

Short communication

Detection of allergens in plantain (*Plantago lanceolata*) pollen

Background: Allergens in *Plantago lanceolata* have not been characterized yet. The objective was to characterize some plantain-pollen allergens and to investigate the cross-reactivity between plantain and grass pollens.

Methods: Sera from four patients monosensitive to plantain pollen and from eight grass-pollen-allergic patients showing strong skin reactivity to plantain pollen in the skin prick test (SPT) underwent immunoblot analysis with both *Plantago* and grass mix extract. Moreover, immunoblot inhibition experiments were done with grass mix extract as inhibitor.

Results: All four sera from plantain-allergic patients reacted to two distinct bands at 17 and 19 kDa, and 2/4 sera showed further reactivity to a 40-kDa protein, which in one case represented the most prominent IgE-binding allergen.

Plantain-monosensitive subjects did not show any reactivity to grass-pollen extract, and preabsorption of their sera with grass-pollen extract did not cause any loss of reactivity to plantain pollen. Sera from all eight grass-pollen-allergic controls reacted to a 30-kDa protein in plantain pollen, and some sera showed cross-reactivity to higher and lower molecular-weight structures as well. In all cases, plantain reactivity was totally abolished by preabsorption of sera with grass-pollen extract. A preliminary investigation by immunoblot showed that polyclonal IgG anti-Phl p 5 (but not polyclonal Phl p 1) from rabbit reacted to a 30-kDa protein in plantain pollen.

Conclusions: Three specific allergens (of 17, 19, and 40 kDa, respectively) have been detected in plantain pollen. Further studies on a larger number of patients will determine whether these proteins may be considered major allergens. Cross-reactivity between grass and plantain pollen is mainly caused by a 30-kDa protein in plantain pollen. Group 5 grass-pollen allergen is probably responsible for most grass/plantain cross-reactivity.

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Plantain (*Plantago lanceolata*) pollen is generally considered a minor cause of airborne allergy in Europe. A study by Spieksma et al. concluded that plantain may contribute to pollinosis but only under exceptional conditions (1), and a recent position paper by the EAACI did not include plantain among the relevant causes of pollinosis in Europe (2). Most patients positive to the skin prick test (SPT) with plantain-pollen extracts are hypersensitive to grass (3, 4), a fact which suggests that at least one allergen in grass and plantain pollens cross-reacts. In a series of 242 consecutive grass-pollen-allergic patients seen at one of the outpatient allergy clinics of this area during the last 3 years, 71 (29%) were positive in the SPT with plantain-pollen extract. Few studies have tried to identify the allergens in *Plantago* pollen. In 1980, Baldo et al. (5) detected at least six IgE-binding allergens in the molecular weight range of 10–300 kDa. More recently, Dreborg et al. (6) found at least 13 allergens by immunoblotting with one component of 15 kDa with a high degree of specific IgE binding. The present study

aimed to detect the allergenic proteins in plantain pollen and to determine more clearly the cross-reactivity between grass and *Plantago* pollen.

Material and methods

Patients

The study was carried out on 12 patients (M/F 5/7; mean age 29.8 years, range 19–56 years) with pollinosis. Four subjects reported rhinoconjunctivitis, in one case associated with asthma, from the end of June to the beginning of August and showed a strong reactivity to plantain pollen in the absence of grass-pollen reactivity in the SPT. The remaining eight subjects (controls) reported seasonal respiratory symptoms from the end of April to the beginning of July, and showed a strong skin reactivity to both grass and plantain pollen.

Skin tests

All patients underwent SPT with commercial extracts of

grass (ryegrass, timothy, and Bermuda grass), plantain, mugwort, ragweed, pellitory, and birch pollen (50 000 BU/ml; Allergopharma, Reinbek, Germany), standard 1-mm-tip prick lancetters being used (Bayer). Readings were taken at 15 min, and results were evaluated by comparison with the wheal induced by histamine 10 mg/ml (7). All the patients included in this study showed a strong (4+) reactivity to grass and/or plantain pollen, and did not show any skin reactivity to other pollen grains tested.

Pollen extracts

After microscopic examination to check purity and to exclude contaminations, plantain pollen and grass pollen mix (Allergon, Engelholm, Sweden) were defatted with ether before the extraction at 10% w/v

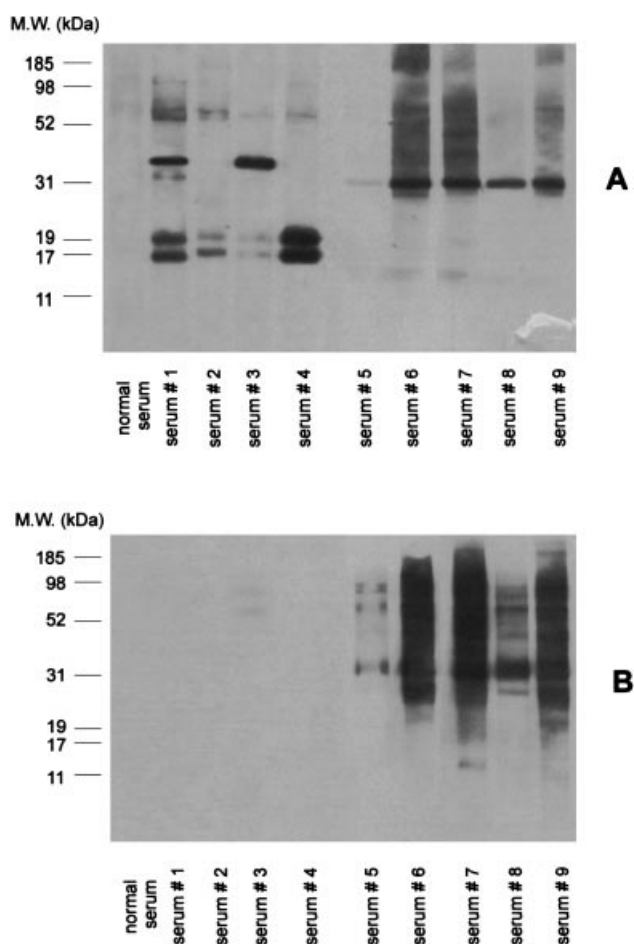


Figure 1. A) Immunoblot with plantain-pollen extract. All sera from plantain-monosensitive patients (nos. 1–4) reacted to two proteins at 17 and 19 kDa, respectively. Two of them (nos. 1 and 3) strongly reacted to 40-kDa protein as well. All sera from grass-pollen-allergic patients (nos. 5–9) reacted to 30-kDa protein in plantain pollen. Normal serum did not show any reactivity. B) Immunoblot with grass-pollen extract. Sera from plantain-monosensitive patients (nos. 1–4) did not show any reactivity to grass pollen. Sera from grass-pollen-allergic patients (nos. 5–9) reacted to several allergens. Normal serum did not show any reactivity.

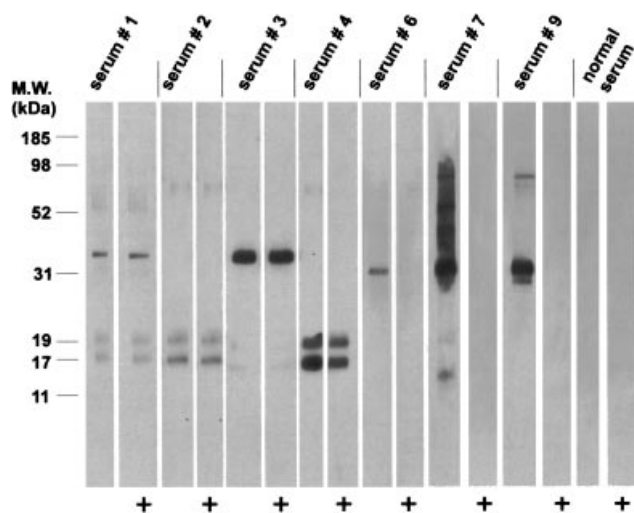


Figure 2. Detection of plantain reactivity before and after (+) absorption of sera with grass-pollen extract. Sera from plantain-monosensitive patients (nos. 1–4) did not show any loss of reactivity to plantain pollen, whereas sera from grass-pollen-allergic subjects (nos. 6, 7, 9) showed total loss of plantain reactivity.

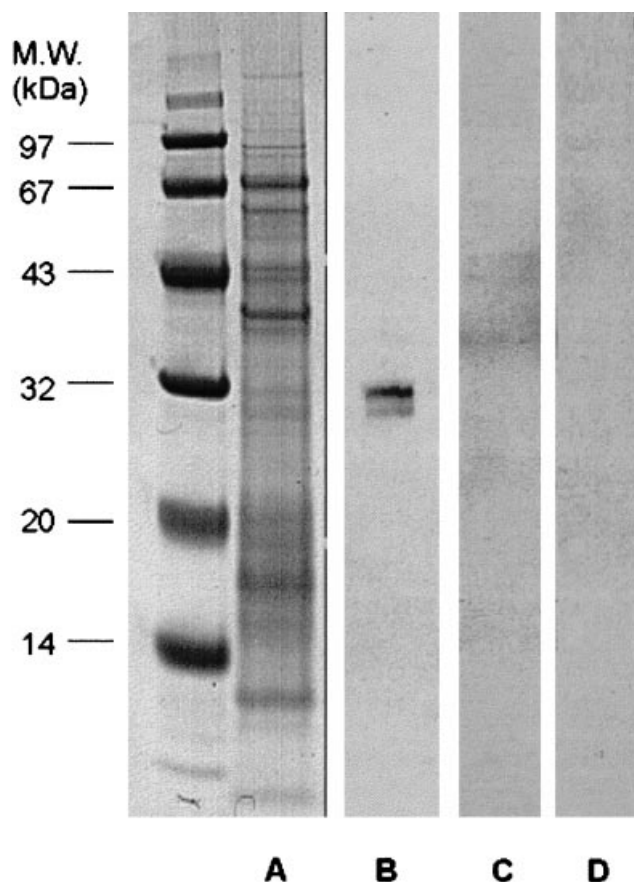


Figure 3. Detection of grass/plantain cross-reacting allergen by immunoblot with plantain-pollen extract. *P. lanceolata* pollen SDS-PAGE profile is shown in lane A. Immunoreactivity of polyclonal IgG anti-Phl p 5, polyclonal IgG anti-Phl p 1, and normal rabbit serum is shown in lanes B, C, and D, respectively.

in saline containing 0.01% thimerosal for 16 h at 4°C. Debris and insoluble materials were removed by centrifugation at 10 000 rpm for 20 min, and supernatants were dialyzed against saline and filtered through a 0.22-µm membrane before lyophilization. After dialysis, the protein content of the extracts was determined by the Bio-Rad method (Prodotti Gianni, Milan, Italy) according to Bradford (8), and they were 0.66 and 1.8 mg/ml for plantain and grass pollen, respectively.

SDS-PAGE, immunoblotting, and inhibition studies

Electrophoresis of the samples was carried out in a 10% polyacrylamide precast gel (Nupage Bis-Tris, Novex, Prodotti Gianni, Milan, Italy) at 180 mA for 1 h, and the resolved proteins were transferred onto a nitrocellulose membrane, according to Towbin et al. (9). The membrane was stained with 0.1% Coomassie brilliant blue or 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 was detected by peroxidase-conjugated antihuman IgE serum. Alternatively, after the transfer of *Plantago* proteins as described above, rabbit polyclonal IgG antisera specific for Phl p 1 or Phl p 5 were used. The polyclonal probes, produced by the research department of Lofarma, were affinity purified and both diluted at 1 µg/ml in saturating buffer. A subsequent incubation with peroxidase-conjugated antirabbit IgG was carried out. In all cases, an ECL Western blotting kit (Amersham, Milan, Italy) was used as substrate.

In immunoblotting inhibition experiments, the proper dilution of selected sera was preincubated for 2 h with 300 µg of grass mix extract before being tested against plantain pollen extract fixed to nitrocellulose. In control experiments, grass mix extract was replaced by saturating buffer. IgE binding was detected as described above.

Results

Patients

The immunoblot profiles of patients' sera are shown in Fig. 1A (nos. 1–4). Sera from all four plantain-monosensitive patients were positive on immunoblot analysis with plantain extract. All sera reacted to two distinct bands at 17 and 19 kDa, and two sera showed further reactivity to a 40-kDa protein. One serum (no. 3) showed predominant IgE binding to this 40-kDa protein. Patients' sera showed a further weaker reactivity at 53 kDa; however, the specificity of this band was uncertain, since a normal serum showed some weak reactivity in this area as well (Fig. 1A). Sera from plantain-monosensitive subjects did not show any reactivity to grass-pollen extract on immunoblot

(Fig. 1B), and preabsorption with grass-pollen extract did not cause any loss of reactivity to plantain pollen (Fig. 2).

Controls

On immunoblot analysis, sera from all eight controls reacted to both grass-pollen proteins (sera from five subjects are shown in Fig. 1B) and to a 30-kDa protein in plantain pollen (sera from five subjects are shown in Fig. 1A). Sera from some controls showed some reactivity to other structures in plantain pollen at higher and lower molecular weight (Fig. 1A and Fig. 2). In all cases, plantain reactivity was totally abolished by preabsorption of sera with grass-pollen extract (Fig. 2).

Detection of the 30-kDa cross-reacting allergen

To detect the cross-reacting 30-kDa allergen in plantain pollen, the reactivity of polyclonal IgG specific for Phl p 5 and for Phl p 1 (both major, 30-kDa grass-pollen allergens) from rabbit to plantain-pollen extract was assessed by immunoblot, as previously described. Anti-Phl p 1 did not show any reactivity, whereas anti-Phl p 5 reacted to a 30-kDa allergen in plantain pollen (Fig. 3).

Discussion

True plantain monosensitivity is rare; therefore, few patients were included in this study. On immunoblot analysis, IgE from plantain-monosensitive patients detected three distinct proteins at 17, 19, and 40 kDa. Sera from all patients included in this study reacted to the two low-molecular-weight allergens. Reactivity to low-molecular-weight allergens in plantain pollen has already been observed in previous studies (6, 10). In contrast, the 40-kDa seems to represent a novel allergen; 2/4 patients reacted to this allergen. Another plantain-monosensitive patient was very recently seen at the allergy center; his serum also reacted to the allergens of 17, 19, and 40 kDa on immunoblot (not shown).

All eight patients with hypersensitivity to both grass and plantain on SPT reacted to a 30-kDa protein in plantain, and sera from some controls reacted to other structures at higher and lower molecular weights as well; inhibition studies showed that such reactivity was the result of cross-reactivity with grass-pollen allergens. Interestingly, none of these eight patients reported clinical symptoms during the period when genuine plantain allergies suffered from rhinoconjunctivitis (from the end of June to the beginning of August), a fact which suggests that the cross-reactive allergen in plantain might show little or no clinical relevance. Low levels of plantain pollen can be detected from the end of April to the end of August in northern Italy (11); the

symptomatic period reported by true plantain allergics is most probably associated with the peak of *P. lanceolata* pollens in this area.

Two potential culprits for cross-reactivity between grass and plantain were group 1 and group 5 allergens in grass pollen, both showing a molecular weight of about 30 kDa (12). The results of our preliminary investigations suggest that the group 5 grass-pollen allergen is probably responsible for most grass/plantain cross-reactivity. Previous studies showed that up to 90% of grass-allergic patients react to group 5 allergen (13, 14). The fact that the group 5 allergen bears at least two

IgE-reactive epitopes (15) might explain why only some grass-allergic patients show plantain reactivity as well (see the first paragraph).

Further studies on larger populations of true plantain-allergic patients will determine more clearly whether the plantain-pollen allergens detected in this study may be considered major allergens.

Acknowledgment

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