

THE INDIGENOUS AFRICAN ALLERGEN RESEARCH LABORATORY



HISTORICAL PERSPECTIVE

Laboratory research into the biochemical and immunological characteristics of allergens causing symptoms in South African allergic subjects was first conducted in South Africa by Dr Ann Orren in 1977 in the Department of Clinical Science and Immunology, University of Cape Town (UCT) as part of an

MD thesis, in which total and specific IgE antibodies were determined in different population groups in the focusing on Bermuda grass (*Cynodon dactylis*).¹ Bermuda grass is now considered an 'introduced' non-African pollen, but a major cause of allergic symptoms in the region.

The first truly indigenous grass pollen allergen, however, only identified in 1993, was kikuyu grass, for which pollen grains were painstakingly collected by hand by Dr Ann Cadman in Johannesburg and studied in the Cape Town laboratory using Western blots and serum from patients with seasonal allergies.²

This report was followed by the identification of buffalo grass (*Stenotaphrum secundatum*) as the second important indigenous aeroallergen.³ These studies were supported and made possible by research grants from the Allergy Society of South Africa (ALLSA) and the Medical Research Council (MRC).

Indigenous allergen identification and characterisations continued intensively over 6 subsequent years, supported by ALLSA and ALK-Abello, in the Department of Clinical Science and Immunology laboratory, and then for the past 14 years in the laboratories of the Allergy Diagnostic & Clinical Research Unit (ADCRU) of the UCT Lung Institute, supported by Laboratory Specialities, and the National Research Foundation (NRF) in the Department of Medicine at Groote Schuur Hospital, Old Main Building H47 laboratory.

Over the past 15 years research into mouse models of allergy have been undertaken in the Division of Infectious Immunology at UCT Medical School, Institute of Infectious Disease and Molecular Medicine (IIDMM), and also seafood allergies. Natalie Nieuwenhuizen⁴ obtained her PhD for her work on 'Exposure to the fish parasite *Anisakis* causes allergic airway hyperreactivity and dermatitis'. Seafood allergy research was conducted by Andreas Lopata, and Mohamed Jeebhay⁵ also utilised the services

of the facility at the IIDMM, collaborating with Andreas Lopata. Molecular and immunological characterisation of the major allergen of abalone Hal m 1 in 1997 by Andreas Lopata culminated in a PhD degree.⁶

Currently Dilys Berman⁷ is doing a PhD on her cumulative studies of aerobiology in the Cape Peninsula now spanning 30 years. This laboratory/clinical service started at the Red Cross Children's Hospital Allergy Laboratory established by Professor Eugene Weinberg, but moved to the Department of Medicine at Groote Schuur, now supported financially by funds granted by ADCRU, UCT Lung Institute.

Over 20 allergy research projects have been conducted in these UCT allergy research units over the past 22 years. These have been highlighted in the accompanying overview article in this issue by PC Potter. The laboratory has also investigated allergies from Namibia and Botswana. Laboratory staff included Ruth Prescott, Andreas Lopata, Bartha Fenemore, Pam Lockey, Sumaya Salie, Barbara Nurse, Najmeeyah Brown and Jean Fletcher, and administrative support staff included Angela Phillips, Ruwayda Adams, Reuben Mqambeli and Jacqueline Higgins. The unit has now produced over 40 international and 48 South African publications reporting on research conducted on our allergens encountered in South Africa over the past 22 years. Laboratory support is illustrated in the papers by Shiang-Ju Kung, Christo Buys, Mike Levin and Dilys Berman, published in this issue of our journal.

The variety of types of projects includes Western blot investigation of unusual cases, referred with 'suspected' allergies to an unknown, or unusual allergen, the determination of house and bedding Der p 1 levels by enzyme-linked immunosorbent assays (ELISAs) in dust samples, the determination of total and specific IgE, or tryptases in specific clinical study cohorts, or the extraction, purification and isolation of specific molecular-weight factors from specific seafood or mammalian or plant samples suspected of being allergenic and submitted to the unit for investigation using polyacrylamide gel electrophoreses, Western blotting and ELISAs.

Other techniques include iso-electric focusing, immunofluorescence gel diffusion, haemolytic assays, cytokine assays, or specific staining using monoclonal antibodies and setting up necessary controls for new assays to ensure reproducibility and credibility of the data submitted for publication.

ESSENTIAL REQUIREMENTS

To function as an allergology research unit, specialised skills and techniques are required. A qualified technologist and trained laboratory assistant is essential. Competency is required in routine total IgE determination, specific IgE determination by ImmunoCAP and ELISA serum tryptase, sputum microscopy, cellular allergy stimulation test (CAST) ELISA (or flow CAST) as a baseline for routine clinical specimens. To investigate novel allergens competence is required in allergen extraction, homogenisation purification, endotoxin detection, polyacrylamide gel electrophoresis, Western blotting, iso-electric focusing, immunodetection by immunofluorescence and freezing and thawing of samples for stability and reproducibility testing.

Essential equipment includes standard laboratory bench-top equipment, e.g. Gilson micro pipettes, cryo-vials, 4°C, -20°C and -80°C refrigerators, cold centrifuges, Page gel sets, Western blotting sets and power packs. Also essential are ImmunoCAP auto-analysers, water baths, shakers, a chemical balance, backup generators, timers and alarms and an up-to-date computer system to control, analyse and store collected data. Photographic equipment is essential. The laboratory also has to subscribe to external quality control and international reference systems for IgE determinations (e.g. Nequas). A Burkard spore trap and a high-quality microscope are essential for aerobiology.

CHALLENGES FOR FUTURE RESEARCH INTO INDIGENOUS ALLERGY RESEARCH

The two greatest challenges to sustain the productive research which has taken place over the past 22 years are staff and funding. Although in the past, for several years, an allergy technologist post was funded by ALLSA on a year-to-year basis, for general basic allergy research, this practice changed to 'project-specific funding', based on competitive application for research funding, for specific well-motivated projects. Although this has worked well for some projects, laboratories cannot be maintained by ad hoc projects, but need to be run by stable staff, readily available to assist with clinical case evaluations as they arise, who maintain assays, quality control, care of equipment and reagents and have highly specialised experience to investigate and identify novel allergens. Therefore, if there are no funded projects, these units would have to close down.

The laboratory technologist and aerobiologist at the Cape Town Indigenous Allergy Research Unit are currently supported by funds generated from clinical trials or laboratory work at the University of Cape Town Lung

Institute. Specific projects funded by the ALLSA research grants, or specific MRC- or NRF-funded projects are essential to sustain this service.

An allergy research unit also needs postdoctoral students and knowledgeable supervisors with both clinical and laboratory skills to evaluate projects with potential collaborators, to develop cost-effective protocols to investigate specific allergological questions in the laboratory, to answer the clinical questions posed. The Clinician Scientist Supervisor needs to be able to submit proposals for ethics approval, and to be knowledgeable about all the laboratory techniques employed in the fields of immunology, biochemistry and molecular biology, to ensure that the allergology laboratory is able to conduct competitive and publishable research, in international peer-reviewed journals.

It is now in our specialty's interest to prioritise the continuation of what has been a unique indigenous research programme on the African continent for allergologists in Africa. This will require funding of basic research trained staff, at technical, doctoral and postdoctoral levels and the encouragement of young investigators to become interested in, and devote time to, the exciting world of laboratory-based investigative allergy research. The challenge should be a priority for all stakeholders in allergology.

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