

Short communication

Bahia grass pollen specific IgE is common in seasonal rhinitis patients but has limited cross-reactivity with Ryegrass

Background: Perennial Ryegrass is a major cause of rhinitis in spring and early summer. Bahia grass, *Paspalum notatum*, flowers late into summer and could account for allergic rhinitis at this time. We determined the frequency of serum immunoglobulin (Ig)E reactivity with Bahia grass in Ryegrass pollen allergic patients and investigated IgE cross-reactivity between Bahia and Ryegrass.

Methods: Serum from 33 Ryegrass pollen allergic patients and 12 nonatopic donors were tested for IgE reactivity with Bahia and Ryegrass pollen extracts (PE) by enzyme-linked immunosorbent assay (ELISA), western blotting and inhibition ELISA. Allergen-specific antibodies from a pool of sera from allergic donors were affinity purified and tested for IgE cross-reactivity.

Results: Seventy-eight per cent of the sera had IgE reactivity with Bahia grass, but more weakly than with Ryegrass. Antibodies eluted from the major Ryegrass pollen allergens, Lol p 1 and Lol p 5, showed IgE reactivity with allergens of Ryegrass and Canary but not Bahia or Bermuda grasses. Timothy, Canary and Ryegrass inhibited IgE reactivity with Ryegrass and Bahia grass, whereas Bahia, Johnson and Bermuda grass did not inhibit IgE reactivity with Ryegrass.

Conclusions: The majority of Ryegrass allergic patients also showed serum IgE reactivity with Bahia grass PE. However, Bahia grass and Ryegrass had only limited IgE cross-reactivity indicating that Bahia grass should be considered in diagnosis and treatment of patients with hay fever late in the grass pollen season.

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Perennial Ryegrass is a major source of pollen early in summer in temperate zones and is one of the major clinically important grass pollen allergens (1, 2). It has been suggested that *Paspalum notatum*, Bahia grass, which flowers throughout summer and autumn, could be responsible for triggering allergic rhinitis late in the hay fever season (3). Bahia has been noted as a trigger of seasonal rhinitis but its role relative to other clinically important and abundant grasses needs further investigation (4, 5). Bahia grass is allergenic by nasal challenge but was not cross reactive with Timothy grass pollen extract (PE) (6).

Current commercial grass pollen immunotherapy preparations do not usually contain Bahia grass nor is it commonly included in skin prick testing (SPT) panels for diagnosis of seasonal allergic disease. The degree of immunoglobulin (Ig)E cross-reactivity between Bahia grass and other clinically important allergenic grasses, such as Ryegrass, remains to be determined. Such information would clarify whether current diagnostic and treatment regimens satisfactorily provide for diagnosis and treatment of Bahia grass allergy. In this study we tested the frequency of specific serum IgE reactivity with Bahia grass pollen and assessed the capacity for IgE

cross-reactivity between Bahia grass and the major allergens of Ryegrass pollen.

Methods

Subjects

A panel of 33 patients presenting to the Alfred Hospital Allergy Clinic with seasonal rhinitis, with or without asthma, were all positive by SPT and EAST for IgE reactivity with Ryegrass pollen (7). Nonatopic controls ($n = 12$) were all negative in skin prick tests with a panel of common environmental aeroallergens including Ryegrass pollen. Approval was obtained from the Alfred Hospital Ethics Committee with informed written consent from each patient.

ELISA for IgE reactivity

Soluble protein extracts were prepared in phosphate buffered saline from various grass pollens purchased from Greer Laboratories Ltd (Lenoir, NC, USA) (8). Roasted peanut extract was prepared as described by de Leon et al. (9). Natural Lol p 1 and Lol p 5 fractions were purified by single dimension SDS-PAGE gel extraction (10). Microtiter plate wells were coated with 0.5 µg of grass PE or 50 ng of peanut extract and serum IgE reactivity was determined by standard enzyme-linked immunosorbent assay (ELISA) protocols (9). Inhibition ELISA were performed, in triplicate, by preincubating serum

dilutions with allergen extracts at three concentrations for 90 min prior to addition to coated and blocked microtitre plate wells (9).

Western blotting

Bahia grass PE (20 µg) was electrophoresed on 14% sodium dodecylsulphate polyacrylamide minigels (SDS-PAGE) and transferred to nitrocellulose. Vertical strips were blocked and probed (9). Briefly, serum was diluted 1 in 5 and positive reactions were detected using a two-step procedure with rabbit anti-human IgE (Dako, Glostrup, Denmark) followed by goat anti-rabbit immunoglobulin conjugated to horseradish peroxidase (Promega, Madison, WI).

Affinity enrichment of antibodies against grass pollen allergens

Ryegrass or Bahia grass PE (200 µg) was electrophoresed on 12% SDS-PAGE and transferred to nitrocellulose. The proteins were visualized with Ponceau S (Sigma, Saint Louis, MI) staining and the major allergen bands were cut out and blocked. Vertical strips from the Ryegrass blots were probed with monoclonal antibodies FMC-A1 (anti- Lol p 1), and FMC-A7 (anti- Lol p 5), to confirm the location of the Lol p 1 and Lol p 5 allergens (8, 11, 12). The allergen

bands were incubated with a 1 in 5 dilution of pooled serum from 10 grass pollen allergic donors. Bound antibodies eluted with glycine/HCl pH 2.3 and neutralized with Tris pH 9 (13) were tested for IgE reactivity with a panel of allergen extracts by ELISA and western blotting.

Results

Reactivity of grass pollen allergic sera with Bahia grass

The frequency of IgE reactivity with Bahia grass was 78% but the mean reactivity with Bahia grass [1.31 optical density (OD) units, SD 0.93] was lower than with Ryegrass (2.31 OD units, SD 0.80) (Fig. 1A). None of the 12 nonatopic control sera reacted with Bahia (mean 0.08 OD units, SD 0.10) or Ryegrass (mean 0.11 OD units, SD 0.10).

The 12 sera with the highest IgE reactivity by ELISA were used to probe blots of Bahia grass PE to visualize the IgE reactive allergen bands (Fig. 1B). Each of the sera had IgE reactivity with a broad band at 26–28 kDa, albeit

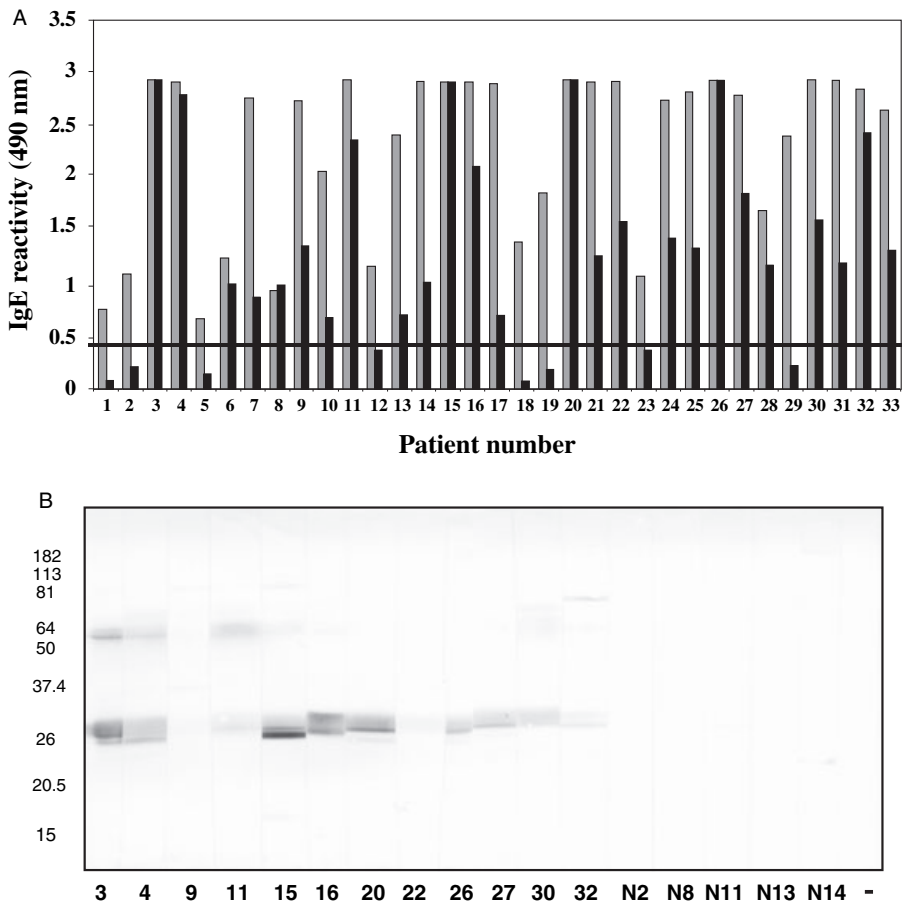


Figure 1. Serum immunoglobulin (IgE) reactivity with Ryegrass and Bahia grass pollen extract. (A) Enzyme-linked immunosorbent assay data for IgE reactivity with Ryegrass (grey bars) and Bahia grass (solid bars) of 33 Ryegrass allergic donors with seasonal rhinitis. The cut off of three standard deviations above the mean reactivity of 12 nonatopic individuals is shown by the horizontal bar (0.40 for Ryegrass and 0.39 for Bahia PE). (B) Western blot data for 12 allergic donors (strips 3–32), five nonatopic donors (N) and a no serum control (–). Molecular weights in kDa are shown on the left.

only weakly in two sera, corresponding with group 1 allergens of other grasses. Eight sera also reacted with bands at 24 and/or 25 kDa, corresponding to group 5 allergens. The IgE reactivity was also detected with bands at 60 and 65 kDa in five of the sera.

IgE cross-reactivity of antibody eluted from allergen bands of Ryegrass and Bahia grass

The pooled serum antibody eluted from natural Lol p 1 and Lol p 5 allergen bands had IgE reactivity with Ryegrass and Canary grass but not Bermuda grass or peanut extracts by ELISA (Fig. 2A). The IgE reactivity of these eluted antibody fractions with Bahia grass was weak (0.3 OD units), equating to 30% of the reactivity of the whole serum pool with Bahia grass, and only 13% of the reactivity of the eluted antibodies with Ryegrass. By western blotting, the antibodies eluted from Lol p 1 and Lol p 5 had IgE reactivity predominantly with group 1 and 5 allergen bands respectively of Ryegrass and Canary grass (Fig. 2B). There was some overlap in the group 1 and 5 reactivity of the eluted antibodies which could be attributed to their close molecular weights. Importantly, there was no IgE cross-reactivity of the anti-Lol p 1 and anti-Lol p 5 antibody eluates with Bahia and Bermuda grass. In the reciprocal experiment, antibodies eluted from the major allergen band of Bahia grass showed no detectable IgE reactivity by western blotting (data not shown). However, by ELISA there was low IgE reactivity of eluted anti-Bahia antibodies with Bahia, Timothy, Bermuda and Johnson grass PE (~0.35 OD units) but no reactivity with peanut extract. Interestingly, the IgE reactivity of these anti-Bahia antibodies with both Ryegrass and Canary grass was 0.60 OD units (data not shown).

Bahia grass does not inhibit IgE reactivity with Ryegrass

Timothy, Canary and Ryegrass PE inhibited IgE reactivity of pooled grass pollen allergic sera with Ryegrass and Bahia grass by ELISA in a concentration dependent manner. Bahia, and to a lesser extent, Johnson and Bermuda grass PE inhibited pooled IgE reactivity with Bahia grass (Fig. 3A), but Bahia, Johnson and Bermuda grass did not inhibit IgE reactivity with Ryegrass (Fig. 3B). The negative control antigen, peanut extract, did not inhibit IgE reactivity of the grass pollen allergic serum pool with either Ryegrass or Bahia grass. These observations were replicated in three separate experiments.

Discussion

Ryegrass pollen is a major seasonal aeroallergen in temperate climates. Our data shows there is also a high frequency of serum IgE reactivity with Bahia grass in

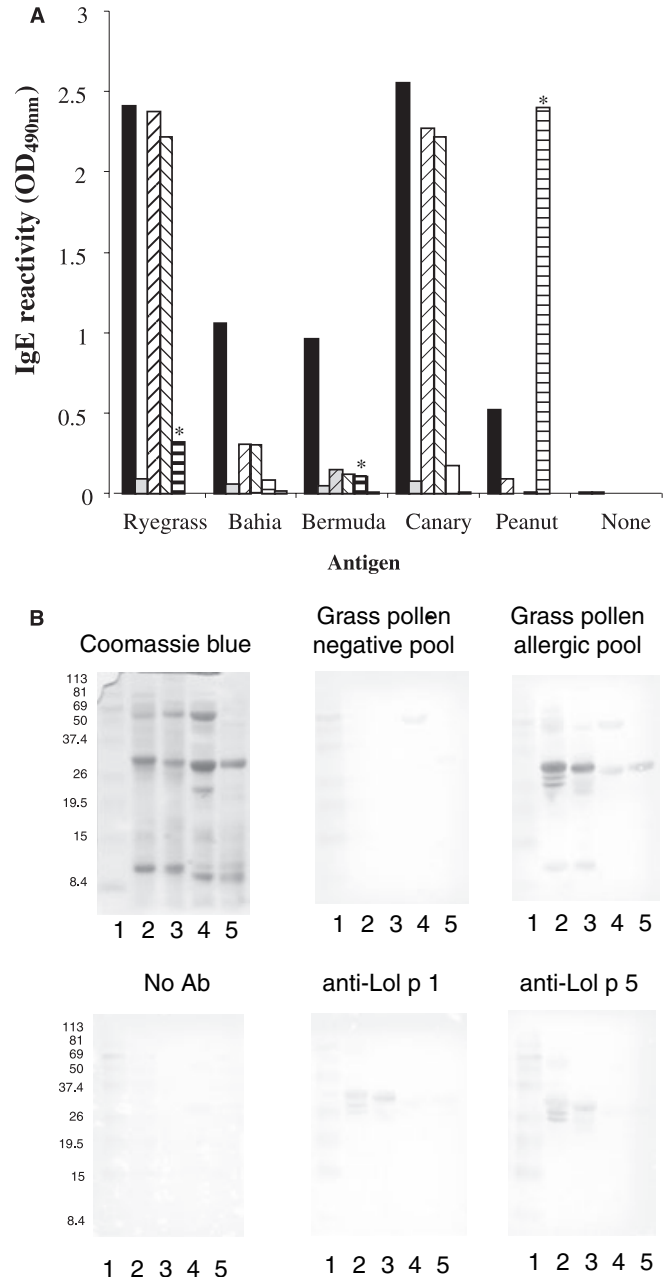


Figure 2. Immunoglobulin (Ig) E reactivity of antibodies eluted from the major allergens of Ryegrass pollen extract (PE). (A) Enzyme-linked immunosorbent assay data for the reactivity of allergic serum pool (solid bars), nonallergic serum pool (grey bars), affinity purified anti-Lol p 1 (forward hatch bars) and anti-Lol p 5 (back hatch bars) antibody preparations, and a peanut and grass allergic serum donor (horizontal hatch bars) and a no primary antibody control (unfilled bars). (B) Coomassie Blue stained SDS-PAGE and western blots of prestained molecular weight markers (1), Ryegrass (2), Canary (3), Bahia (4) and Bermuda (5) grass PE probed serum pools, affinity purified antibody preparations or no primary antibody control (No Ab). Molecular weights in kDa are given on left. *Difference in weight of horizontal hatch bars.

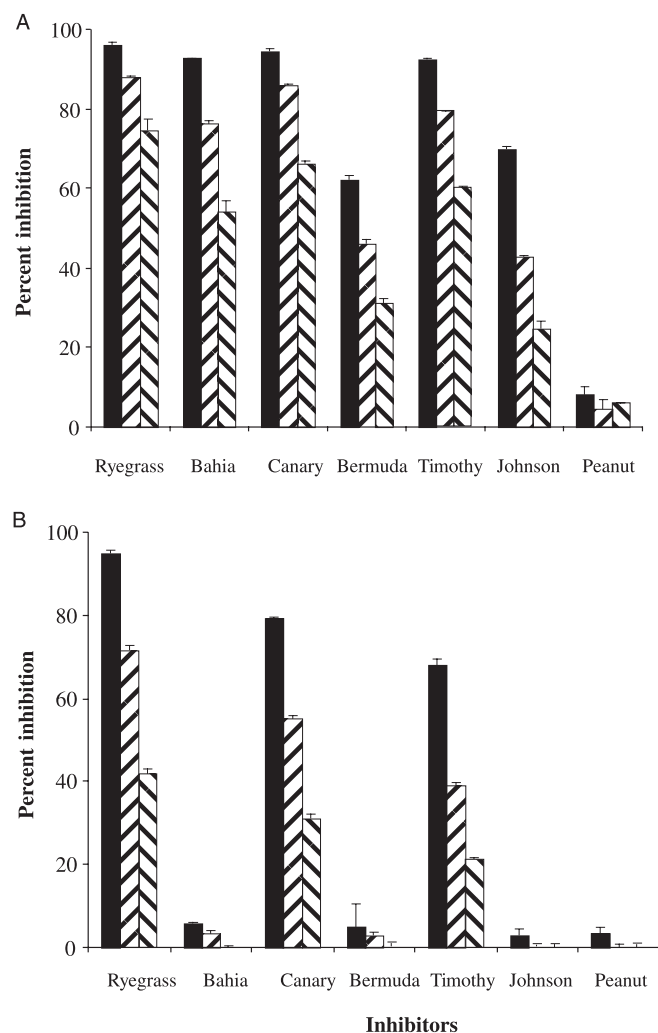


Figure 3. Inhibition of immunoglobulin (IgE) reactivity with Bahia grass pollen extract (PE) (A) and Ryegrass PE (B). Allergen extracts were used as inhibitors at 100 (solid bars), 10 (forward hatch bars) and 1 (back hatch bars) µg/ml. The allergic serum pool was diluted 1 in 20 for (A) and 1 in 50 for (B) to give optical densities (OD) in the linear range of antibody titration curves for IgE reactivity with Bahia and Ryegrass PE. The average OD of IgE reactivity in 24 wells without inhibitor was 1.24 (SD 0.03) with Bahia grass PE and 2.08 (SD 0.06) for Ryegrass PE. SE bars shown.

grass pollen allergic patients with seasonal rhinitis. However there was only limited IgE cross-reactivity between Bahia grass, Ryegrass and other clinically important grasses in contrast to the cross-reactivity evident between Ryegrass, Canary and Timothy grass. The observed pattern of cross-reactivity fits with the

phylogenetic relationship between the grasses and previous studies demonstrating IgE cross-reactivity between Timothy, Canary and Ryegrass but not Bermuda grass (8, 14).

Previous nasal challenge studies showed no *in vivo* cross-reactivity between Bahia and Timothy PE (6) and here we demonstrate only limited serum IgE cross-reactivity between Bahia grass and other grasses including Timothy and Ryegrass. As all the preparations tested here were natural extracts, any glycosylation of the allergens ought to have been preserved. Consequently shared carbohydrate determinants of group 1, 4 and 12 pollen allergens could contribute to the low level of serum IgE cross-reactivity, the clinical relevance of which is debatable (14–17). Despite the low serum IgE cross-reactivity, it cannot be excluded that exposure to Bahia grass pollen could elicit allergic symptoms in some Ryegrass allergic subjects. Indeed there were five of the 33 subjects with similarly high levels of serum IgE reactivity with Ryegrass and Bahia grass (Fig. 1). Moreover, most of the 12 sera tested by western blotting showed IgE reactivity with multiple allergen bands of Bahia grass indicating a broad recognition of Bahia grass, as reported by others (18, 19). As yet none of these Bahia pollen allergens has been cloned or characterized further.

Future studies are required to survey the frequency of *in vivo* sensitivity to Bahia grass pollen by skin prick testing in areas where Bahia grass is present, in particular in patients presenting with symptoms late in summer coinciding with pollination of *Paspalum* species. This approach could identify patients with Bahia grass allergy who may require the inclusion of Bahia grass extracts in immunotherapy reagents.

This is the first study to investigate in detail serum IgE cross-reactivity between Bahia and other grass pollens. We conclude that grass pollen allergic subjects frequently show IgE reactivity with Bahia grass but to a lower level than with Ryegrass. There was only limited serum IgE cross-reactivity between allergens of Ryegrass and Bahia grass. Bahia appears to be largely immunologically distinct from other clinically important allergenic grasses and should be considered in diagnosis and treatment of patients presenting with rhinitis late in the summer.

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