Clinical significance of aero-allergen identification in the western Cape

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Summary

Positive identification and documentation of the seasonal variation of aero-allergens and the immune responses to them has important implications for the timing of allergen avoidance measures and the selection of patients suitable for immunotherapy. The relative abundance of Aero-allergens in the Cape Peninsula during 1984 - 1987 was measured by continuous volumetric air sampling, using a Burkard spore trap. Mould spore counts of > 3000 spores/m3 were found three times per year and were only exceeded by pollen grains in the months of September and October (range 4 800 - 7 400 spores/m3). Gramineae and Compositeae spores were found perennially in significant numbers. Pollen from allergenic trees peaked at fixed times each year: oak in August; plane in September and pine between August and October. Grasses found on the Peninsula include sweet vernal, Bermuda grass, ryegrass, common reed, Johnson grass, brome grass, canary grass, annual meadow and kikuyu. In vivo skin tests in 209 children with known allergic disease were positive to Derma­tothyngoloides pteronyssimus (73%), South African grasses (38%), tree pollens (22,4%), flower and weed pollens (19,6%), cat (27%), dog (12%) and feathers (18,6%). One-third of the 1372 children screened at Red Cross War Memorial Children's Hospital Allergy Service had positive specific IgE responses to environmental allergens. Investigation of 62 children pos­itively allergic to grass using the radio-allergosorbent test revealed positive results in 25 (41%). Of these, 92% were positive to Timothy grass, a grass not occurring in the Cape Peninsula. Knowledge of cross-reactivity profiles for local allergens minimises the number of tests required in allergy diagnosis. 5

Subjects and methods

Aero-allergen sampling

A 7-day Burkard spore trap mounted at a height of 3 m from the ground at the Red Cross War Memorial Children's Hospital, Rondebosch, CB, was used. Pollen grains were collected on a cellulose strip fixed to a drum that completes one revolution in 7 days at a speed of 2 mm/h with an air sampling volume of 10 l/min. The strip was removed, divided into 7 sections (each representing 1 day), stained with basic fuchsin in 32% ethanol/16% glycerine aqueous solution and the pollens were then identified and counted by light microscopy (by D.B.) for the period 1 January 1984 - 31 December 1987. Daily pollen counts of > 50 spores/m3 were regarded as being clinically significant. 6

Local grass identification

Several field trips were conducted during which grasses were sampled from sites throughout the Cape Peninsula and positively identified.

In vivo allergen tests

Skin prick tests (Bencard) for 31 different allergens were performed on 209 children aged 4 - 12 years attending the Allergy Clinic at the Red Cross War Memorial Children's Hospital. Skin-prick test responses in 104 white children were compared with those obtained in 105 coloured children. All
children who had skin prick tests performed had clinical evidence of asthma, rhinitis and/or eczema. Drops of allergen extract were placed on the skin of the flexor aspect of the forearm at 2 cm intervals. The skin was pricked through the drop without causing any bleeding. The test solution was wiped off after 8 - 10 minutes and reactions recorded after 15 minutes and graded as follows: + = no wheal, 3 mm flare; ++ = 2 - 3 mm wheal with flare; +++ = 3 - 5 mm wheal with flare; ++++ = > 5 mm wheal with or without pseudopodia. Reactions greater than + were regarded as positive. Positive (histamine) and negative (saline) controls were included in each patient.

**RAST**

The frequency of positive IgE immune responses in 1 372 children presenting at the General Paediatric Outpatients Department with symptoms and signs suggestive of respiratory allergy was determined.

IgE specific immune responses to 8 common allergens encountered in the Cape Peninsula were also measured in *vitro* using RAST. This panel consisted of: house-dust mite (*Dermatophagoides pteronyssimus*), Bermuda grass, *Cladosporium herbarum*, *Aspergillus fumigatus*, Alternaria, *Milk*, cat epithelium and dog dander.

In order to study immune responses to grass allergens, three individual grass families with low levels of cross-reactivity were used (tribe Panicoideae — *Cynodon dactylon* (Bermuda grass), tribe Festuceae — *Phleum pratense* (Timothy grass), and tribe Hordeae — *Secale cereale* (cultivated rye grass)) for RAST in 62 children with histories suggestive of grass allergy.

**Results**

**Aero-allergen sampling**

Results are expressed as the mean number of spores/m³/mo. over the 4-year period. Tables of actual pollen counts are available from the authors. Mean total pollen counts and fungal spore for the period are shown in Fig. 1. With the exception of the months of October and November, fungal spore counts exceeded pollen counts. The seasonal variation and relative abundance of the three most common fungi, i.e. Alternaria, *Cladosporium* and Epicoccum, are shown in Fig. 2. *Alternaria* occurred most commonly, showing three distinct peaks in February, July and October/November each year.

Weed and grass pollen counts followed the seasonal variation of total pollen counts shown in Fig. 1, being most abundant in October, November and December, but significant grass pollen counts (> 50 spores/m³) were found perennially. Allergenic tree pollens appeared as early as June with oak peaking in August, plane in September and pine in October (Fig. 3). Significant pollen counts were also found for acacia and cypress trees but not willow or poplar.

**Fig. 1. Mean pollen and mould counts expressed as spores/m³/mo. obtained for the 4-year period 1 January 1984 -31 December 1987 (— mould; - = pollen).**

**Fig. 2. Comparison of the mean spore counts (grains/m³/mo.) for allergenic moulds *Alternaria* (-), *Cladosporium* (---) and *Epicoccum* (-----) for the period 1 January 1984 - 31 December 1987.**

**Fig. 3. Comparison of the seasonal variation and allergenic load (mean count) for major allergenic tree (pine (—), plane (-----) and oak (-----)) pollen for the period 1 January 1984 - 31 December 1987.**

Weed pollens occurring in significant numbers included Chenopodiaceae (goosefoot family), Compositae (daisy family), Cyperaceae (herbs), Polygonaceae (large group, includes knot grass), Plantago (plantain) and Urticaceae (nettle).

The pollinating times of the major flowering species are shown in Fig. 4. While the majority of pollens demonstrated a short seasonal pattern, the Compositae were perennial. Grass species found during the field study of the Cape Peninsula included sweet vernal grass (*Anthoxanthum odora-*)
Immune responses to allergens

**Skin tests**

Positive skin test results obtained in both groups are shown in Fig. 5. There was no difference between the frequency of a positive skin test to any of the 34 allergens tested when coloured children were compared with whites. Mixed symptoms of atopy (asthma and rhinitis) were present in 130 of the children (62%), 8 (3.8%) had only eczema, 31 (14.8%) had pure dependent asthmatics.

Indoor allergens accounted for the majority of the positive skin reactions: 73% of the children were positive to *D. pteronyssinus*, 47% to *D. farinae* and 56% to Bencard's housedust extract. Of the 14 steroid-dependent asthmatics tested, 13 were positive to housedust mite. Positive reactions to feathers (chicken) were present in 18%, dog in 12.4% and cat in 27.2%. Of the positive reactions, 38% were to Bencard mixed South African grasses but only 8% of patients were positive to Bermuda grass. Tree pollens accounted for 22.4% of the positive results. These reactions included responses to pine (6.2%), acacia (6.2%), oak (5.6%), syringa (3.3%) and plane (0.95%). It was noteworthy, however, that only 6 out of 209 children (2.8%) had allergies to single pollen species.

Flower and weed pollens accounted for 19.6% of the positive results. Reactions to plantain accounted for half these reactions. Positive reactions were also found to Compositae (3.3%), nettle (2.3%) and pyrethrum (3.8%). All children in this series who were positive to flower and weed-pollen, also reacted to the South African mixed grass skin testing solution.

Allergic responses to individual fungi were made up as follows: *Epicoccum* 8.1%, *Cladosporium* 8.1%, *Aspergillus* 10%, *Botrytis* 13.8%, and *Alternaria* 18.6% and invariably children were sensitive to more than one mould.

**RAST**

One-third of the 1372 children (aged 0 - 12 years) attending outpatients with symptoms involving the upper and lower respiratory tract had specific IgE responses to environmental allergens. Positive results were as follows: *D. pteronyssinus* 31%, dog epithelium 19%, milk 17%, cat epithelium 16%, Bermuda grass 15%, *Alternaria* 15%, *Aspergillus* 12% and *Cladosporium* 8%.

Investigation of the 62 children with histories suggestive of grass allergy revealed specific IgE responses to one or more of the three grass tribes tested in 25 (41%). Sixteen of the 25 (64%) were positive to all three grasses, 23/25 (92%) had positive responses to *P. pratense* (Timothy grass), 20/25 (80%) were positive to *S. cereale* (cultivated rye grass) and 20 (80%) were positive to *C. dactylon* (Bermuda grass). None of the children reacted to Bermuda grass alone but 2 (8%) reacted to either Timothy or rye alone.

**Discussion**

Accurate identification of allergens causing allergic disease in patients is the cornerstone of good allergy practice. In order to take an adequate clinical history, the physician should have a sound knowledge of known allergens in the local environment. These allergens may vary quite markedly between geographical areas. In South Africa it is well known, for example, that housedust mite thrives at the coast but not on the highveld.6 Grasses on the highveld differ from those at the coast.7

This study demonstrates the abundance and seasonal variation of Aero-allergens found in the Cape Peninsula. In addition, a number of findings have emerged that have important implications in patient management. The most abundant Aero-allergens occurring perennially were the fungal spores. Here the relative abundance of *Alternaria* contrasts with results obtained in Johannesburg8 and Windhoek9 where *Cladosporium* was the dominant fungal allergen. The cyclical variations in *Alternaria* peaks were consistent in this region during the 4-year study period. No explanation for this occurrence is known. Of the children studied, 20% were allergic to fungi with *Alternaria* accounting for the largest number of responses. A strong clustering of immune responses to *A. fumigatus, Alternaria* and *Cladosporium* using the RAST system has been reported.2

Vaccines for immunotherapy to fungi are not available in South Africa. Systemic reactions have been reported with
newly developed fungal vaccines in Europe. Extreme care would be required if these were to be introduced here in view of the perennial presence of the common allergenic fungi.

Allergenic tree pollens showed clear and well-defined peaks. Reactions to tree pollens accounted for 22,4% of the positive results among allergic children. Since the flowering time of oak and plane trees is well defined it is possible to advise sensitive patients to avoid exposure by, for example, planning vacations to areas away from these trees during these periods.

The perennial presence of Compositae and grass pollens in significant amounts in the atmosphere provides an explanation for the prolonged symptoms experienced by most grass-sensitised rhinitis subjects. The accurate identification of the grass families to which grass allergic patients react is of importance when immunotherapy is contemplated, since vaccines are usually made up of a mixture of grasses. The diagnosis of grass allergy is usually confirmed by performing skin tests or RAST that uses a mixture of grasses. This is quite in order for screening purposes, but when immunotherapy is contemplated a more precise profile of the relevant grass antigens is required. Our patients are heterogeneous in their sensitivity to the major grass families. Twenty per cent of the grass sensitive patients tested did not react to Bermuda grass using RAST. Both of the currently available standard grass mix vaccines contain Bermuda grass (Special Grass Mix and South African Grass Mix; Dome-Hollister Stier) and would not be suitable for these patients. Only 8% of the clinic patients reacted to the Bermuda grass extract using the Bencard skin testing solution. Bencard UK have recently withdrawn their allergen testing extracts from this country and extracts from other companies may vary considerably in their potency. Ideally, patients should be skin tested with allergen extracts from the same source as those used in desensitising vaccines.

Direct comparison between the RAST results and skin test results were not possible, since these tests were performed on different patient groups for different reasons. Controlled comparisons between in vitro and in vivo IgE immune responses have been the subject of previous reviews. 7-12 Good correlation between the tests occurs if skin test extracts and RAST allergens are produced by the same manufacturer. Although skin testing is accurate and inexpensive, RAST is useful when screening large numbers of children in the outpatient situation. RAST may also be used selectively to detect responses to several allergens (e.g. grass subfamilies and occupational allergens) to areas away from these trees during these periods.

An important observation was that only 6 of the allergy clinic patients (2,8%) reacted to single pollen allergens. As immunotherapy for patients with multiple allergies is not advisable, the number of patients qualifying for grass immunotherapy will be small.

An interesting finding was that 90% of grass-sensitive patients were positive to the Timothy grass RAST, although Timothy grass does not occur in the Cape Peninsula. These responses probably represent cross-reactivity with other grasses belonging to the same subfamily as Timothy grass, 13 i.e. L.perenne, P. annua, H. lanatus, A. odoratum, Avena sativa and Phalaris arundinacea, all of which occur in the Cape Peninsula. Thus, Timothy grass RAST is extremely useful for screening for grass allergies in the western Cape. Although atmospheric sampling will indicate an overall trend for grass allergies, identifying a patient's specific allergy to a particular grass must be done by consulting the South African flowering calendar 14 since grass pollens are all identical on light microscopy.

Of the 8000 floral species present on the Cape Peninsula, only a small percentage are of known clinical importance. Compositae pollen is found in significant amounts throughout the year (Fig. 4), yet appears to be of little importance as a cause of allergic disorders. The allergenicity of some important local grasses, e.g. kikuyu, is at present still unknown and requires further investigation. As with some of the grasses, it is possible that several other pollens of the Cape are in fact allergenic, but studies of their allergenicity have not yet been performed.

The importance of Aero-allergen allergy in Cape Town is underscored by the finding that one-third of the children presenting with recurrent upper and lower respiratory tract symptoms at the Outpatient Department had positive responses to environmental allergens. The most important allergen to which the children react is the house dust mite. 14 Mites are probably responsible for perennial symptoms, but studies of seasonal variation of house dust mites have not yet been performed. In a contrasting study on the western coast of Peru, Lopez 15 found that fungal allergy accounted for the majority of positive skin tests and marginally exceeded the incidence of house dust mite allergy. Ordman 16 found that perennial asthma in persons living in the coastal areas of South Africa and in Israel was related to 'climatic factors' such as relative humidity and temperature. It is likely that these 'climatic factors' directly regulate the fungal pollen and mite Aero-allergens, which in turn induce symptoms via an IgE-mediated mechanism.

Joubert 17 has recently emphasised the importance of contributory factors such as cigarette smoking and has also reported the effect of parasites such as Ascaris lumbricoides 18 in enhancing IgE responses to Aero-allergens in the western Cape. Viral infections 19 are known to play an important role in precipitating acute severe asthma attacks in allergic children in this area. The cytopathic effects of viruses expose airway mucosa, mast cells and nerve endings to allergen penetration and can thus initiate subsequent allergic pathophysiological reactions.

The overriding prevalence of house-dust mite allergy in our population emphasises the need for measures directed towards the effective elimination of this allergen from the houses of allergic patients. Without these measures most of our allergic children will depend on the protection provided by prophylactic drugs such as sodium cromoglycate and ketotifen.

The Aero-allergen prevalence data presented here should assist clinicians in identifying possible causes of a patient's allergic symptoms. A good clinical history will help to identify the allergen which can be confirmed using skin tests or RAST. The Aero-allergen prevalence in the atmosphere in the Cape Peninsula is unique and differs from reports 6-9 from other parts of South Africa. A knowledge of the immune responses encountered to a panel of allergens and the intelligent use of cross-reactivity profiles can minimise the number of further allergy tests that need to be performed.

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REFERENCES

6. Ordman D. Respiratory allergy in coastal areas of South Africa: significance

A. CADMAN

Summary

Atmospheric pollen and spores have been monitored continuously in the Pretoria-Witwatersrand-Vereeniging region (PWV) since 1987. Two Burkard 7-day recording traps were used in Johannesburg and Pretoria, and 11 gravity samplers were installed at various sites in the PWV. An analysis of the pollen component and pollen calendars average data for 1987 and 1988 are presented. Grass pollen was the most significant contributor in absolute and relative terms, comprising 52% of total pollen. The season peaked between October and March, but grass pollen was found throughout the year. Pollen of Northern Hemisphere trees was abundant, relative to that of indigenous trees. The flowering season for these exotics was very short and intense, possibly due to the greater plant population. Seasonal taxa started flowering earlier relative to that of indigenous trees. The flowering season for these exotics was very short and intense, possibly due to the greater plant population. Seasonal taxa started flowering earlier in Pretoria than in Johannesburg. Herbaceous taxa were well represented in the atmospheric sample. The major contributor was the daisy family. The greatest proportion of pollen, 58.8%, was non-seasonal. Species in this category flower consecutively throughout the year. Of the taxa, 28.4% were spring-flowering. The allergenicity of pollen encountered in the atmospheric sample is discussed.


The elegant aerobiological studies of Ordman have offered the only published South African data in the field of atmospheric pollen and spores to date. It is highly likely that these have changed considerably since his surveys were undertaken. Not only are there the effects of industrialisation and changing land-use patterns, but also the results of altered gardening fashions. This article focuses on pollen of the Pretoria-Witwatersrand-Vereeniging (PWV) metropolitan area.

Pollen is recognised world-wide as one of the major causes of upper respiratory tract allergy. It is broadly divided into two categories: entomophilous (insect-dispersed) and anemophilous (wind-dispersed). The former is generally larger, heavier, stickier and produced in smaller quantities than the latter. Pollen ranges in size from about 7 μm to 100 μm, with a median of around 20 μm. It is produced in vast quantities. For example, Travers calculated that 1 ha of maize (Zea mays) could produce about 300 litres of pollen per season. Most plants flower seasonally so that related allergies are also seasonal.

Materials and methods

From January 1987 continuous atmospheric sampling has been conducted in the PWV area. Two sampling methods were used. Simple gravity samplers were located at 11 sites distributed throughout the study area. These trapped pollen on petroleum-jelly-coated microscope slides. Two Burkard samplers were installed at 2 of these sites, 1 at the South African Council for Scientific and Industrial Research (CSIR) in Pretoria and 1 at the research station of the University of the Witwatersrand at Frankenwald in Johannesburg. The latter two localities comprise several hectares of natural vegetation surrounded by suburban gardens. Here, cultivated taxa include annuals and trees predominantly of the Northern Hemisphere. The vegetation across the range of sites is sufficiently varied to ensure that the entire spectrum of taxa commonly found in the study area was sampled.

Slides were collected weekly. The petroleum-jelly was dissolved and the residue was acetolysed following the method of...