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Pрактическое руководство по обработке текста.
The prevalence of allergic rhinitis and asthma in urban areas is increasing worldwide. Susceptibility in children is associated with atopy, and is characterised by increased immunoglobulin E (IgE) production in response to common allergens. Aero-allergens are prime initiators of an immunological response that culminates in airway inflammation. Grassland and savannah constitute important biomes in southern Africa, and a long summer period, high temperatures and wind are major factors contributing to the production of large amounts of anemophilous grass pollen for most of the year. Grass pollen was reported by David Orman to be the major aero-allergen responsible for upper airway allergic disease in South Africa.

In southern Africa there are 967 species of the grass family Poaceae. Of these, 847 species are indigenous, and 115 are naturalised. The related subfamilies of the Panicoideae and Chloridoideae have a wide distribution in South Africa, comprising 566 species, compared with the Pooidae with 133. Panicoids comprise many of the grasses cultivated for lawns, such as Pennisetum clandestinum (Kikuyu), Cynodon dactylon (Bermuda), and Stenotaphrum secundatum (buffalo); for pastures (Digitaria erianthe); garden ornamentals (e.g. Pennisetum villosum); and for erosion control, Pennisetum and Eragrostis. Lolium perenne (rye grass), of the subfamily Pooidae, has been introduced and is widespread in the Cape Peninsula, where it has adapted to the Mediterranean climate.

Both urban and rural populations are exposed to these pollens for the greater part of the year, although interestingly very little pollen allergy is seen in rural areas. The importance of Bermuda grass in this region was first reported by Orren and Dowdle. A study by Potter et al. showed that 43% of patients had a clinical sensitivity to Kikuyu grass (Pennisetum clandestinum), a Panicoid naturalised from Kenya. In the present study we have examined the allergen profile of the local indigenous grass, buffalo grass (Stenotaphrum secundatum), of the same tribe, namely Paniceae. This is a hardy, drought-resistant grass found down the east coast of Africa, as well as on Mauritius and other islands. It grows extensively over the Cape Peninsula and is commonly used for lawns in this region. The flowering period begins in late October and continues through February. It is believed to play a major role in seasonal rhinitis in late spring and summer. Also included in the study is a related and common grass, Eragrostis curvula, known as the weeping love grass, which is of the Chloridoideae subfamily, tribe Eragrostideae, and is related to Bermuda grass (Cynodon dactylon) (Fig. 1). It has a wide distribution throughout South Africa, readily colonising wasteland and verges. It has been introduced as a pasture grass throughout the tropics and East Africa. It forms large perennial tusfts, and has an exceptionally long flowering period, starting to produce pollen in August, peaking after the rainy season, and continuing through the summer into the winter months of June and July.

Bermuda grass (Cynodon dactylon, subfamily Chloridoideae), an important source of allergens in subtropical climates, has been shown to have limited immunological cross-reactivity with the clinically significant grasses of the Pooidae, and therefore separate diagnostic tests and immunotherapy extracts are required for allergic patients. It is important to establish which diagnostic testing panels are appropriate for the taxonomically related and clinically unrelated indigenous grasses of this region in order to prepare the most appropriate immunotherapy extract. In this communication we report the results of our studies on the prevalence of specific IgE responses to buffalo grass pollen in allergic individuals, and our evaluation of the presence of epitopes that are cross-reactive between this grass and other common indigenous grasses of southern Africa.

**METHODS**

**Sera**

There were four patient groups: (i) group 1 – sera from 100 allergic patients, children and adults confirmed in the laboratory to have positive CAP radio-allergosorbent tests (RASTs) to the Pharmacia grass mix g2 and/or specifically to Bermuda g2, Cynodon dactylon (Bermuda grass); (ii) group 2 – a cohort of 32 adult volunteers from the Cape Town area who had presented with seasonal allergic rhinitis and/or conjunctivitis with confirmed sensitivity to Bermuda grass using CAP RAST, and who had not undergone previous immunotherapy; (iii) group 3 – a cohort of 14 laboratory staff with no clinical history of allergic disease, with negative CAP RAST and skin prick tests to Bermuda grass pollen; and (iv) group 4 – 5 cord blood sera and sera from 8 non-atopic individuals, confirmed by skin prick tests (SPTs) and CAP RASTs, were used as controls for immunoblotting.

![Classification of grass species investigated.](image-url)
RASTs and SPTs

SPTs were performed in groups 2 and 4 using the Bayer of rye, timothy, orchard, red top, Johnson and Bahia pollens.

RASTs for Bermuda, rye and timothy grasses, and gx2 grass mix were performed on the UniCAP system for groups 2 and 4. Grass mix gx2 consists of Bermuda (Cynodon dactylon), rye (Lolium perrenne), timothy (Phleum pratensis), Kentucky blue (Poa pratensis), Johnson (Sorghum halpe num) and Bahia (Paspalum notatum) grasses.

Preparation of grass pollen proteins

Pollens were stored as dry material at -80°C and extracted in a 1:10 (weight/volume) dilution in 100 mM ammonium bicarbonate buffer, pH 8.3, plus 1 mM phenyl methyl sulphonyl fluoride (PMSF), 0.015M sodium azide (NaN₃), by rotation overnight at 4°C, centrifuged at 12,000 g for 30 minutes, and the supernatant recovered. Protein concentrations were determined using a Pierce BCA-protein assay kit with bovine serum albumin (BSA) as the protein standard.

Preparation of in-house antibodies

Polyclonal rabbit anti-buffalo and anti-Eragrostis antibodies were raised by injecting crude pollen extracts in complete Freund's adjuvant, boosting after 4 and 8 weeks, and screening for antibody by enzyme-linked immunosorbent assay (ELISA).

Monoclonal antibodies to buffalo pollen were generated by the method of Kohler and Milstein, using myeloma cells. Fifty μg of pollen extract in complete Freund's adjuvant were injected intraperitoneally into male Balb/c mice, with boosting at 2-week intervals in incomplete Freund's adjuvant, and a final booster after 1 week. The spleen was removed and the fusion performed with SP2 myeloma cells.

Characterisation of natural grass pollen extracts by immunoblotting

Ten μg/lane of each pollen extract were loaded onto 12% weight/volume polyacrylamide gels, in sodium dodecyl sulphate (SDS) sample buffer, under reducing conditions and after boiling for 90 seconds, and run for 3 hours. The separated proteins were electroblotted onto polyvinyl difluoride (PVDF) membrane (Amersham International, Buckinghamshire, England) in a semi-dry system at 200 mA for 1 hour. The membranes were probed with patient serum, and the bound IgE antibodies detected using the enhanced chemiluminescence (ECL) technique.

Two-dimensional polyacrylamide gel electrophoresis (PAGE) was performed by iso-electric focusing in the first dimension, using a pH gradient of 3 - 10. This tube was then run horizontally on a 12% SDS gel, and electroblotted onto Hybond-PVDF membrane (Amersham). Immunoblotting with a pool of positive serum was used to indicate the allergenic isoforms.

ELISA and ELISA inhibition

Pollen extracts were coated onto microtitre plates (Nunc Polysorp, Denmark) overnight. Plates were blocked with 2% weight/volume bovine serum albumin (BSA) in phosphate-buffered saline, 0.1% Tween 20 buffer (PBS-T); 100 μl/well of patient serum, diluted 1:10 in 0.5% BSA blocking buffer, was added for 2 hours at room temperature. Bound IgE antibodies were detected by adding a monoclonal anti-human IgE, followed by alkaline phosphatase-labelled rabbit anti-mouse antibody. The colour reaction was developed by the enzyme substrate p-nitrophenyl phosphate.

Shared epitopes were identified by inhibition of IgE binding. Pre-absorption of serum from a grass-sensitive individual with an appropriately diluted pollen extract, overnight at 4°C before incubation in the natural extract-coated plates or immunoblots, removed cross-reacting IgE antibodies to shared allergens.

Identification of common epitopes

Monoclonal antibodies to Bermuda grass (Cyn d 1) and rye grass (Lol p 1, clones 2 and 14) were kindly supplied by ALK-Abello (Denmark), and were used to investigate the presence of these epitopes in indigenous grasses by ELISA, using plates coated with extracts of buffalo, Kikuyu, Eragrostis and Bermuda pollens. Inhibition of binding by pre-absorption of the monoclonal antibodies with local pollen extracts was used to demonstrate the presence of cross-reactive and unique epitopes.

Basophil histamine release

To confirm the allergenicity of buffalo and Eragrostis pollens in the in vivo situation, fresh blood basophils from confirmed grass-sensitive patients with IgE antibodies and non-allergic, control subjects were subjected to incubation with different concentrations of pollen extracts in pipes/calcium buffer for 60 minutes at 37°C. The histamine released was measured by radio-immunoassay (Pharmacia).

Lymphocyte proliferation of peripheral blood mononuclear cells of grass-sensitive patients and non-atopic controls was performed, using pollen extracts of buffalo and Eragrostis.

RESULTS

Characterisation of pollen allergens

SDS-PAGE and immunoblotting of buffalo pollen extract revealed a spectrum of IgE-binding bands, ranging from 10 to 90 kilodaltons (kD), of which the dominant one was a 34 kD MW band. Other IgE-reactive bands on SDS-PAGE were found at MWs 11, 14, 39, 46, 52, 60 and 68 kD (Fig. 2). Two-
dimensional PAGE was used to resolve the major 34 kD band of buffalo grass into its component isoforms, each with a different iso-electric point. About 9-10 isoforms in the pH range 4 - 7, of the major 34 kD group 1 allergen were revealed by immunoblotting using a pool of positive sera. The basic group V allergen, found in Pooid but not Bermuda pollens, was not present. Isoforms were also found in the 14 kD, 44 kD and 57 kD bands.

Distribution of specific IgE antibodies to buffalo grass pollen

In group 1, immunoscreening on Western blots revealed IgE antibodies to buffalo pollen extracts in > 95% of the patients in this group. Further screening showed that more than 90% had concordant sensitivity to *Eragrostis* pollen extract.

In group 2, IgE sensitivity to buffalo pollen extract was found in all but 1 of the cohort of 32 volunteers with seasonal symptoms, and in 30:32 with sensitivity to *Eragrostis*.

In group 3, 12:14 of this clinically asymptomatic group with negative Bermuda grass SPTs surprisingly showed IgE binding on immunoblots of buffalo and *Eragrostis* pollen extracts. Subsequently 6:14 reacted to the Bayer 5 grass mix SPT extract, with a wheal diameter of 3 - 4 mm, and had a positive gx2 CAP RAST.

Negative controls showed negligible IgE binding.

Cross-reactivity

A high degree of concordance of patient IgE binding between the two Paniceae grasses, Kikuyu and buffalo, and the two Chloridoideae grasses, *Eragrostis* and Bermuda, was evident in ELISA inhibition experiments. All four extracts were able to produce inhibition, in all the combinations, as solid phase or as inhibitor, although greater than 75% inhibition was not achieved. Equivalent IgE inhibition by *Eragrostis* and Bermuda at the lower concentrations confirms the presence of cross-reactive epitopes (Fig. 3), but Bermuda did not inhibit all the epitopes on *Eragrostis*.

We also identified a subset of patients who were Bermuda-negative, but whose IgE bound strongly to *Eragrostis*, buffalo and Kikuyu. Binding to these indigenous grasses was not inhibited by pre-absorption with Bermuda pollen extract, confirming that these members of the Panicoideae have unique IgE-binding epitopes not found on Bermuda.

Inhibition of the highly specific Bermuda CAP RAST by buffalo and *Eragrostis* extracts, using a pool of Bermuda-reactive sera, is shown in Fig. 4.
Attenuation of IgE binding by all four pollen extracts was obtained in immunoblot inhibition experiments, in particular to the major 34 kD allergen. Buffalo was able to demonstrate effective inhibition of binding to all the grasses on immunoblots in most sera. When Eragrostis was used as the inhibitor, abrogation of IgE binding was shown to all the blotted extracts in many grass-sensitive sera. In a subset of sera, however, Bermuda was not able to inhibit the major buffalo allergen, even at a concentration of 100 μg/ml (Fig. 5).

**Identification of epitopes common to buffalo and Bermuda**

Cross-reactivity was further detected by testing the ability of the whole buffalo extract to inhibit the monoclonal Cyn d 1 antibody binding to the local grass extracts. The anti-Cyn d 1 Mab showed some binding to extracts of buffalo and Kikuyu grass (Fig. 6), which was not inhibited by pre-absorption with buffalo extract up to concentrations of 10 μg/ml. Also shown is the binding of the anti-Lol p 1 (clone 2) monoclonal antibody to the four extracts, with reduced values for buffalo and Kikuyu, while the anti-Lol p 1 (clone 14) reacted to Kikuyu and buffalo antigens with greater affinity than to Eragrostis or Bermuda-coated allergen.

**Basophil histamine release**

Basophils of grass-sensitive individuals, when incubated with different concentrations of pollen extract, demonstrated a specific and dose-dependent histamine release to pollen extracts of Buffalo, Kikuyu and Eragrostis grasses (Fig. 7).

Sensitisation to the local grasses was confirmed by the dose-dependent proliferation of the T-cells of grass-allergic individuals when stimulated by pollen extracts of buffalo and Eragrostis, in short-term culture, compared with that of non-allergic controls (Fig. 8).

**Discussion**

Increasing urbanisation has resulted in a concomitant increase in allergic diseases in southern Africa. Quality of life may be severely impaired in allergic rhinitics and asthmatics. The effective diagnosis of the causes of upper airway disease is cost-effective in the context of health care delivery. With this in mind, we have identified major sensitising allergens important for the development of appropriate allergen diagnostic panels and effective immunotherapy in the Cape.

Our study has shown that 95% of grass-sensitive individuals have IgE antibodies to buffalo pollen extracts on immunoblots. These patients have a concordant IgE response to Eragrostis,
Interspecies allergens have been proposed to account for the cross-reactivity between unrelated fruits and vegetables, such as the ubiquitous parallegren profilin, an actin-binding protein, which plays an important role in pollen germination and tube growth, which is recognised by 20% of pollen-sensitive patients. Reactivity to the 14 kD protein in buffalo and Eragrostis extracts has been shown by many of our allergic sera. The presence of interspecific calcium-binding allergens was also demonstrated by inhibition of patient IgE binding in the presence of ethylenediamine-tetraacetic acid (EDTA) to Kikuyu, buffalo, and Eragrostis pollen extracts (data not shown).

The rising levels of pollution, comprising diesel exhaust particles (DEPs), industrial emissions and wood smoke from the burgeoning informal settlements, are reported to enhance both IgE production and cytokines associated with airways inflammation. DEPs also increase the availability of allergens of respiratory size, as pollen grains have been shown to aggregate on these airborne particles, while gaseous pollutants facilitate the release of the allergenic molecules.

This confirms the important role played by buffalo and other indigenous grass pollens in pollinosis in this region. The cross-reactivity demonstrated between all four grasses underlies the importance of the dominant Paniceae family as a pollen sensitizer in southern Africa. Current testing panels and immunotherapy vaccines for this region are deficient in representatives from these important grass pollen families. Diagnostic and therapeutic strategies should take this into consideration. We propose that buffalo, Kikuyu and Eragrostis be included in the SPT panels, and further studies are underway to develop appropriate desensitising vaccines for the region.

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References

OBJECTIVE. To investigate whether there is an association between the length of time lived in an urban area and selected adolescent risk behaviours.

DESIGN. Cross-sectional survey in which students completed an anonymous, confidential questionnaire.

SETTING. Four high schools in black communities in the Cape Peninsula, South Africa.

PARTICIPANTS. A sample of 1,296 students obtained by multistage cluster sampling.

MAIN OUTCOME MEASURES. Selected risk behaviours.

RESULTS. There is a relationship between urbanisation and certain risk behaviours. The following risk behaviours were associated with urbanisation: use in the previous month of alcohol, cannabis, and cannabis mixed with Mandrax; being a victim of violence; perpetration of an act of violence; and solvent sniffing in the previous month were not associated with urbanisation.

CONCLUSION. Urbanisation is associated with an increase in the prevalence rates of some risk behaviours. Mental health promotion efforts may be informed by further research aimed at the identification of: (i) the characteristics of risk behaviour that determine whether it is associated with urbanisation; and (ii) where applicable, the specific aspects of the urbanisation process that contribute to an increase in risk.

Widespread migration from the countryside to the city is a key social characteristic of the developing world. In 1975, about one-quarter of the global population lived in urban areas. This proportion was expected to increase to about 40% by the year 2000, an increase of 60%.

When compared with other age groups, young people are disproportionately likely to have migrated from rural to urban areas. The proportions of migrants aged between 15 and 29 years from rural areas of Punjab, Sudan and Ecuador have been

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