



educating the children and their families about optimal asthma management.

Use of pre-exercise prophylaxis was low in this cohort. Only 4 patients (9%) were using bronchodilators before exercising. None of them was on a long-acting bronchodilator (Serevent), which may eliminate the need for exercise prophylaxis. Of the remaining 39 participants, 8 were on Serevent. While this pattern of pre-exercise bronchodilator use suggests a prevalence of 9 - 28% of exercise-induced asthma in this cohort, this figure is low compared with figures from other studies in which exercise-induced asthma was found to occur in up to 90% of asthmatics.¹⁸ Indeed, on reviewing the records of 69 of the 80 patients enrolled in the study, as many as 52 cases (75%) had reported exercise-induced cough or/and wheezing.

In conclusion, the use of the WW decreased the utilisation of bronchodilators and offered a more rational approach to asthma management. It also provides cost benefits to a health service that is chronically underfunded. Furthermore, the study underlies the need for doctors to improve their communication skills with both asthmatic children and their parents.

We thank Haroon Saloojee, Division of Community Paediatrics, University of the Witwatersrand who provided helpful comments on earlier and final drafts of the manuscript. We also thank Nokuzola Mqoqi, Reproductive Health Research Unit, Baragwanath Hospital who assisted us with the statistics.

References

- Cote J, Cartier A, Robichaud P, et al. Influence on asthma morbidity of an asthma education program based on self-management plans following treatment optimization. *Am J Respir Crit Care Med* 1997; 155: 1509-1514.
- Hetzel MR, Williams IP, Shakespeare RM. Can patients keep their own peak flow records reliably? *Lancet* 1979; 1: 597-599.
- Ehrlich RI, Jordaan E, Du Toit D, Volmink JA, Weinberg E, Zwarenstein M. Underrecognition and undertreatment of asthma in Cape Town primary school children. *S Afr Med J* 1998; 88: 986-993.
- Terblanche E, Fourie FR, Wessels JA. Assessment of a peak flow whistle in nonasthmatic children. *Pediatr Pulmonol* 1999; 27: 428-431.
- Ehrlich RI, Du Toit D, Jordaan E, et al. Prevalence and reliability of asthma symptoms in primary school children in Cape Town. *Int J Epidemiol* 1995; 24: 1138-1145.
- Hoek G, Wypij D, Brunekreef B. Self-reporting of acute respiratory symptoms of children and their relation to pulmonary function and air pollution. *Int J Epidemiol* 1999; 28: 293-299.
- Sly PD, Landau LI, Weymouth R. Home recording of peak expiratory flow rates and perception of asthma. *Am J Dis Child* 1985; 139: 479-482.
- Guyatt GH, Juniper EF, Griffith LE, Feeny DH, Ferrie PJ. Children and adult perceptions of childhood asthma. *Pediatrics* 1997; 99: 165-168.
- Fritz GK, Klein RB, Overholser JC. Accuracy of symptom perception in childhood asthma. *J Dev Behav Pediatr* 1990; 11: 69-72.
- Lara M, Duan N, Sherbourne C, et al. Differences between child and parent reports of symptoms among Latino children with asthma. *Pediatrics* 1998; 102: E68.
- Sly PD, Cahill P, Willet K, Burton P. Accuracy of mini peak flow meters in indicating changes in lung function in children with asthma. *BMJ* 1994; 308: 572-574.
- Pinzone HA, Carlson BW, Kotses H, Creer TL. Prediction of asthma episodes in children using peak expiratory flow rates, medication compliance, and exercise data. *Ann Allergy Asthma Immunol* 1991; 67: 481-486.
- Li JT. Home peak expiratory flow rate monitoring in patients with asthma. *Mayo Clin Proc* 1995; 70: 649-656.
- Klein RB, Fritz GK, Yeung A, McQuaid EL, Mansell A. Spirometric patterns in childhood asthma: peak flow compared with other indices. *Pediatr Pulmonol* 1995; 20: 372-379.
- Miles JF, Tunnicliffe W, Cayton RM, Ayser JG, Miller MR. Potential effects of correction of inaccuracies of the mini-Wright peak expiratory flow meter on the use of an asthma self-management plan. *Thorax* 1996; 51: 403-406.
- Autio L, Rosenow D. Effectively managing asthma in young and middle adulthood. *Nurse Pract* 1999; 24: 100-113.
- Van Ganse E, Leufkens HG, Vincken W, et al. Assessing asthma management from interviews of patients and family physicians. *J Asthma* 1997; 34: 203-209.
- Milgrom H, Taussig LM. Keeping children with exercise-induced asthma active. *Pediatrics* 1999; 104: e38.

Accepted 3 June 2000.

ALLERGENICITY AND CROSS-REACTIVITY OF BUFFALO GRASS (*STENOTAPHRUM SECUNDATUM*)

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Background. In the subtropical climate of South Africa, grasses of the subfamily Panicoideae are predominant. Bermuda grass has previously been shown to be an important local allergen, and immunoglobulin E (IgE) epitopes of Bermuda grass extracts are known to be distinct from those of the Pooid pollen extracts. Following our demonstration of sensitivity in 43% of patients grass-allergic to the Panicoid, Kikuyu grass, we have studied the closely related buffalo grass, *Stenotaphrum secundatum*, indigenous to the Western Cape region, the east coast of Africa and the oceanic islands such as Mauritius; and *Eragrostis*, another common indigenous grass with a wide distribution.

Objective. To partially characterise the allergens of buffalo pollen, and examine its immunological relationships with local common grasses such as *Eragrostis* and Kikuyu.

Methods. Grass-allergic patients were evaluated clinically, and skin prick tests (SPTs) and radio-allergosorbent tests (RASTs) to Bermuda and grass mix were performed. Sera of timothy grass-sensitive patients from Belgium were also included in this study. Pollen extract from buffalo grass was characterised by specific IgE binding by means of immunoblotting and enzyme-linked immunosorbent assay (ELISA). Cross-reactivity between the grasses was studied by means of inhibition of IgE binding.

Results. More than 90% of grass-sensitive patients were found to have IgE antibodies to Buffalo and *Eragrostis* pollen. Inhibition of ELISA and immunoblots revealed that extracts of these grass pollens could significantly inhibit IgE binding to the local grass pollens, Kikuyu, buffalo, *Eragrostis* and Bermuda on solid phase, but 100% inhibition was never achieved, indicating that cross-reactive but also unique epitopes are present. We also identified a subset of patients with negative RASTs to Bermuda, and minimal inhibition by Bermuda pollen extract.

Conclusion. Buffalo and *Eragrostis* are important aero-allergens in the Cape, dispersed during the long dry, windy summer. Our data suggest that the local grasses are major sensitisers, and that South African diagnostic panels should include extracts of buffalo and *Eragrostis* grasses.

S Afr Med J 2001; 91: 237-243.

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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 Ordman¹ 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 Pooideae with 133.² Panicoideae 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 Pooideae, 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.³ A study by Potter *et al.*⁴ showed that 43% of patients had a clinical sensitivity to Kikuyu grass (*Pennisetum clandestinum*), a Panicoideae 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 *Eragrosteae*, 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 tufts, 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

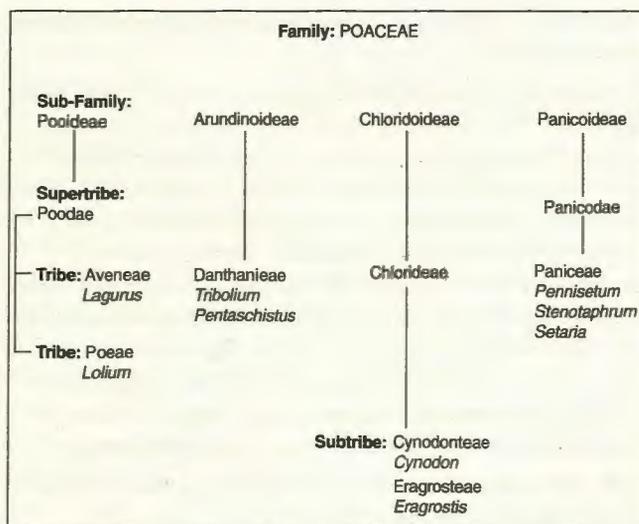


Fig. 1. Classification of grass species investigated.

been shown to have limited immunological cross-reactivity with the clinically significant grasses of the Pooideae,^{5,7} 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 gx2 and/or specifically to Pharmacia 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.



RASTs and SPTs

SPTs were performed in groups 2 and 4 using the Bayer Bermuda grass and Bayer grass mix (M5), consisting of extracts 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 perenne*), timothy (*Phleum pratense*), Kentucky blue (*Poa pratensis*), Johnson (*Sorghum halepense*) 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 (NaN_3), 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,⁹ 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 polyvinylidene 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-

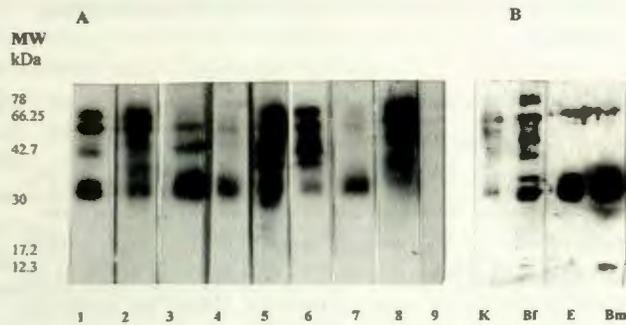


Fig. 2. A: Profiles of IgE binding to blotted buffalo extracts. Lanes 1 - 8 represent different patients and lane 9 is a non-allergic control, detected by autoradiography. B: Analysis of IgE binding to transferred Kikuyu (K) (lane 1), buffalo (Bf) (lane 2), Eragrostis (E) (lane 3) and Bermuda (Bm) (lane 4) pollen extracts, detected by enhanced chemiluminescence.

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-

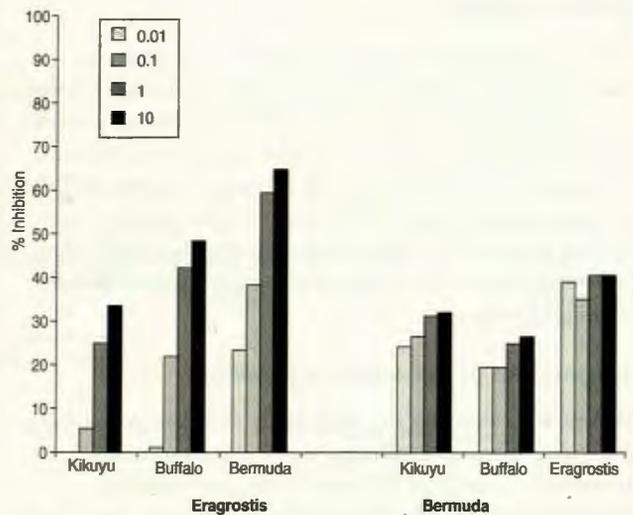


Fig. 3. Comparison of IgE inhibition of *Eragrostis* pollen extracts, using a pool of Bermuda-positive serum. Concentrations of inhibitor ranged from 0.001 to 10 µg/ml.

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.

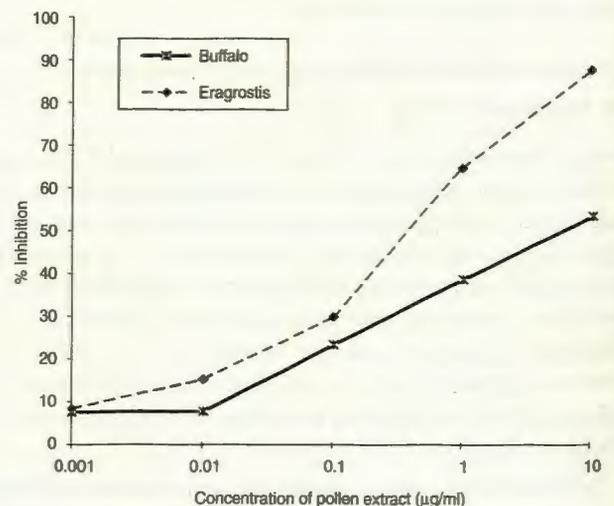


Fig. 4. Inhibition curves of Bermuda CAP RAST by buffalo and *Eragrostis* pollen extracts, using a pool of Bermuda-positive serum. Concentrations of inhibitor ranged from 0.001 to 10 µg/ml.



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).

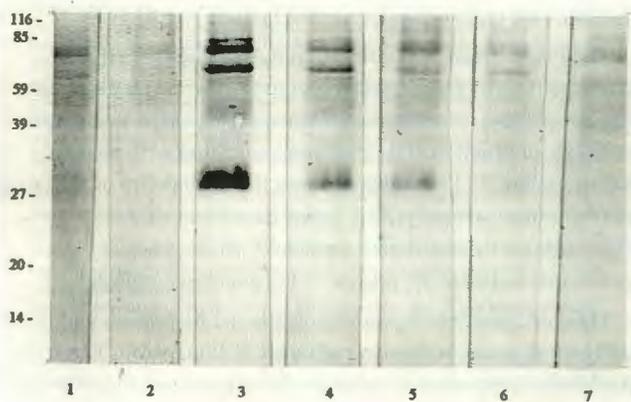


Fig. 5. Inhibition of immunoblot of buffalo allergen with 5 and 50 µg/ml of Bermuda and Kikuyu pollen extracts. Lanes 1 and 2 are cord and non-allergic control sera; lane 3 shows serum binding in the absence of inhibitor; lanes 4 and 5, in the presence of 5/100 µg/ml Bermuda as inhibitor; lanes 6 and 7 with 5/100 µg/ml Kikuyu as inhibitor.

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

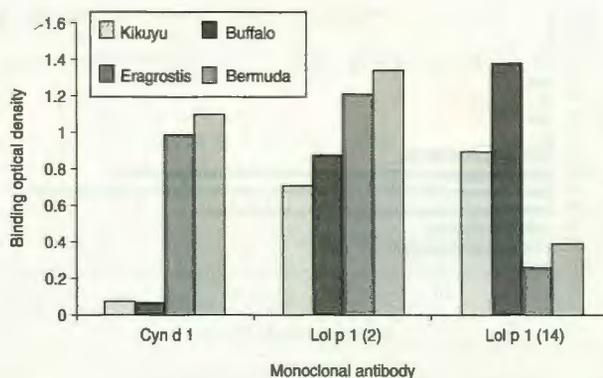


Fig. 6. Cross-reactivity of monoclonal antibodies, α-*Cyn d 1* and α-*Lol p 1*, clones 2, 14 to four local pollen extracts as solid phase, shown by ELISA.

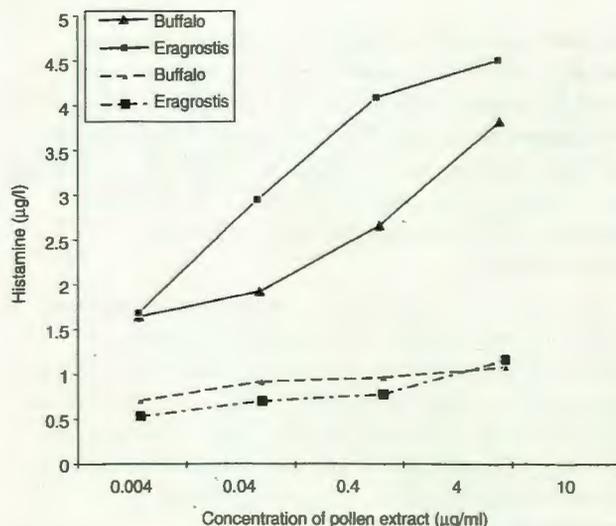


Fig. 7. Histamine release by basophils of one patient when incubated with pollen extracts of buffalo and *Eragrostis* grasses; dose response curve, compared with release by a non-allergic control serum.

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*,



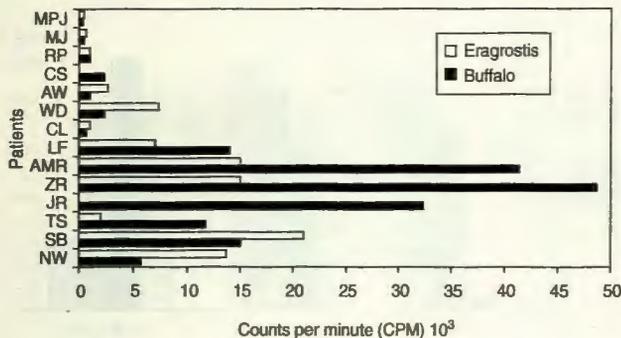


Fig. 8. T-cell proliferation to pollen extracts of buffalo and *Eragrostis* grasses. Values shown are mean cpm of triplicate wells as determined by ^3H -Th incorporation. The top 7 individuals are asymptomatic, SPT +ve, while the rest are grass-sensitive individuals.

and many also react to Kikuyu grass. The major buffalo allergen is a 34 kD periodate-sensitive glycoprotein, pI 5-7, which is consistent with the principal allergenic component of most grasses, the group 1 (Gp 1) allergen.⁹⁻¹² None of the sera exhibited a binding pattern unique to a single species, although 4:32 volunteers, with negative Bermuda SPTs, exhibited negligible binding to a Bermuda grass extract on immunoblotting.

Cross-reactivity among the four species studied has been clearly demonstrated by inhibition of ELISA, RAST, and immunoblotting. *Eragrostis* was able to abrogate IgE binding by most allergic sera, and to abolish binding in some patient sera. It is therefore an important allergenic grass, considering its year-long pollination period and widespread distribution in southern Africa. *Eragrostis* is a logical candidate for a desensitising vaccine for the region.

It was interesting to find that Belgian grass-sensitised individuals had cross-reactive IgE that binds to components on immunoblots of the African indigenous buffalo, Kikuyu and *Eragrostis* grasses. These individuals had not been exposed to the subtropical grasses. It has been found that 80% of the South African patients sensitive to buffalo have concordant sensitivity to rye. Local subjects also have specific IgE to timothy grass pollen in CAP-RASTs, confirming the presence of cross-reactive epitopes since timothy does not occur locally.

Recent reports have implicated cross-reactive sugar molecules in IgE binding,^{13,14} as first reported for the major allergen of Bermuda grass, *Cynd1*,¹⁰ and the 60 kD allergen, BG 60,¹⁵ of Bermuda grass. We have demonstrated carbohydrate moieties in Kikuyu, buffalo and *Eragrostis* extracts, and periodate treatment of these local pollen extracts resulted in reduced patient IgE binding, as well as the monoclonal antibody, anti-*Lol p 1* (2) in ELISAs. The carbohydrate moiety of B-cell epitopes may conceivably contribute to the cross-reactivity between these Panicoid and the unrelated Pooideae grasses.

Interspecies allergens have been proposed to account for the cross-reactivity between unrelated fruits and vegetables,¹⁶⁻¹⁸ such as the ubiquitous panallergen 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 airway inflammation.^{19,20} DEPs also increase the availability of allergens of respiratory size, as pollen grains have been shown to aggregate on these airborne particles,²¹ while gaseous pollutants facilitate the release of the allergenic molecules.²²

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 Panicoid family as a pollen sensitiser 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.

This study was supported by a United Chemicals of Belgium (UCB)-Allergy Society of South Africa research award.

We are very grateful to the following: ALK-Abello Research (Horsholm, Denmark) for supplying the monoclonal antibodies, anti-*Cyn d 1* and anti-*Lol p 1*, clones 2 and 14; Pharmacia and Upjohn Diagnostics for providing the CAP RASTs; Professor W Stevens of Antwerp, Belgium, for the timothy-positive sera from Belgium; Ms M Schinkel for performing the CAP RASTs; and Ms S Salie for the immunoblotting of the other grasses of the Cape region.

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References

1. Ordman D. Pollinosis in South Africa. *S Afr Med J* 1947; **21**: 38-48.
2. Gibbs Russel GE, Watson L, Koekemoer M, et al. *Grasses of Southern Africa*. 2nd ed. Botanical Research Institute, South Africa, 1991: 1.
3. Orren A, Dowdle EB. Studies on Bermuda grass pollen allergens. *S Afr Med J* 1977; **51**: 586-591.
4. Potter PC, Mather S, Lockey P, Ainslie G, Cadman A. IgE specific immune responses to an African grass (Kikuyu, *Pennisetum clandestinum*). *Clin Exp Allergy* 1993; **23**: 581-586.
5. Marsh DG, Haddad MD, Campbell DH. A new method for determining the distribution of allergenic fractions in biological materials: its application to grass pollen extracts. *J Allergy* 1970; **46**: 107-112.
6. Schumacher MJ, Grabowski FJ, Wagner CM. Anti-Bermuda grass RAST binding is minimally inhibited by pollen extracts from ten other grasses. *Ann Allergy* 1985; **55**: 584-587.
7. Lieferman KM, Gleich GJ. The cross-reactivity of IgE antibodies with pollen allergens. 1. Analysis of various species of grass pollens. *J Allergy Clin Immunol* 1976; **58**: 129-139.



8. Harlow E, Lane D. *Antibodies: A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; 139-239.
9. Ford SA, Baldo BA. Identification of Bermuda grass (*Cynodon dactylon*) pollen allergens by electroblotting. *J Allergy Clin Immunol* 1987; 79: 711-720.
10. Matthiesen F, Schumacher MJ, Lowenstein H. Characterisation of the major allergen of *Cynodon dactylon* (Bermuda grass) pollen, Cyn d 1. *J Allergy Clin Immunol* 1991; 88: 763-774.
11. Han S-H, Chang Z-N, Chang H-H, Chi C-W, Wang J-Y, Lin C-Y. Identification and characterisation of epitopes on Cyn d 1, the major allergen of Bermuda grass pollen. *J Allergy Clin Immunol* 1993; 91: 1035-1041.
12. Esch RE, Klapper DG. Identification and localisation of allergenic determinants on grass group 1 antigens using monoclonal antibodies. *J Immunol* 1989; 142: 179-184.
13. Batanero E, Villalba M, Monsalve RI, Rodriguez R. Cross-reactivity between the major allergen from olive pollen and unrelated glycoproteins: evidence for an epitope in the glycan moiety of the allergen. *J Allergy Clin Immunol* 1996; 97: 1264-1271.
14. Calkhoven PG, Aalbers M, Koshte VI, Pos O, Oei HD, Aalberse RC. Cross-reactivity among birch pollen, vegetables and fruit as detected by IgE antibodies is due to at least 3 different cross-reactive structures. *Allergy* 1987; 42: 382-390.
15. Su SN, Lau GX, Tsai JJ, Yang SY, Shen HD, Han SH. Isolation and partial characterisation of the Bermuda grass pollen allergen, BG-60a. *Clin Exp Allergy* 1991; 21: 449-455.
16. Valenta R, Duchene M, Ebner C, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992; 175: 377-385.
17. Van Ree R, Voitenko V, van Leuvene WA, Aalbers RC. Profilin is a cross-reactive allergen in pollen and vegetable foods. *Int Arch Allergy Immunol* 1992; 98: 97-104.
18. Asturias JA, Arilla MC, Gomez-Bayon N, Martinez J, Martinez A, Palacios R. Cloning and high level expression of *Cynodon dactylon* (Bermuda grass) pollen profilin (Cyn d 12) in *Escherichia coli*: purification and characterisation of the allergen. *Clin Exp Allergy* 1997; 27: 1307-1313.
19. Devalia JL, Wang JH, Rsznak C, Calderon M, Davies RJ. Does air pollution enhance the human airway responses to allergen? *Allergy and Clinical Immunology International* 1994; 6: 80-84.
20. Ohtoshi T, Takizawa H, Okazaki H, et al. Diesel exhaust particles stimulate human airway epithelial cells to produce cytokines relevant to airway inflammation *in vitro*. *J Allergy Clin Immunol* 1998; 101: 778-785.
21. Knox RB, Suphioglu C, Taylor P, et al. Major grass pollen allergy: Lol p 1 binds to diesel exhaust particles: implications for asthma and air pollution. *Clin Exp Allergy* 1997; 27: 246-251.
22. Behrendt H, Becker WM, Fritsche C, et al. Air pollution and allergy: experimental studies on modulation of allergen release from pollen by air pollution. *Int Arch Allergy Immunol* 1997; 113: 69-74.

Accepted 27 June 2000.

URBANISATION AND ADOLESCENT RISK BEHAVIOUR

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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 suicidality. Conversely, participation in sexual intercourse 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.

S Afr Med J 2001; 91: 243-249.

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.¹ This proportion was expected to increase to about 40% by the year 2000, an increase of 60%.^{1,2}

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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