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 > 3 000 spores/m^3 were found throughout the year and were only exceeded by pollen grains in the months of September and October (range 4 800 - 7 400 spores/m^3). Gramineae and Compositae 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, rye grass, 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 *Dermatophygoides 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 1 372 children screened at Red Cross War Memorial Children's Hospital Allergy Service had positive specific IgE responses to environmental allergens. Investigation of 62 children possibly 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.

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The identification of the allergen responsible for symptoms in allergic patients is the key to appropriate management of their disease, whether this be avoidance of the allergen, immunotherapy or other treatment. Immunotherapy depends upon the knowledge of the seasonal variation of the allergens in question. It may be dangerous to commence immunotherapy, particularly for pollen allergies, when there is an abundance of pollen in the atmosphere.[1] Previously allergen prevalence in the Cape Peninsula has been loosely attributed to 'spring' for pollens or 'autumn' for moulds, without any accurate data available to guide the clinician.

Specific IgE responses to environmental allergens are not random[2] and depend not only upon the genetically determined immune responsiveness of the individual but are also subject to the dose and intensity of the environmental allergen exposure.[3,4] A detailed knowledge of environmental allergens is essential when attributing a patient's symptoms to a particular allergen. When a clear seasonal variation is known for an allergen, it may also help the clinician to exclude other allergens as a possible cause for symptoms by taking a good, careful allergic history.

An important reason for documenting local grass pollen prevalence relates to the preparation of immunotherapy vaccines. Many grass vaccines contain a mixture of grasses based on European prevalence studies and may contain grasses that do not occur naturally in South Africa. Immunising a patient with a non-relevant allergen has no scientific basis and may be detrimental to their health.

As a first step in evaluating the role of aero-allergens and rationalising the therapy of children with allergic diseases in Cape Town, we have monitored the seasonal variation of aero-allergens in the atmosphere over a 4-year period by continuous volumetric air sampling using a Burkard spore trap. In addition a radio-allergosorbent test (RAST) panel was used to detect IgE responses in children attending Red Cross War Memorial Children's Hospital with recurrent respiratory symptoms. Skin testing using a 31-allergen panel was also carried out to identify the aero-allergens to which allergic children in this area commonly react. Using RAST, we have extended our investigation in grass-sensitive individuals to identify immune responses to the three major grass tribes.

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, CP, 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/m^3 were regarded as being clinically significant.[5]

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

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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 (*Dermatophygoides 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/m^3/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.

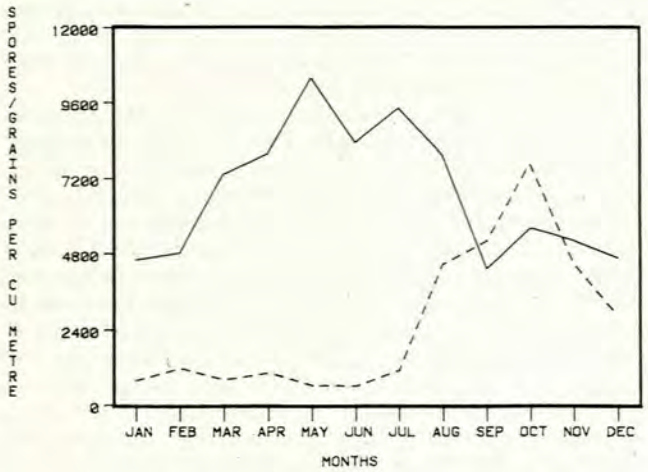

Fig. 1. Mean pollen and mould counts expressed as spores/m^3/mo. obtained for the 4-year period 1 January 1984 -31 December 1987 (— = mould; .... = pollen).

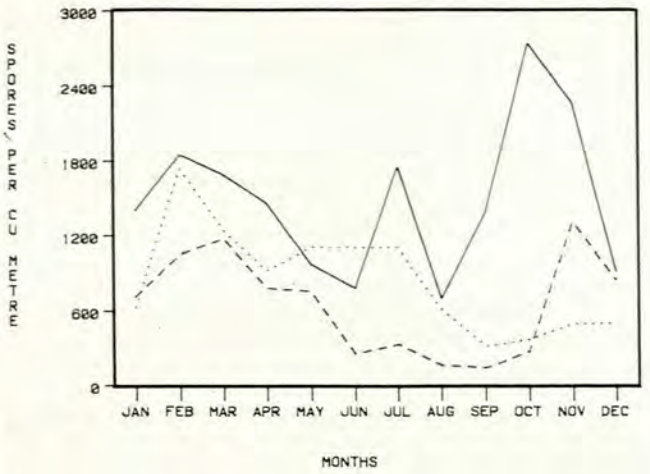

Fig. 2. Comparison of the mean spore counts (grains/m^3/mo.) for allergenic moulds *Alternaria* (—), *Cladosporium* (.....) and *Epicoccum* (.....) for the period 1 January 1984 - 31 December 1987.

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/m^3) 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.

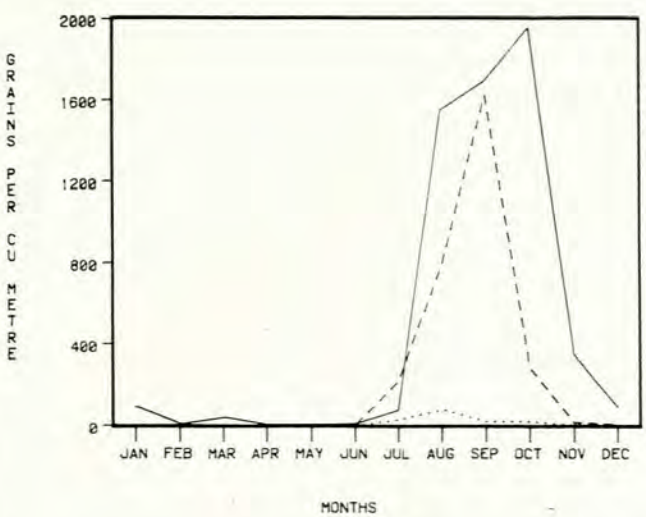

Fig. 3. Comparison of the seasonal variation and allergenic load (mean count) for major allergenic tree (pine (—), plane (-----) and oak (.....)) pollen for the period 1 January 1984 - 31 December 1987.

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-*

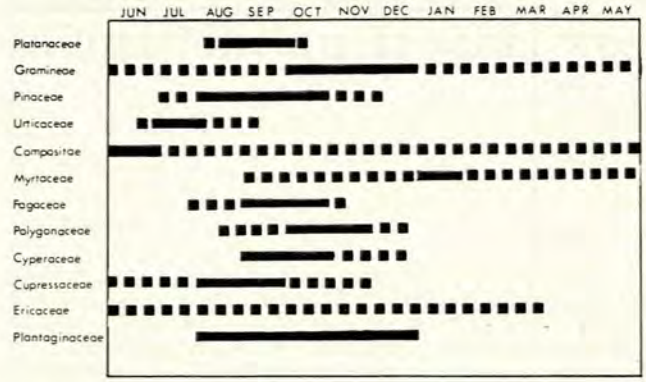

Fig. 4. Western Cape pollen calendar for the major flowering species. Solid lines indicate pollen counts greater than 100 spores/m³; broken lines indicate pollen counts between 50 - 100 spores/m³.

tum), Bermuda grass (*Cynodon dactylon*), rye grass (*Lolium perenne*), common reed (*Phragmites communis*), Johnson grass (*Sorghum halepense*), brome grass (*Bromus inermis*), cultivated rye (*Secale cereale*), velvet grass (*Holcus lanatus*), cultivated oat (*Avena sativa*), canary grass (*Phalaris arundinacea*), annual meadow grass (*Poa annua*), and kikuyu (*Pennisteum clandestinum*). Grass pollens were recovered from the spore trap throughout the year but peaked during October - December.

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 rhinitis, 26 (12%) had asthma and 14 (6,7%) were steroid-dependent asthmatics.

Indoor allergens accounted for the majority of the positive skin reactions: 73% of the children were positive to *D. pteronyssimus*, 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.

Allergenic 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.

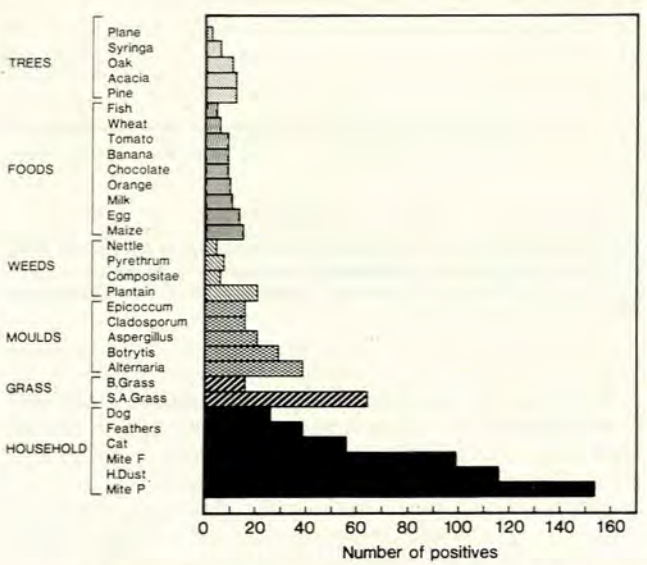

Fig. 5. No. of positive skin tests for specific allergens in 209 children using a 31-allergen screening panel.

RAST

One-third of the 1 372 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. pteronyssimus* 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 Johannesburg[8] and Windhoek[9] 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-sensitive 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.[10-12] 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) not available in skin testing solutions.

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 allergy to grasses in the western Cape. Although atmospheric sampling will indicate an overall trend for grass allergens, identifying a patient's specific allergy to a particular grass must be done by consulting the South African flowering calendar[7] since grass pollens are all identical on light microscopy.

Of the 8 000 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 housedust mite.[14] Mites are probably responsible for perennial symptoms, but studies of seasonal variation of housedust 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 housedust mite allergy. Ordman[14,16] 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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