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Monosensitization to a novel plane pollen allergen

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KEY WORDS

Pollen allergy, allergic rhinitis, Plane pollen, Platanus

SUMMARY

A patient monosensitized to plane pollen is described. On ISAC 103 the patients showed IgE reactivity to both Pla a 1 and Pla a 2, but on immunoblot analysis the serum showed a single IgE reactivity at about 50 kDa allergen. .

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Introduction

Platanus acerifolia or *hybrida*, a hybrid between *Platanus occidentalis* (American sycamore) and *Platanus orientalis* (London plane tree), is frequently found in western Europe, particularly in cities and towns where it is used for ornamental purposes. Plane trees are wind pollinated and produce huge amounts of pollen between mid March and the end of May. Nonetheless, their relevance as a cause of seasonal allergy is still uncertain, and some relevant position papers and review articles of the main pollen sources in Europe do not mention plane pollen at all (1,2). On the other hand, studies carried out in Spain reported plane pollen being a relevant allergen source (3-5), suggested the possible cross-reactivity between plane pollen and plant-derived foods (6,7), and also purified and characterized the major plane pollen allergen, named Pla a 1, as a non-glycosylated 18 kDa protein (8).

Little is known about the relevance of plane pollen in Italy. This allergy center has been performing SPT with plane pollen extract on a regular basis in all subjects presenting for suspect seasonal respiratory allergy for more than 5 years (corresponding to about 3000 individuals finally diagnosed as having pollen allergy). Skin reactivity to plane was often observed, but always associated with hypersensitivity to Fagales (birch, hazel) pollen or to the plant pan-allergens, profilin and polcalcin. The lack of recombinant plane allergens for routine diagnostic purposes, along with the overlap between pollen seasons of plane and other co-sensitizing trees made it impossible to assess the real clinical relevance of the observed plane hypersensitivities. Recently, a patient monosensitized to plane pollen was eventually found and is reported here.

Case report

A 40 years-old man reported rhino-conjunctivitis from mid-April to the end of May for 7 years. Skin prick tests carried out with commercial pollen extracts (Allergopharma, Reinbeck, Germany) showed a clear cut hypersensitivity to plane pollen; SPT with extracts of grass, pellitory, plantain, mugwort, ragweed, birch, hazel, olive, and cypress scored negative.

Methods

An immunoblot analysis was carried out. Plane pollen was homogenized and extracted (5%) in 0.1M phosphate-buffered saline, pH 7.4 (PBS) shaking over-night at 4 °C. After centrifuging the supernatant was harvested and dialyzed against the same buffer. The protein content, measured after Bradford (9), was 1.2 mg/ml. Electrophoresis of plane pollen extract (25 µg/lane) was carried out in a 10% polyacrilamide precast Nupage Bis-Tris gel according to manufacturer's instructions (Invitrogen, Milan, Italy) at 180 mA for 1 h. The resolved proteins were transferred onto a nitrocellulose membrane (Protran BA 85, Schleicher and Schuell, Milan, Italy) according to Towbin (10). The membrane was saturated in TBS buffer containing 5% defatted dry milk, before incubation with patients' sera undiluted or diluted 1:2 in saturating buffer. Bound specific-IgE were detected by peroxidase-conjugated anti-human IgE (diluted 1:1400, Biospecific, Emeryville, CA, USA), using an ECL western blotting kit (Amersham, Milan, Italy) as substrate.

Finally, patient's serum IgE specific for a 103 allergen molecules were measured by a commercial allergen microarray immunoassay (ISAC; Phadia, Uppsala, Sweden) following manufacturer's recommendations. Reaction sites were incubated with 20 µL of patient's serum for 2 hours. After rinsing, washing, and drying, allergen-specific IgE complexes were stained with a fluorescence-labelled anti-human IgE for 1 h. After further washings, a laser scanner took fluorescence readings and results were transformed into numerical data by comparison with a reference serum standardized against ImmunoCAP IgE.

Results

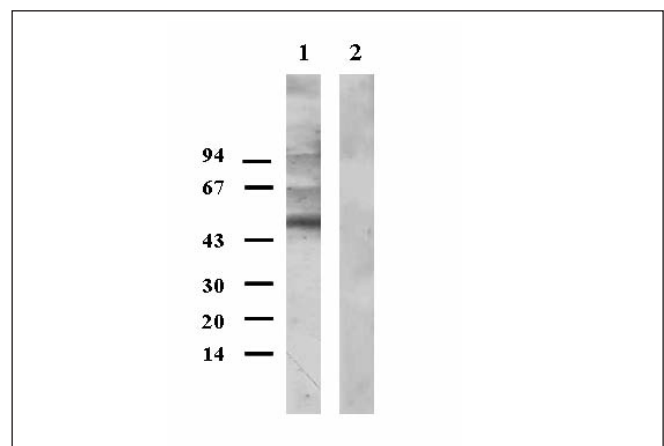
On immunoblot the patient's serum showed a marked IgE reactivity at about 50 kDa (figure 1). On ISAC 103

immunoassay, patients serum reacted to Pla a 1 (3.5 ISU) and Pla a 2 (0,8 ISU). All the remaining 101 allergen molecules scored negative.

Discussion

Plane pollen allergens identified so far include Pla a 1 (18 kDa)(8), Pla a 2 (43 kDa)(11), Pla a 3 (a lipid transfer protein; 10 kDa), and Pla a 8 (profilin; 14 kDa). Both Pla a 1 and Pla a 2 are reportedly major allergens, and recently Asturias and co-workers (12) found that a combination of Pla a 1 and Pla a 2 can be reliably used to diagnose plane pollen allergy instead of the whole pollen extract. Although the patient described in this article showed IgE reactivity to both Pla a 1 and Pla a 2, on immunoblot his serum reacted to a protein showing a molecular mass > 43 kDa. In a previous study Asturias et al (8) detected IgE reactivity at a similar m.w. in some polysensitized patients, whereas no patient monosensitive to plane pollen seemed to react against this allergen. Whether this is a novel plane pollen allergen or a different form of Pla a 2 remains to be established. This is the first study reporting monosensitization to plane pollen in Italy and shows that, although such condition is an extremely rare event in this geographic area, it may be worth testing patients routinely with plane pollen extract. Cross-inhibition studies will be needed to ascertain whether the more frequently observed plane hypersensitivities are genuine or the result of cross-reactivity with other pollen allergens.

Figure 1 - Immunoblot analysis with plane pollen extract. Left lane: patient's serum shows IgE reactivity to a 50 kDa protein. Right lane: a normal control serum does not show any IgE reactivity



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