia*-allergic patients were performed by immunoblotting (Fig. 1). The higher IgE-reactivities were found at 18 and 43 bands, which correspond to Pla a 1 and Pla a 2 allergens with prevalences of 75% and 50%, respectively. Profilin and an IgE-reactive band of 12 kDa were also detected with observed prevalences of 22.7% and 43.2%, respectively. An IgE dot blot was performed with nPla a 1, nPla a 2, and plane profilin using sera from *P. acerifolia* allergic patients. These experiments showed that 72.7% sera reacted with Pla a 1 and with Pla a. 2% and 15.9% with plane profilin. Therefore, we focused on Pla a 1 and Pla a 2 as markers for *P. acerifolia* allergy by skin test and serum-specific IgE determinations. The proteins used in this study were checked by SDS-PAGE and immunoblot against a pool of sera from *P. acerifolia* allergic patients (Fig. 2a). Recombinant proteins were produced in *E. coli* as inclusion bodies and a solubilization/refolding step was necessary in order to obtain soluble proteins. rPla a 2 tended to aggregate as demonstrated by gel permeation chromatography (data not shown).

ELISA inhibition experiments corroborated that Pla a 1 and Pla a 2 are important allergens as both allergens together were able to inhibit 79% of the IgE-binding capacity of a pool of sera from *P. acerifolia*-allergic patients (Fig. 2b).

Platanus extract-specific immunoglobulin levels correlated with nPla a 1 and nPla a 2 reactivity

A high correlation was found between the IgE response of nPla a 1 or nPla a 2 *vs.* *P. acerifolia* extract with a correlation coefficient of 0.80 and 0.79, respectively ($P < 0.001$; Fig. 3). The median IgE response to *P. acerifolia* extract was 18.1 U/mL, with a lower and upper 95% confidence limits of 5.8 and 51.2, respectively. For nPla a 1 and nPla a 2, the medians were 6.7 U/mL (1.3–27.9) and 4.6 U/mL (0.9–15.7), respectively. Slightly better

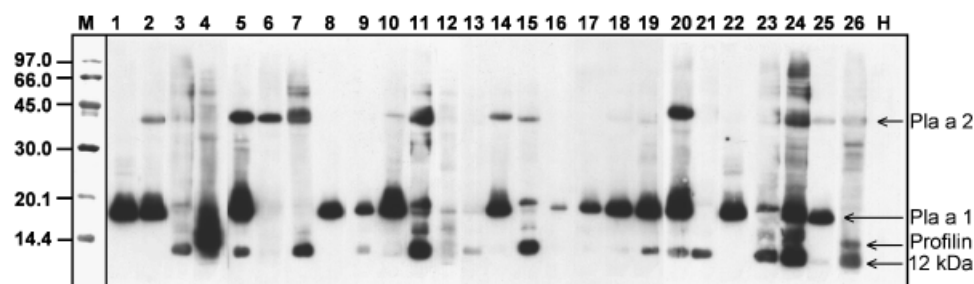


Fig. 1. Representative immunoblot of sera from plane-allergic patients with *Platanus acerifolia* pollen allergens separated by SDS-PAGE. Proteins transferred onto polyvinylidene difluoride membranes were probed with sera from *P. acerifolia*-allergic patients (lanes 1–26) and serum pool from healthy individuals (lane H). Molecular mass markers were stained with Amido Black B (lane M).

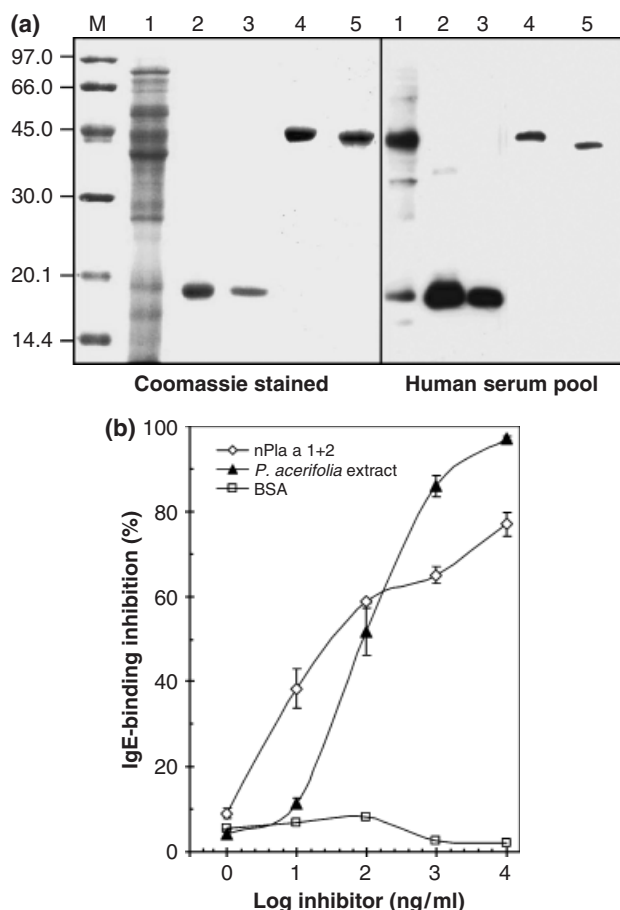


Fig. 2. (a) Coomassie-stained-SDS-PAGE and immunoblot of *Platanus acerifolia* pollen extract (lanes 1) and purified allergens: nPla a 1 (lanes 2), rPla a 1 (lanes 3), nPla a 2 (lanes 4), and rPla a 2 (lanes 5). Extract (20 µg) and purified allergens (2 µg for Coomassie staining and 0.2 µg for immunoblotting) were loaded in SDS-PAGE. Immunoblots were incubated with a serum pool of monosensitized *P. acerifolia*-allergic patients (diluted 1 : 3). (b) EAST-inhibition curves. The binding of human IgE to *P. acerifolia* extract was inhibited using *P. acerifolia* extract, nPla a 1+nPla a 2, and BSA. Values represent mean±SD.

correlations to *P. acerifolia* extract were found among MS patients (0.95 and 0.88 for nPla a 1 and nPla a 2, respectively) than among PS patients (0.76 and 0.78 for nPla a 1 and nPla a 2, respectively). With most of the sera, the values of IgE-reactivity tend to be higher in PS patients.

Regarding the use of purified allergens as an *in vitro* diagnostic for *P. acerifolia* sensitization, and keeping a threshold of 0.6 U/mL (O.D. at 405 nm = 0.060), the sensitivity for nPla a 1 was 84.6% for MS patients and 77.4% for PS patients and 92.3% (MS) and 90.3% (PS) for nPla a 2. These results indicated that, for at least one of the two allergens, the sensitivity was 92.3% with MS and 96.8% with PS *Platanus*-sensitized patients. A sensitivity of 95.5% would be expected when a mixture of nPla a 1 and nPla a 2 will be used for *in vitro* diagnostic for *P. acerifolia* sensitization. The rate of false-positive cases for the extract

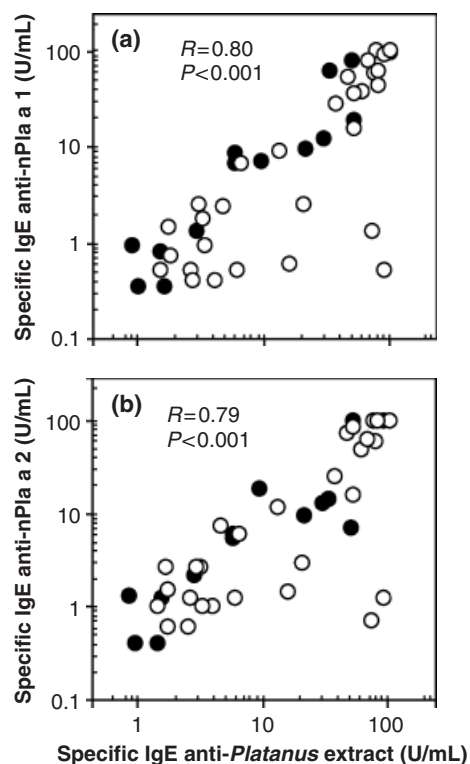


Fig. 3. Relation between specific IgE to *P. acerifolia* extract and nPla a 1 (a) and nPla a 2 (b) in individual patients. Open circles represent sera from PS patients and closed circles represent sera from MS patients.

was 16.7%, and 0% and 20.8%, for Pla a 1 and Pla a 2 respectively. Both allergens together would give a 20.8% false-positive result, with four cases among the atopic control group and one into the healthy group (Table 1).

Component resolved *in vivo* diagnosis using natural Pla a 1 and Pla a 2 allergens

Weal areas of different concentrations of the purified allergens and extract are shown in Fig. 4. The median of the SPT response to *P. acerifolia* extract was 22.2 mm² (18.8–34.2). The median of the SPT response to nPla a 1 at 30 µg/mL was 35.3 mm² (21.6–54.4) and 22.5 mm² (13.4–26.2) to nPla a 2, at 100 µg/mL. No significative differences ($P=0.589$) were found between the weal responses to *P. acerifolia* extract and nPla a 2 at 100 µg/mL, while the weal response of nPla a 1 at 30 µg/mL was higher than that obtained for the whole extract ($P=0.015$). In general, values for skin test tend to be higher among the MS patients with no adverse side effects in all the tested subjects.

The median of the allergen concentrations that induced a weal surface area of the same size as that of the histamine were 3.0 µg/mL for nPla a 1 and 52.3 µg/mL for nPla a 2. The concentration of *P. acerifolia* extract that produces the same effect contains 62.2 µg protein/mL.

The specificity and sensitivity results of skin test and specific IgE determinations are shown in Table 1. In SPT,

Table 1. Sensitivity and specificity of *P. acerifolia* extract and purified allergens by using skin prick test and specific IgE determination

	Group	<i>P. acerifolia</i> extract*		nPla a 1		nPla a 2		nPla a 1 + nPla a 2		rPla a 1		rPla a 1 + nPla a 2	
		SENS [†]	SPEC [‡]	SENS [†]	SPEC [‡]	SENS [†]	SPEC [‡]	SENS [†]	SPEC [‡]	SENS [†]	SPEC [‡]	SENS [†]	SPEC [‡]
SPT [§]	MS	100%		86.7%		86.7%		100%		73.3%		100%	
	PS	100%	100%	84.4%	100%	68.8%	100%	87.5%	100%	65.6%	100%	75.0%	100%
	MS + PS [¶]	100%		85.1%		74.5%		91.5%		68.1%		81.8%	
Specific IgE	MS	100%		84.6%		92.3%		92.3%		76.9%		92.3%	
	PS	100%	83.3%	77.4%	100%	90.3%	79.2%	96.8%	79.2%	67.7%	92.3%	93.5%	79.1%
	MS + PS [¶]	100%		79.5%		90.9%		95.5%		70.5%		93.2%	

*Commercial *P. acerifolia* extract preparation (311 µg protein/mL; Bial-Aristegui)

[†]Sensitivity ($n = 47$ allergic patients for SPT; $n = 44$ allergic patients for specific IgE)

[‡]Specificity ($n = 24$ control patients)

[§]SPT performed with 30 µg/mL of nPla a 1 and 100 µg/mL of nPla a 2 and rPla a 1

[¶]MS+PS: all *P. acerifolia* allergic patients

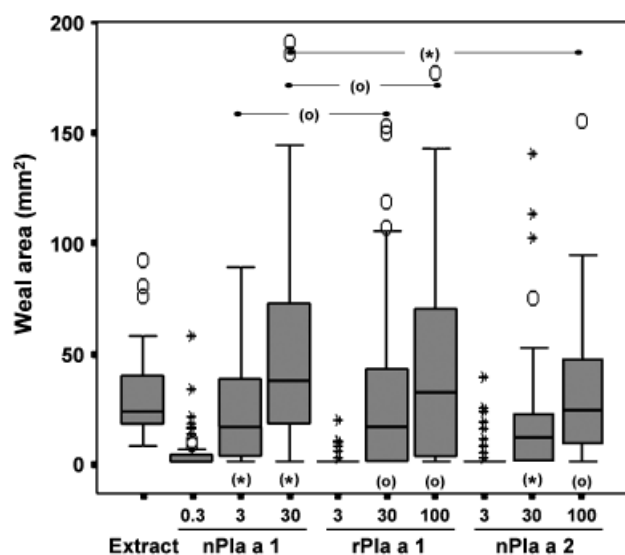


Fig. 4. Skin prick test with *Platanus acerifolia* extract (311 µg protein/mL) and three different concentrations (µg/mL) of purified nPla a 1, rPla a 1, and rPla a 2. Values are given as the average of weal surface areas. Symbols under the box graphs represent statistics using the Wilcoxon's test against *P. acerifolia* extract: (o) no statistical significance, (*) $P < 0.05$.

false-positive cases were detected with purified allergens, confirming the high specificity (100%) of the test. For nPla a 1, a sensitivity of 86.7% in the MS group and 84.4% in the PS patients were found at a concentration of 30 µg/mL, while nPla a 2 at 100 µg/mL showed 86.7% and 68.8% sensitivity for MS and PS patients, respectively. Taking the results together, the sensitivity for the mixture, Pla a 1 + Pla a 2 would be 100% and 87.5% for MS and PS patients, respectively, with no false-positive case. This mixture could diagnose *P. acerifolia*-sensitized patients with a sensitivity of 91.5% and a positive predictive value of 100%.

A significant correlation between SPT and allergen-specific IgE levels for nPla a 1, rPla a 1, and nPla a 2 was

found with the highest correlation coefficients at 30 µg/mL ($R = 0.62, 0.72$, and 0.59 , respectively, and all of them with $P < 0.001$). In contrast, the complete extract showed no significant correlation.

Reactivity of recombinant plane pollen allergens

Specific IgE of rPla a 1 (median 4.55 U/mL with 95% confidence limits of 0.9–15.7) exhibited a high correlation with that of nPla a 1 ($R = 0.89$; $P < 0.001$) and *P. acerifolia* extract ($R = 0.69$; $P < 0.001$). On the other hand, only a weak relationship ($R = 0.48$; $P = 0.001$) was found between rPla a 2 (median 0.9 U/mL; 0.5–2.2) and nPla a 2 (Fig. 5). Using rPla a 1 for *in vitro* diagnostic of *P. acerifolia* sensitization, the sensitivity was 70.5% (79.5% for nPla a 1) and the rate of false-positive cases was 7.7% (Table 1). rPla a 2 sensitivity was 56.8% (90.9% for nPla a 2), with a false-positive rate of 19.2%.

Skin tests using rPla a 1 at 100 µg/mL (median 30.1 mm²; 15.1–46.2) have 68.1% sensitivity with 100% specificity. The median of rPla a 1 that induced a weal surface area of the same size as that of the histamine was 20.7 µg/mL. There were significant differences between rPla a 1 at 100 µg/mL and nPla a 1 at 30 µg/mL ($P = 0.104$), while rPla a 2 was not assayed in the skin test.

Discussion

Purified allergens offer the possibility of component-resolved diagnosis by means of classical *in vivo* and *in vitro* diagnosis [29, 30], and more recently using microarrayed allergens on glass chips for monitoring allergic patients' IgE reactivity profiles in a single measurement [30, 32]. Pla a 1 and Pla a 2 seem to be the best indicators for specific *P. acerifolia* sensitization as both are present in the 92% and 83% of the monosensitized patients [21, 23] and together are responsible for 79% of the total IgE binding activity. *P. acerifolia* LTP, Pla a 3, has recently

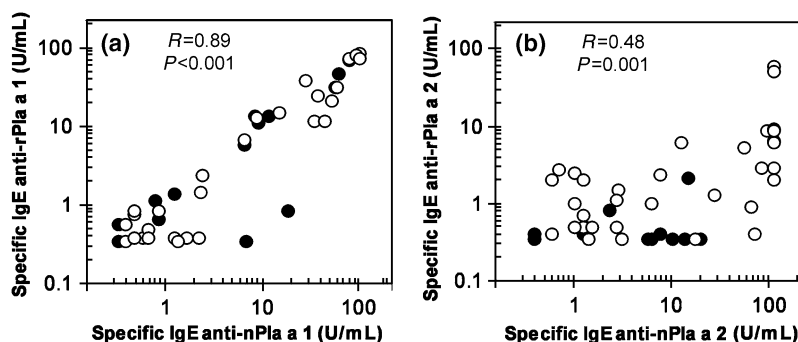


Fig. 5. Relation between specific IgE to natural and recombinant proteins of Pla a 1 and Pla a 2 in individual patients. Open circles represent sera from PS patients and closed circles represent sera from MS patients.

been identified as major allergen in plane tree pollen-allergic patients recruited from the Mediterranean area but a minor one among German patients [33]. Owing to the unavailability of Pla a 3, indirect measurement of IgE reactivity to peach LTP, by dot-blot and EAST, showed that only 11.4% of our patients have IgE against this protein (data not shown). Nevertheless, this result should be considered with caution as no evidence of immunological relationship between peach and plane LTP has been demonstrated. Then, reliable *in vivo* and *in vitro* diagnosis of *P. acerifolia* allergy could be possible using purified Pla a 1 and Pla a 2 allergens.

No adverse reaction was observed at any concentration in patients or control subjects, demonstrating the innocuousness of using purified allergens for *in vivo* testing. Most of the *P. acerifolia* allergic patients, especially monosensitized (sensitivity of 100%), could be detected by skin testing with 30 µg/mL of nPla a 1 and 100 µg/mL of Pla a 2. This study shows that the diagnosis of *P. acerifolia* allergy could be simplified by using two allergens, Pla a 1 and nPla a 2, as a reliable diagnosis of the allergy condition with a sensitivity of >90% and a specificity of 100% has been obtained. The four patients that could not be detected with the mix of Pla a 1+Pla a 2 exhibited, by immunoblotting, IgE reactivity to a 12-kDa allergen, but they showed no reactivity to peach and hazelnut LTP (data not shown). This allergen, which is currently being characterized in our laboratory, had a prevalence of 31% and 48.4% among MS and PS patients, respectively, and therefore, the inclusion of this minor allergen in SPT could detect 100% of the *Platanus*-sensitized patients of our cohort. Although none of our patients showed exclusive reactivity to plane profilin, this panallergen could also be included as it is considered a diagnostic marker for polysensitization [34]. In EAST, the sensitivity for the mix Pla a 1+Pla a 2 was 92.3% (MS) and 96.8% (PS), with a percentage of false-positive (20.8%) comparable with that for the extract (16.7%). All false-positive cases appeared in the atopic group, suggesting the implication of cross-reactive molecules present in

other allergenic sources [13, 16, 17, 35]. Although Pla a 1 and Pla a 2 prevalences are always higher in the MS group, we could not detect any statistical significant correlation between the skin reaction or the IgE level to any of the two allergens and the mono- or polysensitized condition.

Although, more than 400 allergens have been cloned and produced as recombinants, not more than 40 have been validated by skin testing in allergic individuals. Recombinant allergens should be produced in different expression systems (bacteria, yeast, plants, and insect cells) depending on the specific characteristics of each particular allergen. Recombinant Alt a 1, expressed in yeast or bacteria, showed similar performance for *in vivo* and *in vitro* diagnosis in *Alternaria alternata* allergy [6]. Some other recombinant allergens, such as Ole e 1, Cyn d 1, and Der p 1, can only bind IgE depending on the expression system used due to lack of proper folding or glycosylation of bacteria-expressed recombinant proteins [36]. This could also be the case for rPla a 2, a glycoprotein with eight disulphide-bridges [23], that showed low sensitivity and weak correlation (Table 1 and Fig. 3a). On the other hand, rPla a 1 is a smaller and non-glycosylated protein [22] and seems to have the same IgE-binding characteristics as its natural counterpart as suggested by the good correlation shown between natural and recombinant allergen, although the slightly reduced IgE-reactivity of rPla a 1 in several patients (Fig. 5a) could indicate altered protein folding. rPla a 1 could be a good candidate to be included in a component resolved diagnosis panel for detection of *P. acerifolia* allergy. The use of eukaryotic-expression systems is underway in our laboratory in order to enhance correct disulphide-bridge formation and to allow glycosylation to improve the diagnostic performance of recombinant Pla a 1 and Pla a 2.

In summary, this study has demonstrated that two allergens, Pla a 1 and Pla a 2, induce skin prick reactivity comparable with that produced by the *P. acerifolia* extract and are sufficient for a reliable diagnosis of *P. acerifolia* in most patients. A precise patient-tailored diagnosis offered

by purified allergens is required for a specific immunotherapy and may now be available for many allergic sources. Pla a 1 and Pla a 2 could detect all monosensitized patients allergic to plane pollen and therefore future specific immunotherapy based on these allergens could be considered. However, clinical studies using GMP-produced allergens will be required to document therapeutic efficacy of purified allergens compared with current immunotherapy using raw extracts.

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