

Putting Children First

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Division of Cancer Biology

Genes that perturb the thymic DN to DP transition: implications for leukaemogenesis

D Izon and others.

Objectives

The main objective of our research is to study and uncover oncogenes responsible for T cell leukaemogenesis by focusing on the critical CD4-8- (double negative; DN) to CD4+8+ (double positive:DP) transition. Secondly, this research aims to identify the downstream signalling pathways responsible for transmission of oncogenic potential. Ultimately, this knowledge can be used to design leukaemia-specific drugs which have lower side effects and increased efficacy than current therapies.

Aims

1. To establish the role of the Wnt signalling pathway in T cell development.
2. To establish co-operation/antagonism of Wnt and Notch genes in T cell development.
3. To establish co-operation/antagonism of Ras and Notch genes in T cell development.
4. To discover genes required for the CD4-8- (double negative:DN) to CD4+8+ (double positive:DP) transition using retroviral cDNA library rescue:significance for leukaemogenesis.
5. To elucidate specific downstream signalling pathways from Aims 1-4 using transient transfection in cell lines. Developing thymocytes will also be similarly interrogated by DNA particle bombardment into foetal thymic organ culture (gene gun FTOC).

Hypotheses

Based on previous research it is hypothesised that Wnt genes will accelerate the DN to DP transition. Genetic interaction in *Drosophila* has strongly suggested that the Wnt and Notch pathways interact. It is expected that they will also interact in T cell development. However, this will be the first time it has been directly tested in T cell differentiation. It is already known that the Ras and Notch pathways interact. Whether they are antagonistic or complementary in T cell development remains to be determined. The major focus of analysis will be the DN to DP transition as the genes which rescue pre-TCR deficient DN precursors to the DP stage have been demonstrated to induce T cell leukaemogenesis. Consequently, to uncover new genes operating at this important checkpoint we will utilise retroviral cDNA library rescue of pre-TCR deficient precursors to identify their downstream signalling pathways.

Potential Significance

Elucidation of all the genes responsible for the DN to DP transition will help develop a molecular signature of T cell oncogenesis. Additionally, illumination of the signalling pathways utilised by such genes will provide a foundation for

development of leukaemia-specific therapeutic treatments. This rationale has been successfully demonstrated in Gleevec treatment of myeloid leukaemia through inhibition of the abl kinase pathway. Therefore, this research, by focusing on T cell oncogenes and their concomitant signalling pathways, seeks to mirror the success achieved with Gleevec in myeloid leukaemia.

SCL Expression & Function in the Central Nervous System

Anke van Eekelen, Cara Bradley, Elena Takano and Glenn Begley.

SCL is expressed in the central nervous system (CNS) of the mouse during normal development and in adulthood and is highly regulated by a specific 3.8 kb regulatory DNA sequence stretching from -0.9 kb upstream of the SCL coding region to exon 3 (0.9E3 regulatory sequence). We hypothesize that such a highly regulated expression of SCL in the murine brain implies an important, as yet undefined role for SCL in neural cells.

We initially performed a neuroanatomical mapping study to document the precise pattern of SCL expression in the CNS. This investigation was published this year in *Neuroscience* 122 (2003), p 421-436. To further identify the neurochemical phenotype of the SCL-expressing neurons in mid and hindbrain, we utilise brain sections of transgenic mice positive for 0.9E3creER(T) and R26R-EYFP, in which the EYFP fluorescent cells in CNS represent the SCL expressing neurons. These sections are then co-immunostained for other neurotransmitters, peptides and/or specific CNS-receptors and analysed by confocal microscopy. Ongoing morphological analysis of this kind is aimed at the elucidation of a range of cellular phenotypes that SCL expressing neurons in different brain regions may have and which potentially lead to the understanding of the role SCL has in different functional brain circuits.

To directly address SCL function in CNS, we also generated "conditional" transgenic mice, in which we ablated SCL in a tissue specific manner. This spatial control is required, since full SCL knockout mice are not viable beyond embryonic day 10 due to the lack of yolk sac haematopoiesis. It is therefore essential to create conditional transgenics in which SCL can be deleted only in the mouse brain, leaving haematopoietic cells intact. The conditional transgenic mouse model we apply in our research is based on the cre recombinase - loxP system to delete a specific DNA sequence by recombination. Mice with loxP sites flanking the SCL gene (the *floxed* SCL-allele) were crossed with mice that express cre recombinase under control of the nestin promoter (Ncre-mice). The intercross between SCL *floxed* mice and nestincre-mice generated offspring, in which SCL is deleted from the onset of Ncre-expression in the CNS onwards (E8.5-9). Mouse phenotype analysis as a result the specific ablation of SCL in CNS in this loss of function model confirmed that embryonic death could be prevented in this conditional knockout as opposed to the full SCL knockout mouse model, in which SCL null embryos did not survive beyond E10 due to the lack of blood formation. However, survival rate shortly after birth was severely affected: 25% of the conditional SCL knockout pups died at postnatal day 1 or 2 and at least another 35% died around the age of weaning at post natal day 21. Moreover, the surviving pups were significantly growth retarded, revealed

hyperactive and stereotype behaviour and their hindbrain morphology was affected. These results imply a specific role for SCL as a neurogenic transcription factor in brain development and we aim to investigate SCL function during embryogenic neurogenesis into more detail.

Division of Cell Biology

Overview

The Division of Cell Biology continues to focus ongoing research into the aetiology and pathogenesis of immunoinflammatory diseases in childhood, in particular atopic asthma.

This work is driven via the creative efforts of several teams of senior and junior scientists, and involves separate but complementary research streams in areas of human (in particular neonatal) immunology and in experimental animal models. An important element in the Division's research program is the close integration which has been achieved with our colleagues in clinical research, exemplified by our collaborative birth cohort studies carried out in conjunction with the Division of Clinical Sciences. An exciting development at the end of 2003 which stems directly from these collaborations was in-principle approval of funding from the Immune Tolerance Network within the US National Institutes of Health (NIH), for the first international clinical trial on asthma prevention in children using an active "vaccination" approach. The theoretical basis for this prophylactic approach was developed initially from information obtained in the Division's animal-based experimental research program, and further refined in follow-up studies on human infants and young children, involving collaborators throughout the Institute and in departments based in Princess Margaret Hospital for Children. During 2004 the trial protocol will undergo further development involving input from NIH and clinical collaborators in Europe and the US, prior to presentation for final approval to the US Food and Drug Administration. Providing this submission is successful, the trial is scheduled to commence in early 2005.

HUMAN STUDIES

Identification of biomarkers predictive of risk for allergic sensitisation in cord blood

J Rowe, T Heaton, D Suriyaarachchi, M Serralha, H Lehmann, M Sharp, BJ Holt, J Tizard, S Yerkovich M Kusel, PD Sly, P G Holt, in collaboration with D Mallon, Fremantle and Princess Margaret Hospitals, S Johnston, Imperial College London, and J Denburg, McMaster University, Canada.

Studies are in progress on a birth cohort of 240 children at high genetic risk (HR) of atopy/asthma, the aims of which are to chart the development of tolerance or sensitisation to inhalant allergens out to age 5 years, and to relate these outcomes to a variety of factors including infection history. Recent findings from these studies suggest that hyperresponsiveness to staphylococcal enterotoxin B (SEB) in cord blood mononuclear cells is associated with risk for subsequent development of atopic dermatitis (AD) in the cohort. A follow-up study on infants with active AD also indicated this association, in particular in relation to SEB-induced production of IL-13. We have also recently reported that the expression of atopy at 2 years in these HR children is associated with high level production of IFN γ . This finding contrasts with earlier reports

from our lab and elsewhere linking depressed IFN γ responses by CD4 $^+$ T-cells with genetic risk for atopy. However, our recent data pertains to responses by CD8 $^+$ T-cells, and we have shown previously that IFN γ gene expression is developmentally regulated markedly differently in these two T-cell subsets. Further studies are in progress to further investigate the role of IFN γ in atopy in children, in particular its potential contribution to tissue damage at sites of allergen challenge in established asthmatics. Additionally, we have established ongoing collaborations with Sebastian Johnston at Imperial College, London, and Judah Denburg, at McMaster University in Canada, investigating (*inter alia*) the relationship between eosinophil precursor number/function in neonates and susceptibility to respiratory infection. Our preliminary findings suggest that the severity of wheezing RSV infections in early infancy may be directly related to eosinophil levels in individual infants, and follow-up studies are in progress to elucidate this relationship in more detail.

Antenatal cytokine production and risk for atopy

C Macaubas, BJ Holt, C Wee, N de Klerk, PD Sly and PG Holt.

We have recently published a series of findings in *The Lancet* which provide tantalising evidence suggesting that expression of the “low cytokine production” phenotype which is characteristic of infants at HR of atopic disease, is also manifested in the foetal compartment. Notably, circulating levels of IFN γ , IL-4 and TNF α at birth are inversely related to risk for atopic and wheezing outcomes at age 6 years. Given that one of the major sources of cytokines released into the foetal circulation comprises cells in the placenta (in particular trophoblasts), these findings suggest that placental function may be an important factor in the aetiology of atopy/asthma. In this context, it is noteworthy that maternal smoking which is known to be a risk factor for infant wheeze, was demonstrated in this study to markedly reduce foetal cytokine production.

Postnatal maturation of Th1 cytokine response capacity

S Yerkovich, T Heaton, J Rowe, PG Holt in collaboration with F Martinez, University of Arizona.

Ongoing studies in the group are focusing upon mechanisms associated with postnatal maturation of Th1 cytokine gene expression capacity, particularly in response to stimuli such as bacterial LPS. Our earlier investigations indicate that between 12 and 18 months postnatal, maturation of Th1 function as measured by IFN γ gene expression in CD4 $^+$ T-cells most commonly accelerates markedly. However, data on other cell types and other Th1-associated cytokines during this period is sketchy, and we have embarked on a systematic program to broaden our knowledge in this area. Genes under investigation include TNF α , IL-12, IL-17, IL-18 and IL-23. All of these cytokine genes demonstrate a high level of developmental regulation, in particular IL-23. We have also noted that IFN γ production capacity within the innate immune system is high relative to that within the adaptive compartment, and we will focus in future studies on differences in gene regulation in the respective cell types. We have also participated in a recent collaboration which has identified polymorphisms in the TLR-4 gene which are associated with variations in systemic responsiveness

to LPS in adult humans, and will follow up these observations with studies in younger age groups.

Studies on the relationships between immune response phenotypes and asthma-related phenotypes in 11 year olds

T Heaton, J Rowe, D Suriyaarachchi, M Serralha, BJ Holt, E Hollams, S Yerkovich, PG Holt in collaboration with S Turner and P Le Souef, Pediatrics UWA and R Aalberse, Sanquin Laboratories, Amsterdam.

Studies are in progress on a birth cohort of 176 eleven year olds, to elucidate relationships between immune response parameters (allergen-specific T-cell cytokine responses; polyclonal T-cell responses; IgE and IgG antibodies) and clinical phenotypes related to asthma and allergy. It is planned to obtain as large a data set as possible incorporating the widest feasible range of immune/clinical parameters, to provide sufficient statistical power to identify previously covert relationships between atopy and asthma symptomatology. Of particular interest in this study is the possibility, suggested by our data in younger age groups, that Th1 cytokine expression in the period beyond that in which allergen-specific Th-memory is developed, may contribute to the pathogenesis of atopy and asthma, as opposed to antagonising the latter. Additionally, we are focusing in a substudy on the nature of T-cell responses and accompanying IgE/IgG4 responses to cat allergen. The focus here is the so called "modified Th2" response which is claimed to be associated in many nonatopics with tolerance to cat allergen exposure.

Differential gene expression studies in atopy and asthma

A Bosco, K McKenna, C Devitt, A Rate, BJ Holt, WR Thomas, PD Sly, PG Holt in collaboration with R Loh, Clinical Immunology, Princess Margaret Hospital. We have initiated two parallel programs aimed at elucidation of CD4⁺ and CD8⁺ T-cell responses to inhalant and food allergens, utilising Affymetrix microarray technology. This technology offers revolutionary possibilities for identification of covert genes which contribute to atopic responses and in turn to disease expression. However, harnessing the technology for studies on allergen/antigen-specific T-cells is challenging, due to the low frequency of specific T-cells in peripheral blood. During 2003, we have focused on improving methodology for identification and subsequent purification of specific allergen-activated T-cells in culture, aiming at increasing the signal-to-noise ratio in subsequent microarray profiling. This daunting task has been successfully completed and the group is now proceeding to detailed kinetic studies employing Affymetrix microarrays and quantitative Taqman PCR in parallel, to identify relevant time points for identification of both "early" and "late" genes during allergen-specific T-cell activation.

Vaccine Studies

These investigations are carried out as a collaboration involving the Divisions of Cell Biology and Clinical Sciences in TICHR, and the Vaccine Studies Group at Princess Margaret Hospital.

Potential immunomodulatory effects of the MMR vaccine

S Yerkovich, J Rowe, T Heaton, D Suriyaarachchi, M Serralha, BJ Holt, J Tizard,

PD Sly, R Loh, P Richmond and PG Holt.

Our earlier studies have identified the period between 12 and 18 months as one of rapid maturation of Th1 function in many children. The possibility exists that MMR vaccination at 12 months may be a contributing factor to the maturation process over this age range. To test this hypothesis, during 2003/4 we are comparing MMR and MMR-V vaccination in groups of infants, and carrying out kinetic studies on vaccine antigen specific and polyclonal T-cell responses, as well as responsiveness of the innate immune system, following vaccination. The results of these studies will be available in mid 2004.

Interactions between vaccines given at sites with common lymphatic drainage

S Yerkovich, J Rowe, T Heaton, D Suriyaarachchi, M Serralha, BJ Holt, J Tizard, PD Sly, R Loh, P Richmond and PG Holt.

This study is being carried out over 2003/4 in five year olds, and will compare vaccine-specific responses in children given the DTaP and IPV vaccines in the same arm versus one in each arm.

Assessment of the role of maternal antibodies in priming of immune responses against Tetanus Toxoid (TT)

S Yerkovich, J Rowe, T Heaton, D Suriyaarachchi, M Serralha, BJ Holt, J Tizard, PD Sly, R Loh, P Richmond and PG Holt.

We recently reported on TT-specific Th-cell cytokine responses in infants, at the 12 and 18 month points in the standard DTaP priming schedule. This study revealed widespread heterogeneity in response patterns throughout the population, but with the common denominator of a Th2 bias in the overall response. In this follow-up, we have assayed the TT-specific IgG content of cryobanked sera from the infants collected at 2 months (prior to the first priming dose), and have sought statistical associations between these titres and subsequent Th-cell memory responses at 12 and 18 months of age. The results of this study will be published in early 2004, and demonstrate a clear positive association between titres of these maternally-derived antibodies and subsequent development of TT-specific Th-memory. Of particular note was the observation that these stimulatory effects of maternal antibody were restricted to the Th2 component of the TT-specific memory response (IL-4, IL-5 and IL-13), and were not observed in the Th1 (IFN γ) response.

Antigen presenting cell function and allergic sensitisation

A Rate, R Taylor, T Hughes, PG Holt and JW Upham

Dendritic cells (DC) are antigen presenting cells that are fundamental to regulation of the immune response. Our studies have focussed on the way in which DC function changes with age, and how this is related to the development and perpetuation of allergic diseases such as asthma.

We have examined DC subsets at 6 and 12 months of age in a large cohort of children, and have shown that the relative proportions of myeloid and plasmacytoid DC subsets in peripheral blood are independent predictors of Th1 function during infancy. Studies over the next 12 months will examine the relationship between these DC subsets and clinical outcomes such as the risk of

respiratory infection and the onset of allergic sensitisation.

In a recently completed study, we have shown that the ability of cord blood monocytes to up-regulate expression of class II MHC in response to IFN γ is also closely associated with *in vitro* immune responses at birth to both allergens and microbial stimuli.

Development of T-cell memory during infancy

A Rate, PG Holt and JW Upham.

DC are a key regulator of immune memory. Our studies have shown that recall memory T-cell responses to both allergens and vaccine antigens are limited by DC activation and maturity. In 12-month-old children, T-cell memory to the vaccine antigens tetanus toxoid and diphtheria is often difficult to detect. However, we have found that addition of autologous, cytokine derived DC markedly enhances the magnitude of these responses, unmasking covert memory that would not be recognised using standard cell cultures.

Other studies are examining the ontogeny of novel Th1 cytokines in regulating immune responses to paediatric vaccine. The ability to longitudinally examine T-cell memory to defined antigens, and to relate this to clinical endpoints, is a particular strength of these large cohort studies.

Allergen uptake mechanisms

T Hughes and JW Upham.

While there is some information available in the literature concerning the mechanisms by which DC and other antigen presenting cells take up allergens, as opposed to other antigens, this area remains relatively unexplored. A recently commenced PhD project is examining these mechanisms in detail, focussing on the regulation of allergen uptake, the receptors that are involved, and comparing these processes in adults and young children.

ANIMAL MODEL STUDIES

Airway mucosal DC maturation is controlled by local T cell interactions following repeated antigen challenge

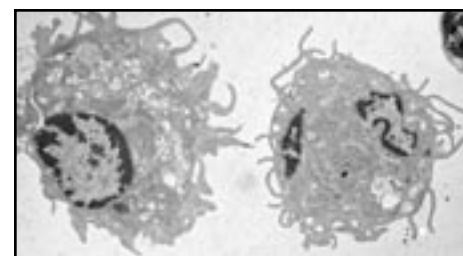
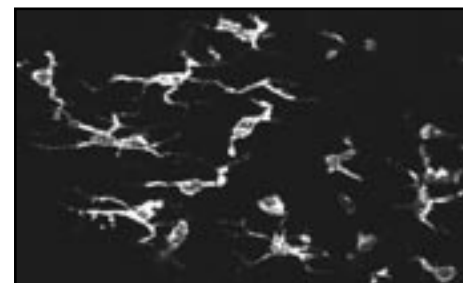
DH Strickland, JA Thomas, PA Stumbles, PG Holt.

A rat asthma model is currently being employed to investigate the effect of repeated antigenic exposure on airway mucosal dendritic cell (AMDC)-T-cell interactions. Consecutive daily OVA-aerosol challenges over a prolonged period (>4 days) prevents the rapid maturation of AMDC previously observed after a single challenge, as assessed by the reduced *ex vivo* expression of the co-stimulatory molecule CD86 after final multi-aerosol. In an *in vitro* model of this process, co-culture of naive AMDC with CD4⁺ T cells from draining lymph nodes of OVA-immunised animals resulted in the up-regulation of CD86 expression within a similar time frame as observed *in vivo*. Furthermore, these increased CD86 levels were abrogated when airway-derived CD4⁺ T cells from multiple aerosolised animals were added to the cultures. These studies demonstrate that AMDC maturation is controlled locally by CD4⁺ T cells in multi-aerosolised animals. Future investigations will focus on the characterisation of putative regulatory T cells within the respiratory tract.

Characterisation of mouse respiratory tract antigen presenting cell (RT-APC) populations and their response during allergic airway inflammation

C von Garnier, DH Strickland, M Wikstrom, M Smith, JA Thomas, S Napoli, PG Holt, PA Stumbles in collaboration with L Filgueira (Dept. of Anatomy, UWA) and D Turner, G Zosky and PD Sly (Clinical Sciences, TICHR).

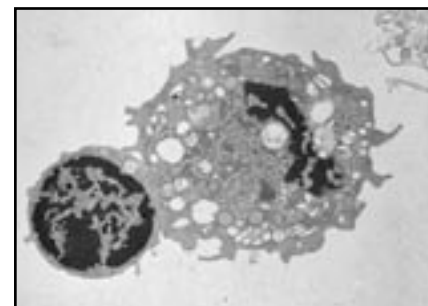
Our previous studies in the rat have shown that RT-APC populations, of which dendritic cells are a key component, are essential in directing the immune response to inhaled antigens. In the mouse, however, the identification of RT-APC subpopulations and their function within the different respiratory tract compartments (conducting airways, peripheral lung parenchyma) remains incompletely understood, both under normal conditions and during allergic airways disease. Our on-going studies of respiratory APC from the conducting airways and lung parenchyma of BALB/c mice have revealed a previously unrecognised diversity of RT-APC populations based on expression of the archetypal DC marker CD11c in conjunction with MHC class II and other cell-surface markers (CD11b, CD2, CD8a, CD40, CD45RB, CD54, CD80, CD86, Ly6G/C, DEC-205, F4/80, B220). Morphological studies by transmission electron microscopy, bone-marrow repopulations studies and functional assays of endocytic potential and T cell activation capacity have revealed these subsets to include rapidly migrating populations of CD11c⁺ and CD11c⁻ dendritic cells, long-lived CD11c⁺ lung tissue macrophages, B cells and potential DC progenitor populations. Furthermore, these subsets show varying degrees of compartmentalisation between the airway mucosal surfaces and lung tissue, suggesting differing capacities for antigen processing and presentation at these sites. Utilising a murine model of allergic asthma, in which increased tissue and central airway resistance is observed after aerosol allergen challenge, the response of RT-APC subsets, both in terms of recruitment kinetics and changes in phenotype, is again compartmentalised and dependent on the time after allergen challenge. Thus at early time points, the principal changes are confined to the mucosal surfaces of the conducting airways, whereas at later times RT-APC of the parenchymal lung tissue are affected. Future work will focus on the role of RT-APC from different lung compartments in regulating local T cell activation.



Tracking RTDC – T-cell interactions following inhaled allergen challenge

M Wikstrom, M Smith, PG Holt, PA Stumbles.

As part of our investigation into the way antigens are handled by RT-APC, we have been using adoptive transfer of ovalbumin-specific TCR transgenic CD4⁺ T cells to visualise the primary T cell response to inhaled antigen. By combining activation markers and intracellular cytokine detection with cellular tracking, we have been able to gain some valuable insights into the early and late events occurring in the draining lymph nodes and the lung. A single dose of allergen (ovalbumin) was sufficient to activate the majority (>85%) of transferred cells in the draining lymph nodes within 24 hours, yet only a fraction (<10%) were ever primed to produce IL-4 or IFN γ . Adding the known airway adjuvants LPS or cholera toxin (CT) did little to obviously enhance effector priming or cytokine production; rather, they increased the amount of proliferation in the local nodes



and the number of antigen-specific T cells migrating into the lungs. However, CT induced the activation of a population of CD4⁺ T cells capable of mediating an eosinophilic inflammatory influx into the lungs following repeated inhaled allergen challenge, suggesting either altered T cell priming in lymph nodes or antigen presentation in the lungs. The majority of antigen-experienced T cells recovered two or three weeks after immunisation was concentrated locally expressing high levels of CD44, and there were more of these persistent cells when adjuvants were used. Our future work will concentrate on how the persistence of antigen-experienced T cells influences local responses in the airways.

Influenza infection, RT-DC function and the post-natal development of RT-DC networks

PA Stumbles, M Smith, I Tobagus, JA Thomas, PG Holt in collaboration with C James (Murdoch University) and D Turner, L Bozanich, PD Sly (Clinical Sciences, TICHR).

Influenza virus is primarily an infection of the mucosal surfaces of the upper and central airways. As such, airway mucosal DC (AMDC) are likely to represent the principal cell type responding to viral infection. In addition, AMDC networks are slow to develop from birth and thus may be susceptible to disruption following early-life infections. To investigate this, we have analysed the response of AMDC to influenza infection in adult mice and also the impact of early-life infection on development of the adult phenotype. Following acute viral infection of adult mice, rapid activation of AMDC was observed as indicated by upregulation of the co-stimulatory molecule CD40. In contrast, DC populations of peripheral lung were slower to become activated: the principal response at this site was the rapid depletion of a population of pulmonary macrophages. Infection of infant mice at 3 weeks of age had an impact on the development of RT-APC, subtly but consistently altering the phenotype and distribution of adult subsets both within the airways and parenchymal lung tissue. Future studies will focus on the T-cell activating function of AMDC during acute infection in adults and infants and on the functional consequences of early life infections in terms of the capacity to develop normal immunological tolerance to inhaled allergens as an adult.

Staff and Students

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Iriani Tobagus BSc(Hons) PhD candidate

Visiting Research Fellow

Christophe von Garnier MD, Respiratory Medicine Unit and Department of Internal Medicine, Basel University Hospital, Switzerland

Invited Presentations during 2003

PG Holt. Keynote Speaker: The hygiene hypothesis: atopy and asthma - Keystone Symposium, Keystone Colorado.

PG Holt. Symposium Speaker: Postnatal maturation of immune function in human infants - Nestle International Workshop on Infant Nutrition, Lausanne.

PG Holt. Symposium Speaker: Development of T-cell memory against inhaled allergens in early life - American Thoracic Society Annual Congress, Seattle.

PG Holt. Symposium Speaker: The role of immunological developmental factors in the aetiology of allergic disease - European Academy of Allergy and Clinical Immunology, Paris.

PG Holt. Symposium Speaker: Heterogeneity of immune response phenotypes amongst atopic children - European Academy of Allergy and Clinical Immunology, Paris.

PG Holt. WAO Symposium Speaker: Early immunological influences in the aetiology of allergic disease - International Congress of Allergology & Clinical Immunology, Vancouver.

PG Holt. Meet the Professor Session: The Th1/Th2 paradigm - International Congress of Allergology & Clinical Immunology, Vancouver.

PG Holt. Workshop Participant: Perinatal events in allergy - APAPARI Workshop on Allergies and Asthma in young children in South East Asia, Singapore.

PA Stumbles. Symposium speaker: Complexity and dynamics of antigen presenting cells in the mouse respiratory tract - 33rd Annual Scientific Meeting, Australasian Society for Immunology, Perth, Western Australia.

PA Stumbles. Symposium speaker: Distribution and Genetic Regulation of Dendritic Cell Subsets in the Mouse - American Association of Immunologists Annual Meeting, Denver, Colorado, USA.

PA Stumbles. Plenary co-chair: New advances in leukocyte activation and differentiation - 33rd Annual Scientific Meeting of the Australasian Society for Immunology, Perth, Western Australia.

External Committees

PG Holt. Scientific Advisory Board, Jenner Institute for Vaccine Research, U.K.

PG Holt. Councillor, International Society for Mucosal Immunology.

PG Holt. International Scientific Board, Pharmacia Allergy Research Foundation.

PG Holt. WHO/IAACI Consulting Group: Primary Prevention of Allergy and Asthma.

PG Holt. Australian Academy of Sciences: Sectional Committee for Biochemistry, Molecular Biology & Immunology.

PA Stumbles. Organising committee, Australasian Society for Immunology Annual Congress, Perth, Western Australia.

JW Upham. Committee Member, Grant Review Panel, National Health & Medical Research Council of Australia.

JW Upham. Convenor, Asthma Special Interest Group, Thoracic Society of Australia & New Zealand.

JW Upham. Committee Member, Program Committee, American Thoracic Society.

Division of Children's Leukaemia and Cancer Research

Overview

Cancer affects approximately 1 in 7,000 children 0 to 14 years of age. Without medical treatment, most childhood cancers are fatal. In contrast to adult cancers, paediatric cancers are of a much wider spectrum, with more than half of them affecting cells of the immune system and the central nervous system, while only a minority involve epithelial cells. Thus, the most common malignancy in children is leukaemia, followed by brain tumours. In order to find better therapies for children with cancer, the Oncology Total Care Unit at Princess Margaret Hospital (PMH) and our division at the institute are both members of the largest study group into these diseases, the US-based Children's Oncology Group (COG).

The research program of the division focuses on childhood leukaemia and brain tumours and comprises three areas. First, it is the identification of genetic alterations which underlie childhood cancers, second, the role of the *HOX11* gene in T-cell acute lymphoblastic leukaemia (T-ALL) and third, the development of a new cancer drug discovery platform. In order to examine the genetic lesions present in the various types of cancer, we make use of the novel microarray technology to determine gene expression profiles. The initial studies involved our panel of established leukaemia cell lines since they are ideal tools for subsequent testing of potential new drugs for the treatment of patients. Such cell lines are essential for the assessment of agents for future cancer therapy, primarily for *in vitro* studies to identify candidate drugs and in xenograft models to measure drug efficacy *in vivo*. Currently, a large study on primary patient specimens is in progress with the ultimate aim to achieve improved risk stratification for ALL patients. The gene expression profiles of relapsing and non-relapsing patients are determined with the goal to identify critical differences in the leukaemia cells at the time of diagnosis. In a parallel study, specimens taken at the time of relapse are being studied in an attempt to understand the genetic basis for chemoresistance. The first phase of the study demonstrated that it is feasible to analyse small patient specimens that were stored in our tissue bank for more than ten years.

The drug discovery technology group is directed by Dr Paul Watt and collaborates with Drs E Golemis and I Serebriiski, Fox Chase Cancer Centre, Philadelphia, USA. The team is using yeast reverse two hybrid screening methods for the development of a platform to identify new cancer drugs. The project is focusing on the commissioning and validation of a genetic system for isolating specific peptide inhibitors of oncoprotein interactions which is sufficiently robust for routine industrial application. The system called '*the discriminator blocker trap*' targets oncoprotein interactions in order to screen libraries coding for peptides for their capacity to block such interactions. This model has potential application for future drug screening for better therapies for cancer as well as other diseases. It is also a particularly valuable tool in

this post-genomic era for the validation of targets which are involved in multiprotein complexes. The competing knockout mouse or RNAi technologies are unable to selectively eliminate particular complexes of a given protein and therefore are not as useful for the validation of target complexes. Another unique feature of our yeast genetic system is the ability to eliminate low affinity blockers genetically using a titration feature known as the 'affinity filter'. These technologies are being commercialized by Phylogica Ltd, the first spin-off company from the Telethon Institute for Child Health Research (<http://www.phylogica.com>). The company has been assigned the IP portfolio of 7 patent families relating to drug discovery technologies, including patents granted in the USA and Australia.

MICROARRAY TECHNOLOGY TO ASSESS GENE EXPRESSION

Oligonucleotide arrays for cancer classification: performance evaluation of Robust Multi-array Analysis (RMA) and Random Forest (RF) algorithm

K Hoffmann, AH Beesley and UR Kees in collaboration with MJ Firth and NH de Klerk, Biostatistics and Genetic Epidemiology, Telethon Institute for Child Health Research

Recent developments in microarray technology have proven to be particularly useful for the diagnosis and classification of cancer. In the case of Affymetrix oligonucleotide arrays most investigators use Affymetrix MAS 5.0 for probe expression measurements and data normalization, coupled with supervised learning algorithms such as support vector machines (SVM), artificial neural networks (ANN) and *k*-nearest neighbours (*k*-NN) for subsequent analysis. However, a number of recent studies reported markedly less variable and more reliable gene expression values using, instead of MAS 5.0, a probe-level data extraction algorithm called the Robust Multi-array Analysis (RMA). Similarly, alternative supervised learning algorithms, such as the Random Forest (RF) have emerged. In contrast to many other supervised analytical methods, the RF approach has a built-in reiterative process for cross validation, thus avoiding the need to remove valuable samples from the study population for the purpose of independent cross validation. To evaluate the performance of RMA in combination with RF we used a published microarray data set of 132 acute lymphoblastic leukaemia (ALL) specimens, representing six different ALL subgroups defined by cytogenetic features and immunophenotype. The image files of this data set were subjected to RMA, which involved quantile normalization, background correction and log (base2) transformation for each probe set. Next we identified the genes that varied most in their expression between these subgroups and implemented the decision-tree based RF algorithm to evaluate the ability of these genes to discriminate between the ALL subgroups. Unsupervised clustering using the top subgroup-discriminating genes identified by RF clearly segregated the 132 specimens into their correct subgroups and our RMA/RF approach achieved equal or higher prediction accuracies than previously reported for this data set, thus validating RMA and RF as suitable alternative tools for the analysis of oligonucleotide arrays.

Gene expression levels assessed by oligonucleotide microarray analysis and quantitative real-time RT-PCR – How well do they correlate?

PB Dallas, NG Gottardo, AH Beesley, K Hoffmann, PA Terry, JR Freitas, JM Boag, AJ Cummings, and UR Kees in collaboration with MJ Firth, Biostatistics and Genetic Epidemiology, Telethon Institute for Child Health Research.

Microarray expression analysis is now used widely in biology, and will likely receive broader application as the technology develops. The verification of gene expression data obtained from microarray experiments using independent techniques continues to be an important component of any well-designed microarray expression study. We have examined the degree of correlation between expression scores for 34 genes obtained in 64 specimens using oligonucleotide microarrays and the expression levels for the same genes measured using quantitative real-time RT-PCR (qRT-PCR). Correlations with qRT-PCR data were obtained using microarray data that were processed using two different procedures, Robust Multi-array Analysis (RMA) and the MAS 5.0 algorithm (Affymetrix). Although the overall correlation was statistically significant for the majority of the genes that we examined, the correlation was not statistically significant using either normalisation procedure for 18% (6/34) of genes. These data indicate that definite conclusions cannot be drawn about changes in gene expression levels based solely on microarray data, emphasizing the importance and continuing requirement for the validation of microarray expression results using independent techniques.

GENETIC ALTERATIONS IN PAEDIATRIC LEUKAEMIA

Prognosis in childhood acute lymphoblastic leukaemia (ALL)

K Hoffmann, NG Gottardo, JR Freitas and UR Kees in collaboration with MJ Firth and NH de Klerk, Biostatistics and Genetic Epidemiology, Telethon Institute for Child Health Research and DL Baker, Department of Haematology-Oncology, Princess Margaret Hospital, Perth, Western Australia

Despite the high cure rates, resistant forms of childhood ALL constitute a leading cause of cancer-related morbidity and mortality in children. The clinical outcome measured as 5 year event-free survival (EFS) has reached up to 85% for patients classified as standard risk (SR) and 64-75% for high risk (HR) patients according to criteria defined by the National Cancer Institute (NCI) comprising age and white blood count (WBC) at presentation. However, a substantial number of patients currently classified and treated as SR patients do not respond to the given treatment and continue to relapse, highlighting an urgent need for a more comprehensive risk stratification at the time of diagnosis. Currently, one of the critical prognostic indicators is the response to the given therapy by bone marrow assessment of tumour burden on day 7 or 14 of treatment, or assessment of minimal residual disease upon completion of induction therapy, implying that the genetic make up of the leukaemic cells governs susceptibility to the chemotherapeutic drugs administered.

To identify prognostic markers that have the potential to discriminate chemotherapy-sensitive (non-relapse) and chemotherapy-resistant (relapse) leukaemia patients at the time of diagnosis, we have generated gene expression profiles from 80 patient specimens using Affymetrix HG-U133A microarrays and

analysed the array data using the Robust Multi-array Analysis (RMA) expression measure in combination with a supervised learning algorithm, Random Forest (RF). The initial analysis using hierarchical unsupervised clustering on all probe sets (22,283) separated specimens into their phenotypic groups, T- and B-lineage ALL. Subsequent analysis on the T- and B-lineage subgroups separately identified probe sets discriminating between patients who later relapsed and those that did not. Importantly, reproducible detection of the differential expression levels for discriminating genes was demonstrated by an independent method, quantitative RT-PCR. Current investigations focus on further verification of discriminating genes identified by microarray analysis using quantitative RT-PCR, validation in independent cohorts of paediatric patients, and *in silico* testing using published ALL microarray data sets.

Our findings demonstrate that a comparison of gene expression profiles at the time of diagnosis can identify genes that discriminate chemotherapy-sensitive (non-relapse) and chemotherapy-resistant (relapse) leukaemia specimens. Such a set of genes will be useful in a more risk-based stratification of patients, by recognizing firstly those patients currently classified as SR, but who are likely to relapse and therefore warrant more intensive therapy, and secondly patients currently stratified as HR who may not require the intensive therapy regimen currently administered.

Gene expression profiling of childhood pre-B acute lymphoblastic leukaemia in comparison to CD34+ haematopoietic stem cells

JM Boag, AH Beesley, A Cummings, J Ford, JR Freitas and UR Kees, in collaboration with MJ Firth and NH de Klerk, Biostatistics and Genetic Epidemiology, Telethon Institute for Child Health Research

Although the 5-year event free survival for children with acute lymphoblastic leukaemia (ALL) has increased in recent years to 64-85%, many aspects of this disease remain poorly understood. To investigate ALL development and biology, and possibly identify novel drug targets, we compared the gene expression patterns of 22 childhood pre-B ALL patient bone marrow (BM) specimens to 5 haematopoietic stem cell samples, enriched from non-malignant BM by CD34 antibody and magnetic bead selection. RNA was extracted from all BM samples and gene expression profiling conducted using HG-133A oligonucleotide microarrays (Affymetrix). Statistical analysis identified the 100 most significantly up and down regulated genes between the ALL and normal specimens. Through extensive data mining we were able to map interactions between greater than 75% of these 200 genes. Genes involved in key cellular pathways such as apoptosis, differentiation, cell cycle and general cell metabolism were concurrently deregulated in ALL specimens compared to the normal specimens. These observations suggest that leukaemogenesis, and possibly malignant transformation in general, is not the sum of random mutagenic events, but a well-organised system that facilitates the evasion of apoptosis and cell cycle checkpoints. The significance of these 200 genes was subsequently confirmed in an independent cohort of 52 pre-B ALL patients. Additionally, expression levels of 8 selected genes were analysed by an independent technique, quantitative RT-PCR, and found in 7 of the 8 genes to correlate strongly with those levels

observed by microarray. Our data reveal some of the underlying molecular principles driving the development of childhood pre-B ALL, which may ultimately assist in identifying more effective treatments for ALL. To ensure our results are not skewed by our choice of normal control tissue, we are currently extending our analysis to include non-malignant pre-B cells (CD19⁺, IgM⁻), obtained from umbilical cord blood.

Gene expression in relapsed childhood acute lymphoblastic leukaemia (ALL)

AH Beesley, JM Boag, A Cummings, JR Freitas, K Hoffmann, and UR Kees, in collaboration with MJ Firth and NH de Klerk, Biostatistics and Genetic Epidemiology, Telethon Institute for Child Health Research and DL Baker, Department of Haematology-Oncology, Princess Margaret Hospital, Perth, Western Australia

Despite tremendous improvements in therapy, resistant forms of childhood acute lymphoblastic leukaemia (ALL) constitute a leading cause of cancer-related deaths in children. The goal of this study is to characterize the genetic profile of drug-resistant leukaemia cells to better define the biology of relapse and identify new therapeutic targets. We performed microarray (Affymetrix HG-U133A) experiments on pairs of cryopreserved ALL bone marrow specimens taken from patients at both the time of diagnosis and the time of relapse. The gene expression profiles of 11 diagnosis and 11 relapse paired pre-B ALL specimens were compared using a decision-tree based supervised algorithm called the Random Forest (RF) that has a built-in reiterative process. The top 20 ranked genes from this analysis were able to perfectly discriminate between diagnosis and relapse pre-B-ALL samples when tested back by hierarchical clustering or principal component analysis. Significantly, the same was found to be true when these genes were tested against independent sets of non-paired pre-B-ALL (n=61) and T-ALL (n=12) samples. However, infant ALL paired samples could not be separated into diagnosis/relapse groups using these genes, suggesting that the different aetiology and therapy in this group of patients may lead to different mechanisms of relapse. Several of the identified genes have functions relating to multi-drug resistance and assays are being designed to confirm their differential expression by quantitative RT-PCR. The expression of these genes will be examined in a panel of leukaemic cell lines for which we have information regarding sensitivity to commonly used chemotherapeutic agents. These cell lines thus form the basis for a model system to experimentally modify resistance phenotypes through manipulation of the genes identified in this study.

THE ROLE OF *HOX11* IN T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA

The search for genes regulated by *HOX11*

K Hoffmann, DN Dixon, J Ford, UR Kees and WK Greene in collaboration with R Taplin, Division of Science and Engineering, Murdoch University, Perth, Western Australia

HOX11 is a homeobox gene originally identified at a chromosomal breakpoint in T-cell acute lymphoblastic leukemia (T-ALL). It is one of the most frequently deregulated genes in T-ALL, although the precise role of *HOX11* in leukaemogenesis as well as in normal development remains to

be further elucidated. *HOX11* encodes a transcription factor that is thought to exert its oncogenic effect through the dysregulation of gene expression. New technologies such as cDNA microarrays and high density oligonucleotide arrays (Affymetrix GeneChips) provide the tools with which transcriptional cascades controlled by *HOX11* can be elucidated. We therefore examined the effect of enforced *HOX11* expression on gene transcription in multiple cell lineages, including T-cell, erythroid and fibroblast. A number of candidate target genes have been identified and confirmed to be differentially expressed in the presence of *HOX11* by Northern blot and/or semiquantitative RT-PCR. Intriguingly, these genes encode proteins involved in cell growth, chromatin remodelling and cell fate. Luciferase reporter assays have revealed that *HOX11* can transactivate gene transcription from the proximal promoter regions of at least two of the identified genes. Future studies will seek to determine whether these genes are oncogenically relevant in an attempt to elucidate the mechanism by which this homeobox protein promotes tumorigenesis.

Regulation of *ALDH1A1* gene expression by *HOX11*

KL Rice, J Ford, I Kng, UR Kees and WK Greene

In childhood T-ALL aberrant expression of the homeobox protein *HOX11*, a transcription factor involved in cell fate decisions, is a frequent event. However, the mechanism by which *HOX11* exerts its leukaemogenic effect remains unclear. Previous studies have identified two target genes of *HOX11*, namely aldehyde dehydrogenase 1a1 (*ALDH1A1*) and *Fhl1/Slim1* (Greene et al, 1998). We have used *ALDH1A1* as model system to dissect the role of *HOX11* in transcriptional regulation and define its responsive element(s). By employing luciferase reporter experiments we have confirmed that *ALDH1A1* is regulated by *HOX11* and requires a region between -91 and -50 relative to the *ALDH1A1* transcriptional start site. *ALDH1A1* is intriguing because of its demonstrated role in synthesizing retinoic acid (RA), a key modulator of several cellular processes including differentiation. This suggests the possibility that the *HOX11* oncoprotein modulates the expression of the *ALDH1A1* gene, which in turns alters cellular RA levels, predisposing tumour development. The possibility of such a *HOX11-ALDH1A1-RA* pathway in T-ALL was further strengthened by bone marrow studies revealing that, similar to *HOX11*, *ALDH1A1* could perturb cellular differentiation in a lineage-specific manner.

HOX11 associates with pericentromeric heterochromatin in leukaemic T-cells

M Heidari, C Elliot, UR Kees and WK Greene

HOX11 was originally described as a transcriptional regulator aberrantly expressed in tumours with an immature T-cell phenotype. Subsequently, it was revealed that *HOX11* is required for normal spleen development since newborn *HOX11*^{-/-} mice exhibit asplenia. In both its normal and abnormal roles, *HOX11* has been postulated to function by binding regulatory elements within specific target genes to control gene transcription. However, very few genomic targets of *HOX11* have been identified and little is known about its mode of action. We therefore sought to further understand the role of *HOX11* in tumorigenesis by determining the identity of genomic sequences that are

directly bound by *HOX11*. Using a whole-genome PCR and ChIP approaches, we revealed that *HOX11* associates with satellite 2 DNA found at pericentromeric heterochromatin. Moreover, this interaction did not appear to require the DNA binding domain of *HOX11*, suggesting that protein-protein interactions were at least partially responsible for this interaction. Together, these results implicate *HOX11* in the restructuring of chromatin, which may be a key feature of this oncoprotein in terms of both its T-cell transformation and transcriptional regulation functions.

PAEDIATRIC BRAIN CANCERS

The identification of deregulated genes and pathways involved in the pathogenesis of primitive neuroectodermal tumours

PB Dallas, D Holthouse, PA Terry, S Egli and UR Kees

Primitive neuroectodermal tumours (PNETs) are the most common type of brain tumour affecting children. Although survival rates for PNET patients have gradually improved over the last 20 years and the prognosis for those classified as average risk is encouraging, the situation for children with high risk PNETs remains dismal. This situation has arisen largely because the molecular biology of PNETs is poorly understood. This lack of knowledge has severely hampered the development of improved treatment strategies that are urgently required.

Chromosomal abnormalities are a common feature of PNET cells, including rearrangements, duplications, deletions, and amplifications. These and other data suggest that multiple genes involved in the coordination of proliferation and differentiation in cells of the developing brain are deregulated during PNET development. As part of the process aimed at identifying these genes, we have analysed chromosomal aberrations in a panel of PNET cell lines using cytogenetic approaches, representational difference analysis (RDA), and microsatellite mapping using 400 markers spread across the entire human genome. This latter work was undertaken in collaboration with the Cancer Genome Project at the Sanger Centre, Cambridge, UK. To further refine our focus to specific regions of the human genome, we have correlated these data with the expression profiles of our five PNET cell lines and a panel of 23 primary PNET specimens, generated using Affymetrix HG-U133A microarrays. These analyses have led to the identification of several genes of interest that play important roles in the regulation of the cell cycle, embryogenesis, and proliferation. We are currently studying these genes in detail to address their role in normal brain cells and in the regulation of proliferation in our PNET cell lines. We anticipate that our studies will lead to a clearer understanding of the molecular pathways involved in PNET pathogenesis, and ultimately to the design of new and improved treatment strategies.

DRUG DISCOVERY TECHNOLOGY

Developing a novel source of structured peptides from natural protein domains

R Hopkins, NM Milech and PM Watt

Conventional yeast peptide screening involves the use of random conformationally constrained 'aptamer' libraries, which typically yield very few

successful blockers of protein-protein interactions. We have generated 2 new peptide libraries that are derived from a bio-diverse set of 19 compact bacterial genomes in order to capture a diversity of naturally structured subdomains present within protein sequences. The first, the Interacting Peptide Library, consists of 63 million peptides fused to the B42 activation domain, and can be used to isolate peptides capable of binding to a target “bait” protein in a forward yeast two-hybrid screen. The second, the Blocking Peptide Library, consists of over 2 million peptides which can be used to screen for peptides capable of disrupting a specific protein interaction using reverse two-hybrid. These Phylomer™ peptides exploit evolution’s structural information to enhance the number and affinity of blocking peptides obtained. Unlike aptamer libraries, these Phylomers range in size from small oligopeptides to polypeptides of approximately 150 residues and encode both natural open reading frames and random peptide structures.

Using the discriminating blocker trap to identify key residues of a target for rational based drug design

PM Watt in collaboration with R Barr and M Bogoyevich, Department Biochemistry, University of Western Australia

Our system has confirmed the interaction of a peptide (named TI-JIP) that interacts with Jun N-terminal Kinase (JNK), inhibiting its interaction with c-JUN, its phosphorylation substrate. The work of our collaborator, Dr Bogoyevich at the UWA Biochemistry department has established that this peptide can inhibit the enzymatic activity of JNK. When used as the ‘bait’ for two-hybrid screening of two different cDNA libraries, TI-JIP did not undergo extensive interactions, indicating that its interactions are relatively specific towards JNK.

In order to map the domains within JNK required for interaction with TI-JIP, we used mutagenesis of the JNK sequence to generate a library of more than 1 million JNK mutants. These clones were screened using our reverse two-hybrid yeast system (the discriminating blocker trap) for mutants that failed to interact with TI-JIP. Sequence analysis of seventeen non-interacting mutants expressing full-length JNK proteins revealed changes to various regions of the JNK molecule. The mutant pool was restricted to those containing five or less mutations, and this analysis revealed a series of mutational “hot-spots” on the JNK structure. We have constructed a series of 9 point mutants to address the importance of these regions and better define the TI-JIP-JNK binding interface. Such a mutation ‘hot spot’ has been identified which maps to the surface of the JNK protein. This should help clarify the mechanism by which TI-JIP inhibits JNK and may highlight a novel region of JNK to target for structure based drug design.

Targeting the Aurora-2 Serine/Threonine Kinase: A novel approach for the treatment of breast cancer

RM Hopkins, D Shaw and PM Watt

Aurora-2 is a kinase associated with cellular components, such as the centrosomes, that are involved in the cell division. It also helps regulate the G2/M checkpoint in mitosis. An important subset of cancer cells has over-ridden

this G2/M checkpoint, and studies in human patients show that Aurora-2 expression is upregulated in a wide variety of cancers, including colorectal and breast cancer. Thus, the targeted inhibition of Aurora-2 promises an important therapeutic strategy.

In yeast cells, overexpression of human Aurora-2 is toxic. Classical dosage suppressor screens have been used to isolate human proteins that bind to and inhibit Aurora toxicity, thereby facilitating cell survival. We propose to take advantage of this phenomenon to screen for Phylomers able to rescue the lethality associated with over-expression of Aurora-2 in yeast. Phylomers isolated from this screen will then be tested for their ability to inhibit cell proliferation of breast cancer cells *ex vivo* and *in vivo* using a nude mouse model.

Targeting telomerase in breast cancer

M Fear and PM Watt in collaboration with P Leedman, Western Australia Institute for Medical Research, Perth, Western Australia

The telomerase holo-complex consists of multiple protein subunits as well as an RNA component to the enzyme. The telomerase complex as a whole elongates the end of chromosomes (telomeres) in cells and prevents cell death. This is required during development, but when telomerase is aberrantly activated in adult cells it leads to tumours. Approximately 90% of human tumours have an active telomerase complex; therefore it is an important target in the prevention of tumours. The aim of this project is to use a novel variation on the yeast three-hybrid technique to identify peptides which block the interaction of two components of the telomerase complex. These are the RNA component (hTR) and the catalytic component (hTERT).

The novel screening system is now in place, and all components have been tested. An interaction between the two components (hTR and hTERT) can be detected in the yeast, and we have successfully selected a positive control blocked interaction from a background of interacting hTR and hTERT. Therefore the screen for potential therapeutic peptides to block this important interaction is now ready, and it is expected that in the coming months peptides will be identified and tested for efficacy in *ex vivo* assays.

Validating protozoa-specific drug targets using peptides from biodiverse gene fragment libraries

F Sotzik, RM Hopkins and PM Watt in collaboration with U Ryan and S Reid, Murdoch University

This project is a collaborative effort between the Department of Veterinary Biology and Biomedical Science at Murdoch University and the Yeast Group within the Leukaemia and Cancer Research Division of the Telethon Institute for Child Health Research. The aims are firstly to identify novel Phylomer™ peptides able to inhibit dimerisation of α - and β -tubulin proteins isolated from three protozoan parasites; *Cryptosporidium parvum*, *Trypanosoma brucei* and *Plasmodium falciparum* (the cause of malaria). The activity of anti-tubulin dimerisation peptides isolated from the yeast screens will then be evaluated *in vitro* and *in vivo* against a range of clinical isolates. Peptide blockers of tubulin

dimerisation will be used to validate the use of tubulins as a target for the generation of novel anti-protozoan drugs.

Targeting PLZF in acute promyelocytic leukaemia

V Cull and PM Watt in collaboration with JD Licht, Mount Sinai School of Medicine, New York NY, USA

A dual bait version of the yeast two-hybrid system, with the aim of creating a more streamlined way to determine blocker specificity, is being developed in the PLZF-ETO project. ETO and PLZF constructs have been tested for autoactivation and interaction, and the optimal interaction and dual-bait pairs in yeast have been ascertained. Each combination has been assessed for the optimal screen conditions, and thresholds have been set. We have recently performed the first PhylomerTM library screen with the new interacting PhylomerTM library. This should create a novel PhylomerTM sub-set that interact with either PLZF or ETO proteins. From subsequent screening with this new pool it should be possible to more rapidly identify blockers of the PLZF-ETO interaction, as well as identify blockers specific to homodimerisation complexes.

Screening for PhylomerTM disruptors of the Jun-Jun homodimer interaction

M Fear, V Cull, D Dixon and PM Watt

We have used a reverse-two hybrid approach to isolate peptide blockers of the Jun-Jun homodimer complex. The Jun-Jun homodimer is one of the best characterised transcription factor interactions and therefore provides an excellent model for proof-of-principle of the PhylomerTM blocking approach. The PhylomerTM library contained 1.5×10^6 peptides, from which a sample of 300,000 peptides was screened to isolate Jun-Jun dissociators. Two independent recapitulation screens in retransformed yeast yielded 60 positive peptides. This equates to a hit rate of approximately 1 in 5000, significantly higher than reported hit rates from screens for dissociative peptides using random peptide libraries. Moreover, when 30 Phylomers were sequenced from this screen, twice as many were found to be expressed in the natural open reading frame as expected from random selection, suggesting an enrichment for natural peptide sequences. The high hit rate of blocking we observed with PhylomersTM is also consistent with recent estimates of the redundancy of protein folds in nature, which suggest that all proteins are assembled from a limited repertoire of only a few thousand structural motifs. The success of this approach may also be attributable to the presence of stable subdomain structures encoded within the PhylomerTM library. We conclude that PhylomersTM may provide a more enriched pool of potent blockers of protein-protein interactions than conventional random peptide libraries.

Acknowledgments

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Patents arising from this work

Patent Applications from Drug Discovery Technology Group

Patent Family No	PCT Number	Patent Name and Description
1	AU99/00018	A method for detecting proteinaceous inhibitors of protein-protein or DNA-protein interactions: Technology patent and therapeutic uses. Granted Australia. Granted USA. National Phase in Europe and Canada.
2	AU00/00414	Isolating Biological Modulators from Biodiverse Gene Fragment Libraries: Technology patent and therapeutic uses. National Phase Examination in USA, Europe, Canada. Received Notice of Allowance in Australia.
3	US01/07669	Improved Reverse N Hybrid Screening Method: Technology patent and therapeutic uses. National Phase Examination in USA, Europe, Canada and Australia.
4	Awaiting PCT No	Methods of Constructing and Screening Diverse Expression Libraries: New developments to Technology patent. Entered PCT Phase – CIP from AU00/00414) (USSN 10/372003).
5	US60/474465	An Improved Genetic Screen for Interaction Interface Mapping: Technology patent and new application. Provisional Application.
6	Awaiting PCT No	Modulating Screening Thresholds for N-Hybrid Screening: Technology patent. Entered PCT Phase.
7	Awaiting application No	Methods of diagnosis and treatment of allergic immune responses: Technology patent and allergic diagnostic applications. New Provisional.

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Theses Passed

Rachael Brake. Identification of transcriptional control elements of the *HOX11* proto-oncogene in leukaemia cells. PhD University of Western Australia.

External Committees

UR Kees. Chairman of Study COG-B946, Children's Oncology Group, USA.

UR Kees. Chairman of Study COG-B969, Children's Oncology Group, USA.

Invited Presentations

UR Kees. Gene expression signatures in lymphoid tumours. JCSMR, Fenner

Conference, 2003, Australian National University, Canberra, ACT.

K Hoffmann. Array studies in childhood leukaemia: optimization for small specimens and identification of target genes. Technology Advances and Applications of Affymetrix GeneChip Arrays in Medical Research, 2003, Adelaide, South Australia.

PM Watt. Presented for Phylogica at Bio2003 and AusBiotech 2003 in Adelaide and in Washington, respectively.

Division of Clinical Sciences

Overview

2003 has been a year of consolidation for the Division of Clinical Sciences. The second year of our five-year Program grant has seen an increasing number of collaborations between the various groups involved. I am particularly pleased to see collaboration developing between post-doctoral and more senior scientists between the groups. Our major achievements:

a) The genetics of acute asthma

Asthma is the most common presenting complaint for children admitted to hospital in Western Society and respiratory viral infection is the most common precipitating agent for these admissions. Knowledge of how viral infections cause the loss of control of inflammation that characterises acute asthma attacks is therefore essential to providing an understanding of asthma itself. Since both Th1 and Th2 lymphocyte responses are involved in asthmatic inflammation, data is needed that defines how the genetic susceptibility to asthma affects Th1 and Th2 responses during acute attacks. To date studies in this area have been done almost exclusively in induced infections or in cells from asthmatics. In collaboration with Prof Pat Holt, we have commenced the first detailed *in vivo* examination that will assess the genetic, viral, immunological and physiological responses during acute asthma in children. Over 140 children have now been recruited to date. Prof Pat Holt is responsible for the immunological measurements that include assessments of Th1 and Th2 lymphocyte function both during the acute episode and on recovery. Our preliminary results are striking, as for CD14, a gene that is important in the immunological processes leading to asthma, we have been able to show relationships between the C-159T polymorphism, increases in circulating levels of soluble CD14 and the severity of the asthma attack. These data will be presented at the American Thoracic Society meeting in May 2004. The data and biobank that we are establishing will allow many further hypotheses to be tested. This is a unique approach to determining mechanisms in asthma and ours is the first study of this kind.

b) Population genetics relevant to atopy

In collaboration with a Danish group, we have studied genes associated with atopy and asthma in nearly 1,899 Greenland Inuits. We recently found a new polymorphism (ala57thr) in the interleukin-4 receptor that is common in this population with an allele frequency of over 30%. We have screened over 1000 subjects from seven other populations from different locations around the world and found it be present in <0.5% of subjects in five of the populations and completely absent in two of the populations. The new polymorphism is in linkage disequilibrium with ile50val, which has been associated with atopy in several other populations. Interestingly, the new polymorphism has the opposite effect on atopy and gives rise to the possibility that it has arisen in the Inuits to counter the effects of the other polymorphism, although a founder effect could not be ruled out at this stage. Important features of our data are that finding

an allele that has evolved to counter the effects of another polymorphism has not been observed before and the presence of a polymorphisms that is very common in one population, but almost absent in all others is usually not seen in human genetics. Functional studies are now planned.

Early life risk factors for asthma and allergic sensitisation

Based on the work from the Program, we have been successful in obtaining funding from the Immune Tolerance Network, a body established with funding from the National Institute of Allergy & Infectious Diseases (USA) to conduct the first study of primary prevention of asthma and allergic sensitization using Buccal Mucosal ImmunoProphylaxis. This will be a large multi-centre, multi-national study co-ordinated by us (Holt & Sly). The study will use clinical, Physiological (lung function measured by forced oscillation in preschool children) and Immunological methods we have developed.

The demonstrations that levels of cord blood cytokines are vulnerable to environmental exposures, ie. Maternal smoking, and predict asthma and allergic outcomes at six years of age (published in *The Lancet*) have indicated that the materno-placental unit and its responses to environmental exposures is likely to be an area of fruitful research in the future.

Allergen studies

- a) Phage display technology has been used to identify a peptide of Der P2 that has been used as a monovalent immunogen to induce Der P2 antibodies. Since the monovalent peptide cannot crosslink IgE on mast cell receptors it has the potential to be developed as a non-anaphylactic therapeutic agent.
- b) Demonstration that the major dust mite allergens Der P1 and Der P2 produce more Th-2 cytokines but the same level of Th-1 cytokines than minor allergens from the PBMC of allergic and non-allergic subjects.
- c) 34% of subjects with allergic disease produce IgG4 antibody to conserved outer membrane protein antigen P6 of *Haemophilus influenzae*, while non-allergic subjects only make the expected IgG1 Th-1-type subclass. This is the first demonstration of different immune responses to infections in allergic subjects.

Physiological assessments

Major advances in this field have included:

1. Refinement of the measurement of airway tone in mice *in vivo*
 2. Extension of oscillatory methods to detect the first antiresonance in rats and in adult humans. We are developing new parameters that reflect airway properties and have the potential to allow the direct study of the mechanical interdependence of airways and lung tissues.
 3. The ability to measure lung function in neonatal mice as young as 5-7 days old and weighing as little as 5g. These techniques give us the ability to directly measure the effects on viral infections and other environmental exposures on the growth and development of the lungs.
- Σ The translation of measurements of lung function in preschool-aged children from the research to the clinical domain. We have demonstrated that standard spirometry and the standard outcome variable FEV1 is not suitable for use in

these children (American Journal of Respiratory and Critical Care Medicine in press), but that forced oscillation can be used successfully in this age group.

There have been few staff changes this year, however, we are pleased with the addition of Graeme Zosky (post-doctoral scientist), Cathy Pienaar (Research Assistant), Hiliary Patterson (Research Assistant) and Takayoshi Fukushima (Clinical Research Fellow) to our group. In addition we have been lucky to attract Dr. K.E. (Bill) Finucane as an Emeritus Research Fellow. Bill has a wealth of knowledge and experience in classic physiology and is a great addition to our group.

The research directions of the group remain largely the same. We are becoming increasingly interested in Children's Environmental Health and continue to work closely with groups within the World Health Organisation and National Institute of Environmental Health Sciences (USA) in this area. We have also increased our efforts in studying the long-term effects of viral infections in early life, with an increasing focus on host-response factors.

RESPIRATORY PHYSIOLOGY

The relationship between viral lower respiratory infections in early life and subsequent asthma

Rachel Collins, Debra Turner, Zoltan Hantos, Peter Sly.

The aim of this project is to determine the relationship between viral lower respiratory infections associated with wheeze (wLRI) in early life and the subsequent development of asthma. The two most common causes of wLRI in the first years of life are respiratory syncytial virus (RSV) and parainfluenza (PF) virus. Epidemiological studies have suggested that both viruses can cause abnormal lung function in the short term, but that RSV may be associated with long-term abnormalities of lung function and wheezing. Administration of these viruses in a murine model will enable us to examine whether or not there is scientific support for these epidemiological associations. To date, the acute phase of RSV infection has been characterized in mice infected as juveniles (3wk) and adults (8wk). Both adult and juvenile mice were extremely hyperresponsive to bronchoconstrictor challenge at 5 and 7 days post RSV infection. This response had disappeared by 21 days with resolution of infection. The degree of hyperresponsiveness did not correlate with the degree of inflammation in the lungs. Adult mice showed a small but significant increase in inflammatory cells in the lung during the acute phase of infection, however there was no increase in cells in juvenile mice. Neonates have not been studied to date due to difficulties in making accurate measurements in such small animals. We hope to overcome these limitations in 2004.

Long term changes in lung function are currently being assessed in mice infected as neonates, juveniles and adults. Measurement of lung function and airway tone are underway in mice 4, 8 and 24 weeks post infection at each of the three age groups. Preliminary results from these studies indicate that differences in lung function may indeed be present up to 24 weeks following infection.

Mechanisms of respiratory disease following influenza virus infection

Liz Bozanich, Debra Turner, Peter Sly, Phil Stumbles.

The aim of this project is to determine the relationship between viral lower respiratory infections associated with wheeze in early life and subsequent asthma. This project runs in parallel with the RSV and PF project discussed elsewhere. Influenza virus is an important cause of respiratory morbidity and mortality world-wide. However, information is very limited as to the basic mechanisms of the lung disease seen following infection with influenza virus. The hypothesis for this group of studies is that respiratory consequences will be seen in the short term following influenza virus infection but long-term dysregulation of airway function will not be seen. In the parallel studies with RSV, we hypothesise that both short and long term effects will be seen, especially when the infection occurs early in life. Adult (8wk) BALB/c mice were inoculated with influenza A virus (H1N1) and monitored for the duration of the acute phase infection. Clinical illness was induced, measured by weight loss and an assigned clinical score, which was maximal at day 9 post-inoculation and had resolved by day 21. We have mapped the profiles of clinical illness, inflammatory cells, cytokines and the viral load during this time course. Influenza infection was found to elevate baseline airway & tissue mechanics at 4 days post inoculation, with tissue responses persisting into day 9. These changes correspond to the kinetics of viral load and the subsequent clinical symptoms. Further work will concentrate on the effect of age of infection and the long-term effects of virus exposure. Dose ranging studies will also be undertaken in 2004 in order to determine an optimal level of infection for each age group.

Bacterial modification of the allergic response in the sensitized lung

Debra Turner, Jennifer Burchell, Peter Sly.

Environmental exposure to bacterial endotoxins is thought to protect against development of atopic disease. Previous work in both rats and humans suggests that the timing of bacterial lipopolysaccharide (LPS) exposure is very important in terms of how LPS modifies the response to allergen, although the exact mechanisms are unclear. Understanding the cellular and molecular mechanisms that contribute to the apparent immunosuppressive effects of bacterial products on the allergic response has important clinical implications for treatment and prevention of allergic disorders. The aim of this project is to characterize the inflammatory effects of LPS exposure and investigate the modifying role of LPS on allergic responses. Naïve mice were used to characterise the inflammatory response to both single and multiple LPS exposures. Results show that a single LPS aerosol induced an immediate inflammatory response, as early as 2 hrs, accompanied by an altered cytokine profile but no airway hyperresponsiveness (AHR). In contrast, multiple LPS exposures enhanced responsiveness to inhaled methacholine in both the airways and tissues ($p < 0.05$) despite a down regulation of the inflammatory response. To confirm the specificity of this down regulation, mice exposed to multiple LPS aerosols were then challenged with Zymosan, a yeast product which uses TOLL-like receptor (TLR)-2 signaling pathways. Multiple exposure to LPS did not alter Zymosan-induced inflammation, suggesting the down-regulation is confined to the TLR4 pathways. TLR4 depleted mice had decreased inflammatory responses

and attenuated AHR compared to TLR4 intact mice. We were unable to show differences in TLR-2, -4 or -9 expression between LPS and control mice. No differences were seen in nitrite expression between LPS and control mice. We conclude from the work so far that a single LPS exposure induces neutrophilic inflammation and modifies the cytokine profile, while multiple exposures are needed to induce AHR in mice. LPS-induced changes appear to occur via TLR-4 signalling pathways. NOS does not appear to mediate LPS-induced inflammation. Ongoing work will examine the interaction of LPS responses and allergen responses.

Determining airway tone and tissue properties in mice

Rachel Collins, Cindy Thamrin, Debra Turner, Zoltan Hantos, Peter Sly.

Mice are becoming increasingly popular for the study of lung diseases. However, informative measures of respiratory mechanics present special challenges. When studying airway diseases, measurements of airway mechanics, including measurements of airway tone, are needed to adequately explore disease mechanisms. This study involves the use of adult mice in which respiratory impedance (ZRS) was measured during slow constant-flow inflation and during quasi-exponential relaxed deflation. Oscillatory signals were generated by a loudspeaker and delivered to the mice via a wave tube. Various multi-component signals (range 2–38Hz) were evaluated. Mechanical parameters were obtained from single frequencies or by fitting the constant phase model to multi-component spectra. Quasi-static pressure-volume curves were derived from pressure and low-frequency wave tube net flow measurements. Preliminary results show that volume-dependence of airway resistance showed changes consistent with a decreased airway tone after deep inspiration, which suggest that airway tone can be successfully measured in mice *in vivo*. Further measurements have successfully been obtained in mice undergoing states of altered airway tone, via administration of either methacholine (increased tone) or atropine (decreased tone) or by cutting the vagus nerves (decreased tone). The ability to measure airway tone has implications for future measurements of lung mechanics in rodent models of asthma and may also lead to the development of a new technique for determining airway tone in infants.

Measurement of lung function using broadband forced oscillation

Cindy Thamrin, Zoltan Hantos, Peter Sly.

The forced oscillation technique (FOT) is a non-invasive method of measuring lung function which is advantageous over other pulmonary function tests in that it requires little or no participation from the subject. The method lends itself to sophisticated modeling, providing detailed information about the mechanical behaviour of the respiratory system. At present, measurements of FOT are taken during brief periods of apnea in the subject. This is because spontaneous breathing potentially obscures the frequency spectrum obtained from the measurement data. Using higher frequencies allows measurements to be obtained over a shorter period of time. This enables the superimposition of the oscillatory signal upon the natural breathing patterns of the subject, which simplifies the technique and makes it more readily useful in young age groups. In this project we aim to expand the current FOT by examining lung mechanics

over a broader band of frequencies, while studying a phenomenon known as antiresonance. Work so far has been carried out in rats, where it has been noted that the first occurrence of antiresonance is more likely to be relevant to airway and tissue impedances than airway wall properties. Subsequent work will be carried out in human adults and infants to determine the suitability of antiresonance as an assessor of respiratory mechanics.

Mechanisms of persistent airway inflammation and airway remodeling

Debra Turner, Neil Carroll¹, Alan James¹, Prof Rakesh Kumar². ¹Pulmonary Physiology, Sir Charles Gairdner Hospital, ²School of Pathology, University of NSW.

The traditional paradigm of allergic inflammation consists of specialised antigen presenting cells presenting antigen to the immune system in organised lymphoid structures (lymph nodes), distant from the airway wall. This relies on a homing of primed lymphocytes back into the airway under the control of a wide range of cytokines, cell specific chemoattractants, adhesion molecules, blood vessels and lymphatic vessels. We propose an alternative mechanism exists by which inflammation may persist and lead to tissue damage and repair (remodeling) via an amplified local response in the airway itself. We postulate that defined lymphoid aggregates (LAs) develop within the airway wall in response to repeated allergen exposure and serve as a local site for antigen presentation and lymphocyte activation, resulting in an increase in inflammatory cells within the airway wall. These cells release pro-inflammatory cytokines and growth factors and subsequently result in altered airway structure and excessive airway narrowing, such as seen in asthma. This project involves assessment of inflammatory cells and LAs in an established ovalbumin-sensitised mouse model developed by our collaborator Prof Rakesh Kumar (School of Pathology, University of NSW). In parallel to these studies, post-mortem human asthmatic airway tissues are also being examined. This project will allow us to systematically examine LAs in the bronchial tree and to relate inflammatory, structural and functional changes to local immune reactions following repeated allergen challenges. As such, LAs may be a new target for understanding the immunological basis of asthma. If repeated allergen challenges result in the development of localised inflammation, independent of the draining lymph nodes, intervention in early exposures to allergen may be critical in preventing development of sustained airway inflammation.

This project has been funded by a grant from WAIMR (Western Australian Institute of Medical Research) 2002-2005.

Murine models of allergic airways inflammation

Graeme Zosky, Debra Turner and Peter Sly

Murine models have become increasingly popular over recent decades in order to elucidate the pathobiology of asthma. There are a number of variations in the methods for inducing allergic airways sensitisation in mice that involve systemic antigen sensitisation and subsequent antigen challenge of the airways. This study aims to examine airway hyperresponsiveness (AHR) and airway inflammation in variations of a commonly used mouse model of asthma

using ovalbumin (OVA) as the sensitising antigen. Airway hyperresponsiveness to inhaled methacholine (MCh) was assessed using a modification of the forced-oscillation technique (FOT) which is a sophisticated method allowing the separation of changes in respiratory system resistance to flow into airway and parenchymal components. Preliminary results demonstrate that a single OVA challenge induces transient AHR in both the conducting airways and tissue that does not persist beyond 24hrs. Multiple antigen challenges cause massive increases in eosinophil influx into the lungs which translates into AHR that persists until at least 48hrs after the final antigen challenge. This AHR is confined to the parenchyma after three OVA aerosols but is present in both the airways and tissue after six OVA aerosols. Future directions for this research include an examination of the correlation between circulating levels of immunoglobulins (IgE, IgG1 and IgG2a) and AHR in this model.

CLINICAL ASTHMA

Role of early, repeated viral respiratory infections and the development of atopy in childhood (The Childhood Asthma Study).

M Kusel, PD Sly, P Holt, R Loh

This prospective cohort study involving a total of 263 families commenced in 1996. The first phase of the study which followed the children till their 5th birthday was completed in August 2003 with the children undergoing their third skin prick test, and lung function tests. The postnasal aspirates collected when the children were in their infancy have found rhinoviruses to be the predominant virus isolated during acute respiratory infections. Rhinoviruses were responsible for six times the number of Upper Respiratory Infections, more than twice the number of Lower Respiratory Infections (LRI) and three times the number of wheezy LRI compared to RSV.

Analysis is currently underway to investigate the role of these infections with the development of asthma and atopy.

The study team acknowledges the tremendous contribution the study children and their families have made to this unique and important study of respiratory infections, asthma and atopy.

The Family Asthma Study.

RC Mutch, AM Callaghan, GE Kendall, Nicholas De Klerk, PD Sly.

The Family Asthma Study is part of an international collaborative effort funded by Glaxo Smith Kline investigating the genetic basis of asthma and allergy. Begun in 1999, the initial work was completed in 2001 when 100 eligible families completed all aspects of the testing.

Identifying and recruiting eligible families was one of the most challenging aspects of the study initially. To be eligible a family had to have an asthma-affected sibling pair, aged between 7 and 35 years, in addition to both genetic parents willing to participate. Families completed a modified ISAAC questionnaire and formal laboratory measures. Laboratory testing for a family could take up to 4 hours; measures were: weight, height, skin prick testing to 9 common allergens, fraction of exhaled nitric oxide, baseline spirometry, a Cockcroft-method methacholine challenge and a blood test.

Individuals and families made the effort to contact us following study participation, to describe their increased understanding and improved management of their asthma with additional gains in lifestyle.

In 2003, work began on analysing the data from the Family Asthma Study. Initial results proved novel with respect to inheritance patterns of atopy within families. In light of the Perth findings we have been given access to the overall International dataset, in order to apply the same analyses as used on the Perth data. The results of these combined analyses will be available in mid-2004.

In early 2004, ethical approval to write to all participants of the initial Family Asthma Study was obtained. We aim to document the severity of asthma exacerbations experienced in the last 12 months by the initial study participants; this study extension will initially take 6 months.

We continue to acknowledge the generosity, interest and support of all participating families who have made this study possible. Thank you.

Is blunted HPA axis a risk factor for asthma and atopy?

L van Reyk, M Deverall, S Silburn PD Sly.

Epidemiological evidence suggests that stress in early life, including both stress in caregivers and infants pre-date the development of asthma and atopy in later life. Family functioning and infant temperament are emerging as significant risk factors for the development of atopic diseases, such as atopic dermatitis and asthma. The HPA-axis is a key neuroendocrine system that is activated during many forms of stress. There is evidence to suggest that an appropriate HPA-axis response to stressful stimuli is important in the development and control of the immune system. Altered HPA responses have been associated with immune diseases such as rheumatoid arthritis and atopic dermatitis. Data from animal models and from young adults suggest that a blunted HPA-axis response to emotional (and possibly physical) stress is associated with an increased risk of immunologically-mediated diseases, such as asthma. However, such analyses have not been undertaken in children and furthermore, no validated methodology exists for the paediatric age group.

It is therefore of interest to study the role of early life events on the development and/or function of the HPA axis as well as its role in immune development. This study proposes first to develop and validate methods for assessing HPA-axis responses to stress in children and then to use these techniques to test the hypothesis that a blunted HPA-axis response is associated with an increased risk of atopic diseases, including asthma.

The role of viral lower respiratory infections in allergy and asthma

T Fukushima, H Patterson, C Pienaar, J Tizard, B Holt, PD Sly.

The influence of viral lower respiratory infections in infancy, on the development of asthma and allergy, is controversial. Infants hospitalized with bronchiolitis (RSV) and parainfluenza (PF) tend to be recurrent wheezers. Whether this is due to the long-term effects of the virus, or is more due to host-response, is unclear.

Some children outgrow the wheeziness, while others tend to become childhood asthmatics. This study aims to determine how RSV/PF alters lung function during the recovery period, and in the longer term. It also aims to assess the link, between the immunological status, severity of infection, and eventual atopic outcome in the children recruited.

Infants under 12 months admitted to PMH with bronchiolitis and PF are recruited, and assessed at set intervals in relation to the acute illness. Their assessment includes a per nasal aspirate, infant lung function during the recovery phase, and post-recovery, history of symptoms using a questionnaire, and blood samples which determine response to common allergens, as well as genetic predisposition to atopy. Currently 64 children have been enrolled in the study, with the first group of recruits beginning their 4th visit later in 2004.

Risk factors for Asthma that persists into adult life

J. Joseph-Bowen, M. Deverall, T. Douglas, M Kusel, B Holt, J Tizzard, PG Holt, PD Sly.

While a number of wheezing phenotypes are apparent throughout childhood, asthma associated with atopy is more likely to persist into adult life than non-atopic asthma. The early life risk factors for this type of asthma are being studied in a number of longitudinal cohorts, including the Childhood asthma study and Family asthma study cohorts detailed in other projects. In addition, the Raine study cohort, so-called because of the initial funding granted by the Raine Medical Research Foundation, has been an integral part of these studies. Questionnaire data have been collected when the children were 1, 2, 3, 6, 8 and 10 years old and a specific respiratory assessment was conducted when the children were 6 years old. The children are now being assessed again at the age of 13 years with the specific aims of examining factors that will allow us to predict which asthmatic children have the type of asthma that is likely to persist into adult life.

CYSTIC FIBROSIS

Early detection of inflammation in cystic fibrosis

S Brennan, K Winfield, PD Sly

In 2003 this research group continued investigations in the area of early development of inflammation and infection in cystic fibrosis. This project is funded as part of a program grant from NHMRC. This project aims to investigate the following

1. To characterise the inflammatory response in the lungs of infants and young children with CF and to correlate this with bacteriology, clinical status and lung function.
2. To determine whether the inflammatory markers assessed are predictive of long term outcome in these children.
3. To investigate the nature of the relationship between markers of lung disease and the breakdown products of lung tissue excreted by the kidneys.

Our findings to date are outlined below:

- Over two hundred broncho-alveolar lavage fluid samples have been collected from 78 different children with CF. Inflammation is evident in virtually all of the

lavage fluids collected, even in the very young infants (from four weeks of age) with no apparent clinical symptoms or infection.

- It appears that once acquired, inflammation consistently tracks with infection.
- The level of acquisition of *Staphylococcus* and *Haemophilus* is lower in this cohort than compared with other national CF centres for the same age group. This may be a consequence of the prophylactic antibiotic policy in the WA paediatric clinic.
- Whilst there appears to be no difference in the age of acquisition in *Pseudomonas* in our clinic compared with the other national CF centers, the lavage program has demonstrated some success at eradicating *Pseudomonas aeruginosa* in young children with CF.

National Hypertonic Saline Trial

S Brennan, E Balding¹, K Winfield. ¹Princess Margaret Hospital for Children
In 2001, we participated in the co-ordination of a national trial of inhaled hypertonic saline (NHSCF Trial) as an adjunct therapy for CF. This trial was launched nationally in August 2000, and locally in WA in October 2000, This study has now been finalized and the results from this trial are currently being compiled for publication. The results of this study will be fully presented in next years annual report.

Inflammation in cystic fibrosis: Friend or Foe?

PD Sly, S Brennan, K Winfield

In cystic fibrosis, inflammation and infection occur concurrently, the role of inflammation is to attack invading pathogens and to effectively remove them from the host. In CF, for various reasons, inflammation overwhelms the lungs and the abundant neutrophils release excessive levels of enzymes (such as elastase) that can also attack lung tissue proteins elastin and collagen. It is this collateral damage from inflammation and infection that initiates fibrotic lesions, leading to long term irreversible lung damage and pulmonary function decline. In 2001, we initiated a new study that we believe may provide important information to the CF community about when inflammation begins to attack lung tissue. This study may provide a solid rationale for the use of anti-inflammatory therapy in CF and may also provide a non-invasive method that could be used to determine the point in disease when that anti-inflammatory therapy is warranted.

The study involves the recruitment of children with CF and children with no history of lung disease for our control population. We have recruited children for this study from both our Perth clinics and schools, as well as other national CF centres. We have investigated the breakdown products of elastin and collagen fibres found in urine and measured by high performance liquid chromatography (HPLC) to see if they correlate with the inflammation measured from sputum or bronchoalveolar lavage in patients at times of stable clinical health and at times of exacerbation of disease. We are also investigating whether current iv. treatments, or anti-inflammatory therapies currently being trailed in the CF community locally and nationally, will influence these levels.

This study received funding from the National Cystic Fibrosis Association for 2002 and submissions for journals resulting from these studies are currently being compiled.

Two international collaborations have also resulted from this project

- (1) Investigation of the correlation of urine markers of tissue damage with visual evidence of lung damage using high resolution CT scan, working with Dr. Harm Tiddens of Rotterdam. Dr. Tiddens and his team routinely use HRCT scans to assess early signs of structural lung damage in CF. We will work together in 2003 to collect urine from those patients aged 4 and over booked for HRCT to assess urine for biochemical evidence of tissue damage.
- (2) Investigation of correlation of biochemical markers of oxidative stress in patients with CF. Working With Dr. Tony Kettle of Christchurch New Zealand, we have established a collaboration to concurrently assess markers of tissue damage alongside established markers of oxidative stress (tyrosine residues). This will provide us with further information about the process of early inflammatory-led damage in children with CF.

Macrolide Therapy for CF lung Disease: Evaluation of Mechanism of Action

PD Sly, S Brennan, K Winfield, G Ryan¹, P Robinson². ¹Sir Charles Gairdner Hospital, ²Royal Children's Hospital, Melbourne.

In Collaboration with Abbott Australasia, US collaborators (Prof. Bruce Rubin) and with Sir Charles Gairdner Hospital, and the Royal Childrens Hospital Melbourne, we are co-ordinating the trial of macrolide therapy in the cystic fibrosis community.

Macrolides are a class of antibiotics that are not routinely used in cystic fibrosis. The macrolide clarithromycin is being trialed in 90 subjects in total in this study. This study is now completed and results are being compiled. Clarithromycin is being tested for it's ability to reduce inflammation and improve lung function when used in conjunction with current antibiotic therapies. The results of this study will be presented in next year's annual report.

Immune Surveillance in cystic fibrosis- the role of macrophages and dendritic cells

S Brennan, J Upham, M Wikkstrom, PD Sly, S Stick

In collaboration with Dr. John Upham, of the Cell Biology Division, we are investigating the role of antigen presenting cells in the early stages of cystic fibrosis lung development. This study involves assessment of blood dendritic cells and monocytes, as well as macrophages found in the bronchoalveolar lavage fluid of children with CF. Children with CF have recurrent infections, which are often difficult to clear and we hypothesise that one reason for this is that there is a dysregulation of the "surveillance" system, which involves the antigen presenting cells in the airways- the dendritic cells and macrophages. With the assistance of the respiratory fellows in respiratory Medicine (Dr. Andrew Martin and colleagues) we use cells from BAL and collect blood from children with CF undergoing BAL, and will also be collecting blood from non-CF children undergoing surgery for non-respiratory related reasons. This study aims

to investigate the presence, phenotype and activity of macrophages in the lungs and the presence and activity of dendritic cells and monocytes in the blood using flow cytometry and in-vitro culture techniques.

This study began in late 2003, and has received funding from the Australian Cystic Fibrosis Research Trust for 2004.

VACCINE TRIALS GROUP

The Vaccine Trials Group (VTG) was established in 1999 as a collaborative venture involving the Telethon Institute for Child Health Research, Princess Margaret Hospital for Children and the University of Western Australia Department of Paediatrics. Our role is to provide a coordinated approach to the development, delivery, assessment and promotion of vaccines and allergy treatments in our community. The vaccine trials are a series of free vaccinations designed to reduce the level of disease in the community. The development and use of new, effective vaccines and treatment results in reduced frequency and severity of disease for individuals as well as reducing the overall cost of healthcare. The group is also available as a resource for the public and for health care workers. This multidisciplinary group includes paediatricians, immunologists, microbiologists, epidemiologists and research nurses and has been involved in a number of international multicentre studies with paediatric and adult vaccines. The Health Department of Western Australia and vaccine companies are also involved.

Staff and Students

Head of Division

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Senior Principal Research Fellow, National Health & Medical Research Council

Director, Clinical Research & Education, Princess Margaret Hospital for Children

Respiratory Physician, Princess Margaret Hospital for Children

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Raewyn Mutch MBChB DipRACOG FRACP PhD Candidate

Cindy Thamrin BE (Hons) BSc PhD Candidate

Lisha van Reyk BSc Hons PhD Candidate

Research Support

Lauren Bamberger BA (Hons)

David Sly

Theses passed

J Kovar PhD University of Western Australia 2003. Impact of inhaled glucocorticoids on early postnatal lung development.

Awards

A Alessandri. PMH Career Development Award, 2003 - 2005

R Collins. Qantas New Investigator Travel Award.

R Collins. Best Oral Presentation. TSANZ (WA branch) Winter Meeting, York

R Collins. TSANZ Travel Award.

T Douglas. Telethon Fellowship.

C Thamrin. Boehringer Ingelheim Fonds Travel Award

C Thamrin. TSANZ Travel Award.

External Committees

International

Peter Sly. Joint American Thoracic Society - European Respiratory Society Working Party on Infant Lung Function Testing (1990-), Chair (1994-96)

Peter Sly. Joint American Thoracic Society - European Respiratory Society Task Force on Standards for Infant Respiratory Function Tests (1997-)

Peter Sly. European Respiratory Society Task Force on Forced Oscillation (2000-)

Peter Sly. World Health Organisation advisor on asthma and lung diseases in children (2001-)

Peter Sly. World Health Organisation Working Party on Management of Asthma in Children under 5 years in the Developing World, (2000-)

Peter Sly. Long Range Planning Committee, Pediatric Assembly, American Thoracic Society (2000-)

Peter Sly. International Task Force, Pediatric Assembly, American Thoracic Society (2001-)

National

Peter Sly. Asthma Consultative Group, Australian Lung Foundation (1999-)

Peter Sly. NH&MRC Fellowship Peer Review Advisory Committee (2001-)

Regional

Peter Sly. Asthma Foundation of Western Australia Research Sub-committee, Member (1993-95), Chairman (1998-)

Peter Sly. Asthma Foundation of Western Australia Medical Advisory Committee (1991-)

Peter Sly. Human Ethics Committee, Princess Margaret Hospital for Children (1991-)

Peter Sly. Scientific Advisory Subcommittee, Human Ethics Committee, Princess Margaret Hospital for Children, Chairman (1993-)

Peter Sly. Institute for Child Health Research Executive Committee (1994-)

Peter Sly. Princess Margaret Hospital Strategic Management Committee (2000-)

Peter Sly. Research Committee, Arthritis Foundation of WA (2001-)

R Mutch. Thoracic Society of Australia & New Zealand (WA).

R Collins. TSANZ Associates Subcommittee.

Invited Presentations

Peter Sly. The developing burden of asthma and atopy in Asia. World Health Organisation, Pattaya, February 2003.

Peter Sly. Exercise Induced Asthma. 13th Interasthma Congress, Fukuoka, March 2003.

Peter Sly. Paediatric Asthma Guidelines. 13th Interasthma Congress, Fukuoka, March 2003

Peter Sly. Spirometry in young children. Thoracic Society of Australia & New Zealand, Adelaide, April 2003.

Peter Sly. Early childhood exposure and asthma in adults. Thoracic Society of Australia & New Zealand, Adelaide, April 2003.

Peter Sly. Management of infants with CF: detection of early lung disease. Meyer Institute, Florence, May 2003.

Peter Sly. Treatment of acute bronchiolitis. Meyer Institute, Florence, May 2003.

Peter Sly. Basic essentials of assessing lung function in infants and young children. American Thoracic Society, Seattle, May 2003.

Peter Sly. Management of infants with Cystic Fibrosis detected by NBS. American Thoracic Society, Seattle, May 2003.

Peter Sly. The role of objective measurements in diagnosing and monitoring. European Respiratory Society, Vienna, September 2003.

Peter Sly. Difficult to treat asthma bronchiectasis. European Respiratory Society, Stockholm, September 2003.

Peter Sly. Measurement of lung function in pre school children. APAPARI,

Singapore, November 2003.

Peter Sly. Is there a role for primary prevention of allergic diseases in the high risk child? APAPARI, Singapore, November 2003.

Peter Sly. The developing burden of asthma and atopy in Asia. Lady Hardinge Medical College, November 2003.

Peter Sly. Diagnosis and monitoring of asthma in children. Indian Academy of Paediatrics, November 2003.

Peter Sly. Asthma phenotypes and age dependent genetics. Queensland Thoracic Society, Brisbane, November 2003.

Division of Molecular Biotechnology

Overview

The focus of the Division of Molecular Biotechnology has in the past been to elucidate the molecular events in allergic sensitisation and to develop biotechnological strategies for diagnosis, treatment and prophylaxis of asthma. We have now been joined by NHMRC principal research fellow, Associate Professor Prue Hart, who will continue her long-standing research on inflammatory disease previously conducted at the Flinders University. Her studies on mast cells, cytokines, cytokine signalling and cell trafficking will interact well with the Division's investigations of these processes in asthma. Her expertise with the use of adenovirus vectors to deliver genes to non-dividing cells and interests in the immunomodulatory effects of ultraviolet light and its role in skin cancer will introduce exciting new areas of research, the latter being particularly relevant to Western Australia. The project reports below describe how UV irradiation induces systemic immunosuppressive effects involving mast cells and how people with more dermal mast cells have a higher prevalence of skin cancer.

Studies on house dust mite and cat allergy have been directed to characterising not only the allergenic molecules that induce the allergic sensitisation, but also other allergens and antigens with the potential to induce protective regulatory responses. The production of high quality recombinant allergens for T-cell stimulation and IgG subclass antibody assays has been critical for the research strategy so special attention has been devoted to this process. The research to date, including studies with T-cell transcription factors and IgG subclass antibody, reported below point to the major Der p 1 and 2 allergens as being both the dominant effector and regulatory molecules in house dust mite allergy. Our work with cat allergens has now identified several other allergenic specificities besides the well-known Fel d 1 allergen. This will provide the entree to study of immune responses to multiple allergenic specificities where the exposure to the allergen source, the cat, is well defined and where a protective effect against asthma can be induced by high dose exposure to cats. The production of the recombinant polypeptides to represent the newly-identified cat allergens is now a priority, along with the production of recombinant allergen for the heterodimer Fel d 1. Our studies on the development of immunotherapy is highlighted by the development of a new model of sensitisation, which is induced by intranasal exposure to papain. This model that not only relies on injections of antigen or adjuvant, will provide a more relevant evaluation of new strategies as shown by the efficacy of subcutaneous immunotherapy compared to the tissue damage produced by intranasal allergen administration. A new type of immunotherapy to be tested is the immunisation with a small peptide of Der p 2 (68-80) that has been shown to be able to induce anti-Der p 2 responses in mice even when administered as a monovalent peptide on fusion protein.

T-cell transcription factor and cytokine responses to different house dust mite allergens

NNR Chu, BJ Hales, LA Hazell, KL Mills and WR Thomas

While the known major Der p 1 and Der p 2 allergens can be identified as targets for immunotherapy, the importance of other allergens in house dust mite allergy is unresolved. It is also possible that immune responses to these allergens play an important part in the protective responses to allergens. To provide a more comprehensive view of the T-cell responses to the different specificities, the production of mRNA for the Th1 and Th2 transcription factors T-bet and GATA-3 were measured by real time PCR before and after the stimulation of peripheral blood mononuclear cells with allergens.

Paradoxically, as reported by others, unstimulated cells from allergic subjects had lower levels of GATA-3 indicating a feedback mechanism. More of the allergic subjects, however, upregulated GATA-3 after stimulation with the major Der p 1 allergen and house dust mite extract. A higher increase in GATA-3 was also found for Der p 2. No significant changes were found for the less major allergens Der p 5 and 7 although there was a trend. The mRNA for T-bet did not show any consistent pattern.

The IL-5 and IL-13 responses of cells from allergic subjects to the major allergens were larger than those to the less major allergens. There was no pattern of differences of IL-10 and IFN- γ between the major and less major allergens or difference in IFN- γ . There was interestingly a very significant increase in the Der p 1- induced IL-10 release of cells from allergic subjects, but this was not induced by mite extract. There have been mixed reports about the relative production of IL-10 by cells from allergic and non-allergic subjects so the increased release induced by a highly purified allergen could be used to study the source of the variation. An important conclusion is that the less major allergens do not induce increased regulatory responses in allergic or non-allergic subjects.

Subclass-specific IgG responses to house dust mite allergens and mucosal antigens

BJ Hales, NNR Chu, LA Hazell, KL Mills and WR Thomas

IgG subclass antibody can be used as a measure of regulatory responses to the antigens. IgG1 antibodies result from non-allergic Th1 type responses typically induced by infectious agents, and IgG4 antibodies result from Th2 type responses found in asthma. IgG4 antibody in the absence of IgE antibody indicates a Th2 response, which has been modified by immunoregulatory cytokines. An ongoing project is being conducted to measure the differences in the responses induced by different allergens and variations due to clinical groups and exposure. The responses to bystander bacterial antigen, P6 of *Haemophilus influenzae* have also been measured. A solid phase microtitre plate immunoassay that measures antibodies with time resolved immunofluorescence assays using the dissociation-enhanced lanthanide immunoassays (DELFIAs) were employed. The antibody assay have the logistical advantage over cytokine assays in larger sample, and archival sera can be examined. A standardized

immunoassay was used in which recombinant allergens were captured with the same coating of anti-H6 tag. Human-mouse chimeric anti-Der p 2 antibodies were used to provide a quantitative standard curve.

Approximately 50 % of mite-allergic subjects produced IgG 1 and IgG4 antibodies to mite allergens but the response was limited to the major allergens Der p 1 and Der p 2. The less major allergens, Der p 5, 7 and Der p 8 had little IgG antibody reactivity indicating decreased immunogenicity rather than just a lack of IgE. The IgG4 titres did not correlate with the IgE and many sera had high levels of one isotype and low levels of the other. Non-allergic subjects rarely had IgG1 or IgG4 antibody to any allergen.

High titres of IgG1 antibodies to the P6 outer membrane protein of the bacteria *Haemophilus influenzae* were found in the sera of allergic and non-allergic subjects. IgG4 antibodies were found in 35% of allergic patients (to any allergen) but not in the sera of non-allergic subjects. The finding of IgG4 to the bacterial antigen is the first report of altered responses to non-allergenic antigens in allergic subjects and indicates a bystander immunoregulation or a general skewing of responses to the Th2 phenotype.

Monoclonal antibodies to Der p 3 and other allergens

BJ Hales, LA Hazell and WR Thomas

Monoclonal antibodies remain a powerful tool for allergen research. The cDNA cloning for the production of recombinant allergens in the laboratory is therefore being complemented by production of monoclonal antibodies. The antibodies will be used for the purification of allergens, environmental monitoring, the quantitation of serological assays and the development of allergen mimics and analysis of B-cell epitopes. A panel of new monoclonal antibodies have now been produced to the major house dust mite allergens Der p 1 and Der p 2 and more recently the first monoclonal antibodies to Der p 3 have been produced. The study of this allergen has been difficult, at least in part due to its potent trypsin activity, and the new reagents will play a key role in the production of recombinant polypeptides and mutants as well as monitoring its presence in extracts and dust samples and inhaled air.

Component-resolved diagnosis and natural allergens of house dust mites

WR Thomas, KL Mills LA Hazell in collaboration with M Weghofer, G Pittner, F Horak, S Vrtala and R Valenta, Department of Pathophysiology, University of Vienna, Austria

The panel of recombinant allergens produced in the Division have been used to explore the diagnostic potential of allergen arrays. A large study of mite-allergic patients confirmed the very high IgE binding of Der p 1 and Der p 2 with Der p 7 being the next most important. The component resolved diagnosis however differentiated the mite-allergic subjects into a population with IgE predominantly directed to Der p 1 and Der p 2 and a population with IgE-binding to a broad spectrum of allergens, including the cross reactive tropomyosin allergen Der p 10. The patients with the broad spectrum

of reactivity also reacted to more sources of allergens and had higher IgE immunoglobulin levels. The new diagnostic procedure can thus identify allergen cross reactivity and multi-sensitive patients, which have been shown to respond differently to immunotherapy. In another study immunoblotting of 2-D gels has been used in conjunction with the recombinant allergens to identify the contribution of the recombinant allergens to the allergens in the extract. It was shown that the recombinant allergens could absorb out all the IgE binding to all the isoforms of their natural counterparts and account for most of the IgE binding molecules separated by the 2-D electrophoresis. Interestingly however a series of acidic low molecular weight IgE binding spots (probably isoforms) different to the known recombinant allergens were identified.

Arginine kinase allergen of the house dust mite *Dermatophagoides pteronyssinus*

S-K Khoo (UWA Department of Paediatrics), KL Mills, LA Hazell, BJ Hales, WR Thomas in collaboration with DC Holt, K Fischer DJ Kemp, The Queensland Institute of Medical Research and Menzies School of Health Research, Brisbane, Queensland

The enzyme arginine kinase is a prominent allergen produced by moths and prawns. It also has an amino acid sequence, which is highly conserved across disparate species. To test for its possible importance in mite allergy and cross reactivity with other invertebrate arginine kinases, a cDNA clone was constructed by PCR from a cDNA library using a sequence obtained from an expressed sequence tag (EST) library. This was provided by Professor Kemp and his colleagues Drs Holt and Fischer. The clone was expressed as a His-tagged protein and shown to produce a soluble protein in good yield. IgE binding measured by the standardized DELFIA assay showed that 75% of mite -allergic subjects have IgE antibody to arginine kinase although on average the binding was quantitatively a modest 3 ng/ml. This protein provides a frequently reacting, but low potency, allergen that will be used to examine the regulatory events which underpin this type of immune response. No evidence for cross reactivity with prawn extract could be found by absorption experiments.

Non - Fel d 1 Cat allergens

W Smith, AJ Butler, LA Hazell, DG Nickels, BJ Hales, KL Mills, WR Thomas in collaboration with Dr MD Chapman and A Pommes, Virginia, USA

Cats produce a secretoglobin-like protein Fel d 1 which is a dominant allergen for many cat-allergic subjects. Our interest in studying other allergens stems from the instigation (by others) of clinical trials, which use peptides from Fel d 1 for immunotherapy. It is not clear whether therapy with Fel d 1 alone would be expected to downregulate responses to other cat allergens or if people with significant allergies to other cat proteins will respond. It is also apparent from recent quantitative studies that while many people are highly allergic to Fel d 1, many others have low to modest titres of IgE to this specificity. It is also possible that cat dander which is currently used to produce diagnostic skin test reagents could be replaced with a more representative formulation given that this is probably an indirect source of allergen produced by other tissues. A cDNA cloning project was initiated to provide a more comprehensive delineation

of cat allergens. To date the project has described three new allergens. One of them cat salivary lipocalin bound IgE in 65% of allergic subjects but at a low level while the other allergens cat haptoglobin and cat S100A12 bound IgE in 35% and 43 % of cat allergic subjects respectively. Although the IgE binding of cat lipocalin was at a low titre it was often higher than the binding of Fel d 1 in subjects with low responses to this allergen.

IgE binding to isoforms of the major Ara h 2 peanut allergen

BJ Hales KL Mills, LA Hazell, WR Thomas in collaboration with A. Bosco and P G Holt Division of Cell Biology, Telethon Institute for Child Health Research
Allergic responses to peanuts are of importance because they often produce severe and life threatening or fatal immediate reactions. Their risk to health is compounded by the use of peanuts as a "filler" ingredient in a wide variety of foods. The known sequence of the major allergen Ara h 2 was used to PCR clone cDNA encoding the polypeptide sequence reported in the literature and a new isoform with a 12 amino acid insertion. The isoforms were produced in *E. coli* and IgE binding was examined by standardised DELFIA. The larger Ara h 2.0201 isoform showed increased IgE binding and, although the increased size was due to insertion (relative Ara h 1.0101) of a repetitive sequence, absorption experiments indicated the increased IgE binding included new specificities.

Immunogenic epitope isolated by phage display

TK Heinrich, SR Gunn, BJ Hales, JC Lenzo, WR Thomas in collaboration with PM Watt and R Hopkins, Division of Children's Leukaemia and Cancer Research, Telethon Institute for Child Health Research

The monoclonal anti-Der p 2 antibody 10B2 has been used to isolate peptides with antibody-specific binding activity from phage display libraries. The libraries have either been M13 filamentous phage libraries supplied commercially or by Professor Smith or the phylomer libraries constructed by random fragments of bacterial genomes cloned into the T7 bacteriophage T7Select vector. All the peptides show sequence similarity to a region 68-80 of Der p 2 and certain residues appeared in all the sequences. This region of Der p 2 corresponds to the only turn structure in the whole molecule and is highly exposed, being close to the region previously described to be recognised by the anti Der p X monoclonal antibody and by human IgE antibodies. Interestingly a disulphide bond contained within the peptide sequence was not necessary for antibody binding. The Der p 2 peptide and one of the phylomer peptides was able to immunise mice to produce anti-Der p 2 specific antibodies. This was even possible when the Der p 2 peptide was cloned onto the C-terminal of the glutathione-S-transferase of the pGEX vector and used as a monovalent immunogen. It thus is a single epitope that can induce anti Der p 2 antibodies and not be able to cross-link IgE on mast cell and basophil receptors. The IgG anti Der p 2 titre induced by the peptide was high, equivalent to 200 mg/ml of the original monoclonal antibody, but inhibition studies showed it preferred reactivity to the peptide over the intact allergen. The project will continue to discover more epitopes and mimetics of Der p 2 and for other major allergens and to test their therapeutic potential.

Model allergic sensitisation and immunotherapy

JC Lenzo, SR Gunn, WR Thomas in collaboration with PG Holt Division of Cell Biology, Telethon Institute for Child Health Research

The development of experimental models has been a major logistical hurdle in the study of the development of asthma and therapeutic strategies. The repeated exposure of mice to inhaled antigens induces a transient IgE response and Th2 cytokine response but, quite unlike the events in human disease, is followed by a tolerance to further sensitisation. A systematic investigation of different conditions for inducing sensitisation by mucosal exposure has found that intranasal administration of papain, a cysteine protease enzyme homologous to the mite allergen Der p 1, can induce prolonged and boostable IgE responses. Further study has now shown that the mice sensitised with papain produce a lymphocytic and eosinophilic lung infiltrate on intranasal challenge and Th2 cytokines. Enzymatic activity was not absolutely required for the sensitisation but under different circumstances proteolytic inhibitors could be stimulatory or inhibitory. Other allergens do not induce these responses even when administered at the same time as the cysteine protease, but it has now been possible to induce bystander sensitisation by first sensitising the mice with papain and then administering papain and another antigen. The T-cell epitopes for the response to papain have been defined and the model has been used to study immunotherapy. Successful prophylaxis and immunotherapy have been achieved with a protocol of subcutaneous injection. Despite the ability of the intranasal administration of peptide containing the T-cell epitope to induce tolerance to systemic sensitisation, this procedure not only did not downregulate the immune responses to the intranasal administration of allergen, but augmented the tissue damage induced by further exposure to allergen. The papain model will now be used to explore the efficacy and safety of new strategies for therapy and the use of genetically engineered allergens.

Immunomodulatory effects of ultraviolet B (UVB) radiation in mice

PH Hart, JJ Finlay-Jones, J Tan and S Gorman

UVB immunomodulatory effects have been implicated not only in skin cancer development but also in the initiation and progression of autoimmune and infectious diseases in experimental animals. UV rays cannot penetrate beyond the outermost layer of skin. Previous studies by this group when located at the School of Medicine at Flinders University have implicated the dermal mast cell, and its products (in particular histamine) in the mechanisms by which UVB can modulate the immune system. In our studies, the effects of UVB on systemic immunomodulation are examined; the shaved dorsal skin of mice is irradiated whilst the ventral skin provides the site for hapten/antigen sensitisation several days later. The immunomodulatory effects of UVB result in reduced swelling of the ears when they are challenged by surface painting with the same antigen/hapten after a further five days. To better understand the molecular mechanisms involved, lymph node cells draining sites of UVB irradiation and/or hapten sensitisation are isolated and are being examined phenotypically and functionally to analyse of the immunomodulatory properties of UVB. The effects of the mast cell product, histamine, are being determined in parallel to those of UVB.

Mast cell prevalence in sun-unexposed buttock skin biopsies and skin cancer development

PH Hart, JJ Finlay-Jones in collaboration with MA Grimbaldeston, Department of Pathology, Stanford University, Palo Alto, CA, USA

In several strains of mice, the prevalence of dermal mast cells correlates inversely with the amount of UVB radiation required for 50% suppression of a contact hypersensitivity response. However, it is not ethically possible to UV-irradiate volunteers and correlate their responses to sensitisation and challenge with contact allergens, with their dermal mast cell prevalence. Instead we hypothesised that volunteers with pathologically-confirmed skin cancers may have a higher dermal mast cell prevalence and be immunologically compromised upon strong intermittent bursts of sun exposure. Buttock skin was chosen for analysis as mast cell prevalence in buttock skin does not vary with chronological ageing and avoids the effects of photoageing. Further, we hypothesised that the prevalence of dermal mast cells in buttock skin is similar to that in dorsal skin when one is a child and at an age when most susceptible to skin cancer initiation. There was a significantly increased dermal mast cell prevalence in the skin of patients with basal cell carcinoma than in age and sex matched controls for both a Danish and an Adelaide population, ie groups with different latitudinal and cultural exposures to UV. More importantly, we have found a significant increase in dermal mast cell prevalence in buttock skin of patients with melanoma and this difference was not related to variations in skin and hair colour. These findings support UVB-induced immunomodulation dependent on dermal mast cell prevalence as contributory to human skin cancer initiation and development. Why different individuals have different mast cell prevalences in buttock skin is not known.

Use of adenoviral vectors for dissection of cytokine mechanisms in activated human monocytes and macrophages

PH Hart, JJ Finlay-Jones with GC Mayne and KA Williams, Flinders University, Adelaide, South Australia

Due to their phagocytic and poorly proliferative nature, it has been difficult to transfect human monocytes and macrophages isolated from human peripheral blood. This has been a stumbling block for use of primary monocytes and macrophages for study of cytokine signalling pathways relevant to the development and resolution of inflammation. Adenoviral vectors have recently allowed transduction of a high percentage of human macrophages but only after CSF-upregulation of the integrins, $\alpha_v\beta_3$ or $\alpha_v\beta_5$, during culture for 48 h, a time allowing significant monocyte to macrophage differentiation. In our hands, after 24 h incubation with M-CSF (20 ng/ml) and a further 24 h incubation with an adenoviral vector encoding green fluorescent protein (AdV-GFP, MOI 50:1), only 35% of CD14-positive cells express GFP. However, centrifugation of these cells with AdV-GFP at 2000 x g for 1 h at 37°C significantly enhanced the number of cells expressing GFP (to 65%) and the level of GFP expression per transduced cell (5-fold). The viability of the cells was not compromised. Centrifugation allowed efficient transduction of monocytes and macrophages with an MOI at least ten-fold lower than otherwise required and did not activate the transduced cells or

affect their ability to produce TNF_α or IL-1_α in response to lipopolysaccharide. This methodology was also suitable for transducing large numbers of *in vitro* monocyte-derived macrophages and macrophages isolated from synovial fluids with up to 75-80% of CD14-positive cells transduced after 24 h exposure AdV-GFP (50:1) and centrifugation (2000 x g). This methodology should provide significant expression of transgenes in human monocytes and macrophages.

Cytokine gene polymorphisms in Caucasoid Australian women and risk of preterm birth

PH Hart in collaboration with MA Annells and HM McDonald, Women's and Children's Hospital, Adelaide, South Australia

The relationship between preterm birth (PTB) and 22 single nucleotide polymorphisms in genes encoding cytokines and mediators of apoptosis and host defense was examined. Caucasoid women (n=202) with a spontaneous PTB <35 weeks were compared with 185 with term birth(s). Genotyping was performed using the polymerase chain reaction and sequence specific primers. Multivariable analyses included demographic and genetic variables. Alcohol [Multivariable Odds Ratio (MOR) 2.3, P=0.001] and substance use (MOR 3.7, P=0.01) were associated with PTB <35 weeks. Smoking (MOR 2.3, P=0.03), haplotypes *IL10* -1082A/-819T/-592A (ATA) (OR 2.1, P=0.04) and *TNF* +488A/-238G/-308G (AGG) (MOR 2.4, P=0.04), *IL4* -509C/C (MOR 3.4, P=0.02) and presence of *MBL2* codon 54Asp (MOR 2.3, P=0.02) were independently associated with PTB <29 weeks. Homozygosity for *IL10* -1082G/-819C/-592C (GCC) haplotype (MOR 1.9, P=0.02) was more common in women with premature rupture of membranes (PROM). Thus, polymorphisms in immunoregulatory genes may influence susceptibility to PTB or PROM.

Tea tree oil for treatment of histamine-induced inflammation of human skin

PH Hart, JJ Finlay-Jones in collaboration with Z Khalil, University of Melbourne, Victoria and AL Pearce, Flinders University, Adelaide, South Australia

Whilst the anti-microbial properties of tea tree oil (TTO) are established, evidence for the anti-inflammatory effects of TTO in human skin remains largely anecdotal and requires evaluation. When TTO is topically applied 10 min after histamine injection into human inner forearm skin, both the developing wheal and flare are reduced in comparison to that of the other histamine-injected but otherwise untreated arm. If applied 20 min after histamine injection, only the developing wheal is reduced in size. Studies of a rat blister base perfused with the water soluble components of TTO has dissected the mechanisms by which TTO modulates cutaneous vascular responses. 1,8-Cineole, representing 2% of TTO, reduced vascular changes induced by sensory neuropeptides released when the distal portion of a cut sciatic nerve was electrically stimulated. Further, 1,8-cineole was without regulatory effect in a plasma extravasation response to substance P and its pre-terminal modulatory effect was confirmed in tests in sensory-denervated rats. In contrast, terpinene-4-ol (approx 40% TTO) reduced substance P-induced microvascular changes and protein extravasation by a direct effect on post-capillary venules, without sensory nerve involvement. α-Terpineol (3% of TTO) had regulatory properties on both pre- and post-sensory nerve

terminals. In human skin, only terpinene-4-ol applied 10 min after histamine injection, but not α -terpineol or 1,8-cineole, regulated the developing wheal and flare suggesting that the histamine-induced responses in humans are in large part determined by histamine directly affecting the vasculature via post-terminal mediated events. The underlying strength of these studies is the use of a well-established rat physiologic model to differentiate the mechanism of regulation of microvascular changes by modulatory agents.

Tea tree oil for treatment of patients with nickel contact hypersensitivities

PH Hart, JJ Finlay-Jones in collaboration with AL Pearce, Flinders University, Adelaide, South Australia

The effect of topically applied tea tree oil (TTO) on nickel-induced contact hypersensitivity reactions in human dorsal skin has been examined. TTO (100%), a 5% TTO lotion, a placebo lotion (no TTO), or 100% macadamia oil were applied at days 3 and 5 after nickel exposure. The erythema index and flare area were measured on days 3, 5 and 7. The regulatory effects of TTO were also investigated on the proliferative response to nickel or polyclonal mitogens by peripheral blood mononuclear cells from nickel-sensitive and control subjects. TTO (100%) significantly reduced the erythema index and flare area when compared with the nickel-only sites. The anti-inflammatory effects were predominantly, but not exclusively, seen in a subgroup of nickel-sensitive subjects with a prolonged development phase of the nickel-induced contact hypersensitivity response. The 5% TTO lotion, the placebo lotion and the 100% macadamia oil were all without significant effect. TTO significantly inhibited proliferation to nickel but not to non-specific polyclonal mitogens by peripheral blood mononuclear cells from nickel-sensitive subjects. Thus, topical application of 100% TTO may have therapeutic benefit in nickel-induced CHS in human skin. The mode of action of TTO requires further investigation, but may be an effect on the antigen presenting cells or the antigen presenting process in nickel-induced CHS.

Staff and Students

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John Finlay-Jones BSc (Hons) PhD, [from July 2003]

Shelley Gorman BSc (Hons) PhD submitted, [from Nov 2003]

Jamie Tan BSc MSc, [from August 2003]

Theses Passed

Kristina L Mills. Characterisation of the group 4 allergens of the house dust mite. PhD University of Western Australia.

External Committees

WR Thomas. Chairman, International Union of Immunological Societies Allergen Nomenclature Committee

WR Thomas. NHMRC Peer Review Advisory Panel

PH Hart. Member, Medical Advisory Board, Sylvia & Charles Viertel Charitable Foundation

PH Hart. Member, Selection Committee, Amgen Medical Researcher Award

Invited Presentations

WR Thomas. Recombinant allergens: New diagnostic and therapeutic products.

American Academy of Asthma, Allergy and Immunology, Denver 2003

WR Thomas. New strategies in allergen vaccine development. American Academy of Asthma, Allergy and Immunology, Denver 2003 (Hot topic)

WR Thomas. Primary prevention of allergic disease. role of early allergen

exposure. World Allergy Organisation- Congress XVIII ICACI Vancouver 2003

Division of Population Sciences

Overview

The Division of Population Science comprises over 140 staff and students engaged in a diverse range of scientific activities. The research program for the Division seeks to document the burden of disease in children and young people, assess causal pathways that lead to disease or health, and assess the significance of these findings for the prevention of disease and/or the promotion of health.

The scientific work of the Division is carried out by project teams working in epidemiology, biostatistics and computing, genetic epidemiology, and psychosocial sciences, and through extensive collaborations with government and non-government sectors. While there is a large diversity in the range of issues studied in the Division, project scientists achieve a particular focus in the areas of asthma and atopy, cancer, infectious diseases, developmental disorders and innovative methodologies.

Highlights from the past year included:

- The Institute, through its Kulunga Research Network, launched the Rio Tinto Child Health Partnership. This partnership involves the Institute, Rio Tinto, Alcohol Education and Rehabilitation Foundation Ltd and the State Governments for Western Australia, Northern Territory and Queensland. The three projects of this initiative will model data from the Western Australian Aboriginal Child Health Survey assessing their applicability to other Australian jurisdictions; add value to existing initiatives to reduce prenatal exposure to smoking and alcohol, and; develop workforce capacity for "early years" initiatives.
- Associate Professor Nadine Henley, from the Faculty of Business and Public Management, Edith Cowan University (ECU), commenced sabbatical with the Division from 1 July 2003 to 30 June 2004. She is helping to create new alliances between the Division and ECU and brings expertise in the area of social marketing and qualitative research. Nadine presented a seminar at the Institute on "Social Marketing: 'Selling' Child Health" in October 2003.
- The latest phase of the longitudinal Western Australian Pregnancy Cohort (Raine) Study was launched in June 2003. Intensive assessment of these children at 13 years of age includes objective measurement of physical activity, physical fitness, and motor competence, as well as markers of cardiovascular health, low back pain, and mental health. Attending parents are also asked to have their height, weight and blood pressure measured. Response rates to all elements of the 13-year follow-up by study families have been exceptionally high. In its first six months, assessments for more than 200 study children have already been completed.

- Now in its second year, our National Institutes of Health (USA) project, LOOKING at Language, has more than 1400 children involved with the study. Language assessments have already been completed for 370 children (90 sets of twins and 190 single born). A new phase of the study was launched in February 2004, with family members invited to take part in the LOOKING at Language family assessments. This study seeks to document the genetic and environmental determinants of specific language impairment in children.
- The Australian Study of Causes of Acute Lymphoblastic Leukaemia in Children (AUS-ALL) began in July of 2003. AUS-ALL is a national case control study, managed at the Institute. It aims to investigate the interaction between environmental exposures and genotype, in particular the epigenetic effect of folate, in leukaemia. Since July 1, 2003, the study has been ascertaining cases of leukaemia from nine major oncology units in Australia and the first wave of control recruitment occurred in August. Data collection entails a paper based questionnaire about likely exposures in parents and children, a paper based questionnaire designed to rank dietary folate intake, a telephone interview that further explores likely exposures in the family, and DNA collection in the form of blood or buccal cells.
- Writing on the first volume of results from the Western Australian Aboriginal Child Health Survey commenced in February 2003 and at the time of this report these results are scheduled to be released in the first half of 2004.
- The health benefits of swimming pools built in two remote Aboriginal communities was published in the British Medical Journal in September 2003. We found a reduction in ear infections and improvement in skin health. There was also some improvement in school attendance and social indicators. We have also completed the first Australian study of treatment-seeking behaviour for otitis media in Aboriginal children. A paper has been accepted for publication.
- Institute scientists in the Divisions of Population Science and Laboratory Sciences along with our colleagues in the School of Paediatrics and Child Health were awarded a Wellcome Trust/NHMRC grant to investigate safety and immune responses to neonatal pneumococcal conjugate vaccination in Papua New Guinea in collaboration with the PNG Institute of Medical Research.

More details of these and other activities may be found in the following sections.

ABORIGINAL HEALTH

Kulunga Research Network

H D'Antoine, D McAullay, K Butler, J Johnston.

The Kulunga Research Network was set-up as a partnership between the Institute and the Western Australian Aboriginal Community Controlled Health Organisations. It was formed in 1999 to build capacity in Aboriginal research.

The Kulunga business plan, developed in 2002, identified three major strategies – research, communication and workforce development.

- The Aboriginal research program is reported separately. The staff from the Network have played an important role in the development of the new ethical guidelines for Aboriginal research and will play a role in implementing these guidelines.
- The Communication Strategy continues to grow and develop. Kulunga reports on its activity in a regular newsletter, that is growing in distribution, and through conferences and other forums. Kulunga hosted a full-day forum at the Institute on 'Reconciliation and Research' with excellent speakers. This was well attended.
- We have not yet secured funding for the Workforce Strategy. However, the staff continue to provide lectures to health professionals and students.

One of the roles of Kulunga is to act as an advocate for Aboriginal children and youth. This is a very important role and the Aboriginal staff from the Institute provide advice on Aboriginal child and youth health at both a state and national level and participate on several committees at these levels.

Rio Tinto Child Health Partnership

The Rio Tinto Child Health Partnership (RTCHP) is a Partnership involving the Institute, Rio Tinto, Alcohol Education and Rehabilitation Foundation Ltd and the Governments for Western Australia, Northern Territory and Queensland. There are three major projects in the Partnership:

1. Modelling of the Western Australian Aboriginal Child Health Survey

The modelling of the WAACHS data for Northern Territory and Queensland will provide significant child health outcomes (eg information about disorders and risks associated with disorders) to assist policy and decision makers (eg Commonwealth Grants Commission) allocate resources more effectively and improve their understanding of what outcomes they can expect to subsequently deliver.

2. Reducing Prenatal exposure to Smoking and Alcohol

Exposing the fetus to toxins such as smoking and alcohol is associated with adverse outcomes. Prenatal exposure to smoking is associated with an increased risk of spontaneous abortions and low birth weight, and excessive alcohol exposure to brain growth and intellectual capacity. This project will add value to maternal and child health programs that are already in place in each of the states.

3. Workforce Development Strategy

Workforce Development is a key theme in a range of projects involving Aboriginal and Islander communities. In many situations, and particularly those involving health and wellbeing, there is a desperate need to build community capacities. This has been highlighted in major reports such as the Collins Report into Indigenous education in the Northern Territory, the Cape York Justice Study (aka Fitzgerald Inquiry) in Queensland and the more recent

Gordon Inquiry in Western Australia. This project will focus on developing the workforce in the early years.

Launches for the Partnership were held this year in Canberra, Perth, Darwin and Brisbane. The Partnership will be conducted over five years. The Institute is in the process of recruiting a coordinator for the project.

Swimming pool project

D Lehmann, M Tennant, D Silva, Kulunga Research Network (ICHR) H Coates, F Lannigan, (Princess Margaret Hospital), M Hollins (Armidale Hospital) and S Weeks (Disability Services Commission).

The objective of this study was to determine what impact new swimming pools in 2 remote Aboriginal communities in WA might have on skin and ear disease in addition to quality of life for children. We found a reduction in the prevalence and severity of skin sores over an 18 month period after the pools were built. In one community this fell from 62% to 18% and in the other from 70% to 20%. Over the same period prevalence of perforations of the tympanic membrane fell from 32% in both communities in the pre-pool examinations to 13% and 18%, respectively. School attendance improved and crime rates fell in one community whilst residents in both communities reported improved social outcomes for children, a safer location to swim and noticeable improvements in health.

This study is currently looking at the long term sustainability of the health effects of the pools on children in the 2 communities previously studied. Researchers have planned a continuation of the study whereby children will be assessed for a further 3-year period. A third community was included and morbidity records have been examined. This will increase the number of children observed, help researchers to evaluate possible methods of assessing the effects of a pool. Morbidity data (collected by local health staff) will enable us to assess trends in antibiotic use before and after the pools were used as well as any outbreak of disease that may be related to pool use.

Our results have been presented at The Rural Health Conference, The Annual Scientific Meeting of the Royal Australasian College of Physicians and the 15th Conference of the International Society for Environmental Epidemiology, Perth 23 – 26 September 2003. M Tennant was awarded a prize for the most outstanding abstract by a new investigator.

Western Australian Aboriginal Child Health Survey

Zubrick SR, Lawrence DM, Silburn SR, Mitrou F, D'Antoine H, Milroy M, Wilkes T, Eades S, Read A, Martinovich L, Ishiguchi P, Wade-Marshall D, Griffin J, Doyle S, Blair E.

Data analysis on this landmark study of 5,000 Aboriginal and Torres Strait Islander children under the age of 18 years commenced in February 2003. Assisted by a consultancy contract with the Australian Bureau of Statistics, Institute investigators started work on the first volume of findings focussing on the physical health of Aboriginal children and young people. During 2003 the

study has overcome significant analytic challenges through:

1. the development of new methods of measuring levels of relative isolation;
2. data linkage of interview data to population data registers;
3. the development of new estimation techniques for hierarchical data analysis; and
4. detailed reports assessing the impact of residential mobility on sampling designs for Aboriginal populations.

Work is underway to launch the first volume of results in the first half of 2004.

Bibbulung Gnarneep

H D'Antoine, S Eades (early phase), K Hunt, H Lette, A Mahony (early phase), J Nannup, L Slack-Smith, E Tursan d'Espaignet

The Bibbulung Gnarneep Project started in the mid 1990s. The early phase of the project involved the conduct of studies into infant care practices on Aboriginal women living in the Perth Metropolitan Area. This first phase has led to the preparation of two PhD theses by A Mahony and S Eades. Anne Mahony was awarded her PhD in 2003. Sandra Eades has submitted her thesis for examination.

The project team developed a new phase in late 2002 and 2003 known as the "Sharing Stories Phase". Data were collected from ten mothers of Aboriginal children living in the Perth Metropolitan Area using an unstructured qualitative ethnographic method. The study focussed on the hopes, aspirations of the mothers for their children and the strategies that they use to achieve these aims. The interviews were recorded on tape and analysed using NUD*IST. The team is currently analysing the interviews and preparing a paper on schooling that emerged as a major concern of the mothers from the interviews.

Monitoring the Northern Territory's Strong Women Strong Babies Strong Culture Project

E Tursan d'Espaignet, M Carnegie (Australian National University), D Mackerras (Menzies School of Health Research), M Measey (NT Department of Health and Community Services)

Although the Northern Territory's Aboriginal infant mortality rates have declined significantly over the past few decades, the persistent large gap between Aboriginal and non-Aboriginal rates in the Territory indicates there is still much to be done to reduce the inequity between the two population groups.

In the early 1990s, the NT Department of Health and Community Services developed a community-based intervention program known as the Strong Women, Strong Babies, Strong Culture Program (SWSBSC) in the early 1990s. The aim of the program was for senior women within Aboriginal communities to help younger Aboriginal women prepare for pregnancy, and to support pregnant Aboriginal women by encouraging them to visit clinics for antenatal care early in their pregnancy, by providing advice and encouragement about healthy pregnancy management in relation to nutrition (including greater use of bush foods), by promoting the adoption of safe practices such as not taking

alcohol and not smoking during pregnancy, and by reinforcing the need to seek adequate and timely medical help and to take prescribed medicines.

The program began in 1993 as a pilot project in three Top End communities of the Northern Territory. Using data up to 1995, Mackerras reported an increase in average birthweight in the pilot communities compared with the rest of the Top End communities. Between late 1996 and early 1997 the program was expanded to a second group of communities that also had poor perinatal health status.

Using information from the NT Perinatal Collection to the end of 2001, Dr Tursan d'Espaignet and colleagues monitored whether the improvements in birthweight had been sustained in the initial three pilot communities, and examined the data for any change in birthweight in the additional communities. They reported a sustaining of the gains in the first group but found no evidence of a statistical change in the second group of communities. Their paper was published in the December 2003 issue of the *Journal of Paediatrics and Child Health*.

Investigation of causal pathways for otitis media in young Aboriginal and non-Aboriginal children in the Kalgoorlie-Boulder area

D Lehmann, D Elsbury, R Monck, A Stokes, J Finucane, N Pomat, A Arumugaswamy, K Carville, C Jeffries-Stokes in collaboration with D Dunn, R Bonney (Bega Garbarringu Health Services Aboriginal Corporation), Ngunyntju Tjitji Pirni Inc, HLC Coates (Senior ENT Surgeon, Princess Margaret Hospital), TV Riley (Department of Microbiology, University of Western Australia), S Weeks (Audiologist, Disability Services Commission), AW Cripps (Griffith University, Queensland), J Kyd (Faculty of Applied Science, University of Canberra), J Bowman, D Smith (Pathcentre), D Murphy (Public Health Bacteriology Laboratory, Brisbane).

We have completed enrollment of 180 non-Aboriginal and 100 Aboriginal children into a study aimed at identifying the most potent factors predisposing children to otitis media. Funding for the study comes from NHMRC and Healthway. Babies born in Kalgoorlie Regional Hospital are followed closely from birth to age 2 years with specimens collected and clinical follow-up done on seven occasions. All children have now reached age one year. Data on demographic, socio-economic and environmental risk factors are collected. Nasopharyngeal aspirates are collected to investigate upper respiratory tract bacterial and viral carriage. Mucosal immune status is investigated by measuring antibodies in sequential samples of saliva and one breast milk sample at first visit. Ear health is assessed by an ENT specialist on three occasions. Hearing is assessed once by an audiologist in the second year of life and tympanometry is done at each visit from age 4 months onwards. Healthway also fund an adjunct qualitative study described below. We will be able to investigate the impact of pneumococcal conjugate vaccine given to both Aboriginal and non-Aboriginal children on upper respiratory tract carriage. Ear specialists have found that more than 60% of Aboriginal and 20-40% of non-Aboriginal children have otitis media, the highest rate being in children aged 6-11 months. 35% of Aboriginal

children and 10% of non-Aboriginal children carry the pneumococcus before age 2 months. More detailed molecular typing of Haemophilus influenzae, the pneumococcus and Moraxella catarrhalis is being done to give us further insights into the epidemiology of upper respiratory tract bacterial carriage. Rhinoviruses have been identified more frequently in Aboriginal than non-Aboriginal children aged 3-5 months. Findings from this study will be used to develop appropriate interventions to prevent otitis media, which can seriously affect childhood development, school performance and subsequent social and economic well-being.

Treatment-seeking behaviour for otitis media and an investigation of smoking and child feeding practices among the Aboriginal population in the Goldfields

C Jeffries-Stokes, D Lehmann, A Stokes, J Johnston, A Mason, J Evans, F Nichols, D Elsbury, J Finucane, R Monck, D McAullay in collaboration with Bega Garnbirringu Health Services Aboriginal Corporation and Ngunyntju Tjitji Pirni Inc.

We have completed the first Australian study of treatment-seeking behaviour for otitis media in Aboriginal children, which was used to develop a more detailed qualitative study. We have found that (1) there is a limited understanding of the disease process (perhaps not surprising given the frequently asymptomatic nature of the disease until the ear drum has perforated and there is a visible ear discharge) (2) there is a lack of awareness that tobacco smoking may put children at risk of otitis media and (3) exclusive breastfeeding is of short duration. With a grant from Healthway we are now addressing these issues in collaboration with the Aboriginal community through workshops as well as indepth interviews with parents of children participating in the large cohort study as well as key informants in the community, at the same time as analysing data from the cohort study described elsewhere. Workshops have been run through Wongutha Pirni Aboriginal Corporation focusing on the almost diametrically opposed concepts related to smoke –the positive aspects of smoking ceremonies in Aboriginal culture conflicting with the known dangers of cigarette smoke. This activity (also supported by Healthway) culminated in the design of T-shirts worn during a NAIDOC procession in Kalgoorlie and an exhibition of fireplaces and paintings in Perth.

Evaluation of tympanoplasty in Aboriginal children in Western Australian and factors associated with successful outcome

D Mak (Kimberley Public Health Unit), A MacKendrick, H Coates, S Weeks, L Leidwinger, F Lannigan, D Lehmann (ICHR), K Sivwright (ICHR), M Bulsara. The project aimed to assess the outcomes of tympanoplasty (repair of hole in ear drum) performed in Aboriginal children for middle ear disease and to identify factors associated with the success of surgery. We have reported the positive short and long-term outcomes of tympanoplasty in Aboriginal children, reinforcing the need to make surgery more accessible to reduce the high prevalence of deafness and thus improve educational and social outcomes.

CANCER

Cancer epidemiology

E Milne, C Bower, N de Klerk, U Kees, Helen Bailey in collaboration with B Armstrong (University of Sydney), F van Bockxmeer (Haematology, Royal Perth Hospital), D Baker (Princess Margaret Hospital), L Fritschi (Department of Public Health, University of Western Australia), J Thompson (WA Cancer Registry), L Lockwood (Royal Children's Hospital, Brisbane), M Rice (Women's and Children's Hospital, Adelaide), M Stevens (Children's Hospital Westmead, Sydney), E Smibert (Royal Children's Hospital, Melbourne), R Suppiah (Mater Children's Hospital), F Alvaro (John Hunter Hospital), P Downie (Monash Medical Centre), M Haber, M Norris (Children's Cancer Institute Australia for Medical Research), R Scott (Hunter Area Pathology Service), J Attia (University of Newcastle), G Marshall (Sydney Children's Hospital), M Miller (Marg Miller Consulting).

Causes of acute lymphoblastic leukaemia.

Researchers in the Childhood Cancer Epidemiology program were successful in winning a 5-year (2003-2007) NHMRC funded national case-control study into the causes of childhood acute lymphoblastic leukaemia (ALL). The primary hypothesis of this study is that maternal folate supplementation during pregnancy protects against ALL in the offspring, with the effect modified by genetic factors in folate metabolism. This hypothesis arose from the findings of a case-control study undertaken in Western Australia by Dr Judy Thompson and Professor Bruce Armstrong between 1984 and 1992 (published in the *Lancet* in December 2001).

The new study addresses the actions and interactions of supplemental and dietary folate, environmental exposures, and genetic polymorphisms in parents and children in determining the risk of childhood ALL. The team is multidisciplinary, bringing together molecular biologists, geneticists, oncologists and epidemiologists.

Case subjects will comprise 350 children (0-14 years) newly diagnosed with ALL in Australia between 2003 and 2006. They are identified through all the paediatric oncology centres in Australia. Three controls are selected for each case, frequency matched by age, gender and State of residence, a total of 1,050. Controls are identified using random digit dialling. This involves the random generation of a set of potential telephone numbers, using the State area code prefix of the case phone numbers as a seed. The randomly selected numbers are then linked to a name and address wherever possible, and a letter outlining the study is pre-mailed to those addresses. The phone numbers are then dialled, and parents choosing to participate in the control group are mailed information sheets and questionnaires about dietary, residential and other exposure history. A computer-assisted telephone interview is used to record important information on occupational and medical history. Interviewers are blinded to subject status. An occupational exposure expert, blinded to case/control status, will examine all the occupational information and allocate probability and amount of exposure to the chemicals with reference to a custom designed database of jobs and exposures.

Blood and buccal samples are taken from the case child (in remission), and blood samples are taken from his/her parents. Buccal samples are being collected from the first 350 control children and their parents. Genomic DNA is isolated by standard techniques, and polymorphisms in specific folate metabolizing enzymes, xenobiotic metabolising enzymes and DNA repair enzymes are to be examined. The main effects of genetic and environmental factors, and the biological interactions between them, are to be quantified in this study.

In total, there have been 103 cases notified to us, 74 of whom have achieved remission and are thus eligible for inclusion in the study. 53 cases have been invited to participate, of whom 35 have consented, 13 have yet to consent and 4 have declined. DNA collection is complete for 19 cases, while 14 case families have completed questionnaires.

To date, 250 control families have been recruited, 150 of whom have returned their exposure questionnaires and 100 have returned their dietary questionnaires and buccal samples. DNA extraction has been undertaken for all specimens received, but we have not yet commenced genetic analysis.

Other studies commencing in 2003 include an analysis of trends of childhood cancer over the last four decades (1960-2002), and several population based data linkage studies examining possible links between childhood cancer and maternal and birth characteristics, and specific illnesses within immediate family members.

DEVELOPMENTAL DISORDERS

Autism

E Glasson, C Bower, G Dixon, J Wray (State Child Development Centre)

The prevalence of autism disorders has increased over the past decade and current estimates suggest that approximately 6 in every 1000 children are affected. ICHR houses Australia's only prospective autism register, which aims to monitor the number of people receiving a new diagnosis in WA each year. Now into its sixth year of data collection, the Register is invaluable for describing the patterns of diagnoses within the spectrum. The latest report from the Register indicates that in excess of 200 people receive a diagnosis in WA per year, with a median age of diagnosis of 4 years. The Register has attracted local, national and international interest with a wide variety of people requesting summary data.

Using ICHR's unique population databases, we are currently investigating the presence of obstetric complications, congenital defects, and childhood illness and disability for children who receive a diagnosis within the autism spectrum. Using the MCHRDB, we have found children who develop autism had been associated with an increase in obstetric complications compared with population controls. Findings showed that the autism cases were more likely to be first-born, have older mothers, experience fetal distress, and be delivered

by a caesarean section. Future work will focus on the early childhood illnesses and comorbid conditions that commonly occur in children with this puzzling disorder.

Birth defects

Neural tube defects

Bower C, Miller M, Payne J, Serna P, de Klerk N, Stanley F, D'Antoine H, Eades S.

In our case control study, the mothers of 36 infants with neural tube defects (cases) and 578 infants without birth defects (controls) completed questionnaires. We estimated mother's intake of folate from supplements and food (including fortified foods). Almost a third of women took folic acid supplements periconceptionally. Supplement use was associated with a non-significant 4% reduction in risk. For women not taking supplements, dietary sources of folate were protective, and most women obtained at least some folate daily from fortified food. For the two thirds of WA women not taking supplements, fortified food is an important source of folate for the prevention of NTD.

This study also confirmed the relatively high rate of knowledge (over 60% amongst the control mothers) of the association between folate and neural tube defects seen in other WA studies and elsewhere. Better educated, older, and married women who engage in other health-promoting behaviours (not smoking, taking exercise, planning pregnancy) were found to be more likely to know about the preventive effect of folate and to have taken periconceptional folic acid supplementation. Importantly and in contrast, we have shown that there are no such differences for the intake of foods voluntarily fortified with folic acid. This is in spite of limited voluntary fortification in Australia, of mainly breakfast cereals. This finding underscores the importance of fortification in reaching all women in the target group.

In previous studies we have found that neural tube defects (NTD) were 43% more common in Indigenous infants in Western Australia in the 1980s, and that there has been a 30% fall in NTD overall in Western Australia since promotion of folate and voluntary fortification of food has occurred. In order to investigate whether the fall had occurred in both Indigenous and non-Indigenous infants, data on NTD (births and terminations) were obtained from WA Birth Defects Registry, and on all births from the Maternal and Child Health Research Data Base. Knowledge of folate was asked in a survey of Indigenous women interviewed post-partum. Pre-promotion (1980-1992), there was a 42% increase in NTD in Indigenous compared with non-Indigenous infants (prevalence ratio (PR) = 1.42 [95% confidence interval (CI) 1.04, 1.94]); while in the most recent period (1996-2000), the prevalence in Indigenous infants was almost twice that of non-Indigenous infants (PR 1.98 [CI 1.25, 3.15]). 55% of Indigenous women knew about folate in pregnancy. Like sudden infant death syndrome, this study has highlighted health promotion that has been successful in reducing the risk of a childhood condition overall, but has failed to be effective for Indigenous children.

Birth defects and other health outcomes in children born following assisted reproductive technology treatment.

M Hansen, C Bower, N de Klerk, J Kurinczuk (Oxford University), L Milne, Sandy Webb (WA Department of Health), Bev Petterson, Helen Leonard, Lyn Colvin

The risk of birth defects in infants born following assisted reproductive technology (ART) treatment is a controversial issue. We carried out a systematic review to identify all papers published by March 2003 with data relating to the prevalence of birth defects in infants conceived following in-vitro fertilization and/or intracytoplasmic sperm injection compared with spontaneously conceived infants. Twenty-five studies were identified for review. Two-thirds of these showed a 25% or greater increased risk of birth defects in assisted conception infants. Independent reviewers identified 7 papers as appropriate for inclusion in a meta-analysis. The results of meta-analyses of these 7 and of all 25 studies suggest a statistically significant 30-40% increased risk of birth defects associated with assisted reproductive technologies. These results are currently awaiting publication and have implications for the counselling of couples seeking ART treatment.

Analyses of hospital discharge data from our study of health outcomes in children born following ART have begun. We are comparing time to first admission, number of admissions, length of stay, and principal diagnoses in 2106 infants born following ART in WA between 1993 and 2000 with the remainder of spontaneously conceived children born over the same time period (n=177451).

Other health outcomes to be assessed in this study include cerebral palsy, intellectual disability and birth defects diagnosed by 6 years of age as well as birth defects diagnosed in infants born preterm.

Fetal Alcohol Syndrome in Australia

Payne J, Bower C, D'Antoine; Elliott E, Morris A, Rose D (Australian Paediatric Surveillance Unit); and Haan E (Adelaide Women's and Children's Hospital)

Fetal Alcohol Syndrome (FAS) is caused by maternal alcohol consumption during pregnancy and represents the severe end of a spectrum of the effects of exposure to alcohol in pregnancy. FAS has been described as a preventable tragedy. Children with FAS display a wide range of effects, the cardinal features being cranio-facial abnormalities, prenatal and/or postnatal growth deficiency, and evidence of damage to the central nervous system. Estimates of the birth prevalence of FAS range from 0.26-7.2 per 1,000 live births in the USA and Canada, and 39 per 1000 in a community in South Africa. Data from the Western Australian Birth Defects Registry show a rate of 0.18 per 1,000, but this is considered to be an underestimate of the true prevalence. In the Northern Territory, a rate of 0.68 per 1,000 was recently reported.

With a research grant from Healthway, we are conducting a study of FAS in Australia, which has three objectives:

- 1) To ascertain the incidence of FAS diagnosed by Paediatricians and child health specialists in Australia, using the Australian Paediatric Surveillance Unit (APSU);
- 2) To collect information from health professionals to determine knowledge, beliefs, and practices in relation to FAS and alcohol in pregnancy; and
- 3) To collate contemporary Australian data on alcohol consumption in pregnancy.

Based on the first two years of ascertainment through the APSU, the reported birth prevalence of FAS in this study is 1.0 per 100,000 live births (95% CI 0.32-2.36). This is much lower than rates reported in North America and WA. The birth prevalence of FAS reported in the first two years of this study should be regarded as a minimum estimate as FAS is a complex disorder that is most frequently diagnosed between two and ten years of age.

Aboriginal and Torres Strait Islander children who are not living with their parents are over-represented in the notifications that do not meet the case definition used in this study. In many of these instances data that might have allowed a diagnosis of FAS are not available for these children. The strategy for analysis of data at the end of this study will take this into account.

Aboriginal and Torres Strait Islander children may be under-represented as cases in the APSU data due to lack of access to paediatric services and under-diagnosis. Children with FAS are frequently in care, born to mothers with multiple substance use, and are demanding of health and education resources. FAS contributes to significant social, medical and educational burdens to affected children, their families and the community. Raising awareness of FAS amongst health professionals in Australia is likely to facilitate earlier recognition of FAS, the use of appropriate services, and the opportunity to assist in preventing FAS in subsequently born children. Estimates of the size of the problem need to be addressed. The data being collected through the APSU are providing an estimate of FAS seen by paediatricians.

LOOKING AT LANGUAGE

Our ability to communicate is vitally important.

Language impairment is a serious developmental health problem that has long-term consequences for academic, social and behavioural success and adult employment opportunities.

The LOOKING at Language study aims to understand more about the factors that influence language acquisition and Specific Language Impairment (SLI) in children during their preschool and school years.

SLI affects approximately seven per cent of children with otherwise normal development – normal hearing, normal intellectual abilities and normal physical development. Their only difficulty is with language.

Currently we do not know what causes SLI. However, recent twin and family

studies suggest that SLI may be strongly genetically determined.

In collaboration with the University of Kansas, we are investigating possible genetic and environmental causes of language impairment to guide effective treatment and preventative programs.

The study is collecting valuable evidence from a population-based sample of Western Australian families of twins and single-born children aged between one – eight years.

As part of the study, researchers have travelled across the State to meet with the families, in some cases travelling as far away as Broome and Esperance.

Now in its second year, LOOKING at Language has more than 1500 children involved with the study. Language assessments have already been completed for 420 children (95 sets of twins and 230 single born).

A new phase of the study was launched in February 2004, with family members invited to take part in the LOOKING at Language family assessments. Language assessments have already been completed for 55 family members.

The LOOKING at Language study has been made possible through a prestigious grant worth US\$2.2million awarded to the Centre for Developmental Health by the USA National Institutes of Health.

Studies in Cerebral Palsy

L Watson, E Blair, FJ Stanley, J de Groot, J Smith, C Harrison, J Lay, in collaboration with B Petterson (Disability Services Commission), N Badawi (The Children's Hospital at Westmead, NSW), JJ Kurinczuk (University of Leicester, UK).

What is cerebral palsy?

The term 'cerebral palsy' refers to a heterogeneous collection of diseases with the common clinical features of motor impairment resulting from some non-progressive defect or anomaly of the brain acquired early in life. (In WA 'early in life' is defined as before the age of 5 years.)

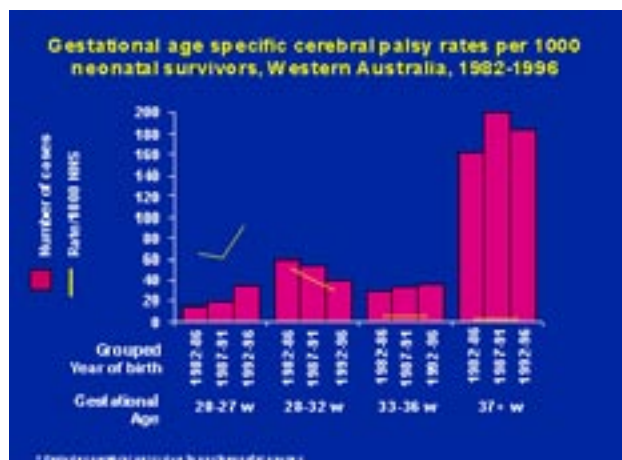
The motor impairment may take a number of forms but the most commonly occurring type, affecting 80% of those with cerebral palsy, is spasticity. This may affect primarily the legs or one side of the body or it may affect the whole body. The motor impairment is sometimes accompanied by epilepsy, intellectual and/or sensory impairments. Additional impairments are more likely if the motor impairment is severe. The impact of cerebral palsy on an individual's functional ability can vary from imperceptible to totally incapacitating.

How often does cerebral palsy occur?

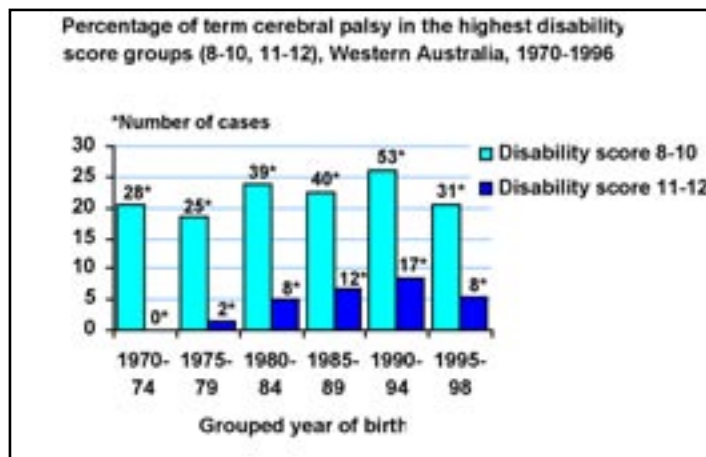
Since 1979 the WA Cerebral Palsy Register has actively identified cases of cerebral palsy born or living in WA since 1956, using multiple sources

of ascertainment. It records identifying data, clinical descriptions of all impairments, limited pregnancy and delivery data and cause if any is recorded.

In conjunction with the Maternal and Child Health Research Data Base (MCHRDB) this allows us to measure trends in birth prevalence, which are published in occasional reports available from the Institute. The most recent was published in December 1999 and reports statistics to the 1994 birth cohort. Data are now complete to birth year 1996. Overall birth prevalence has remained between 2 and 2.5/1000 for the life of the Register, but the frequency varies inversely with the gestational age of the infant at birth. During the 1980s the frequency in very preterm cohorts increased, and continues to increase in those born before 28 weeks, though the majority of cases are still found among the more numerous term births.

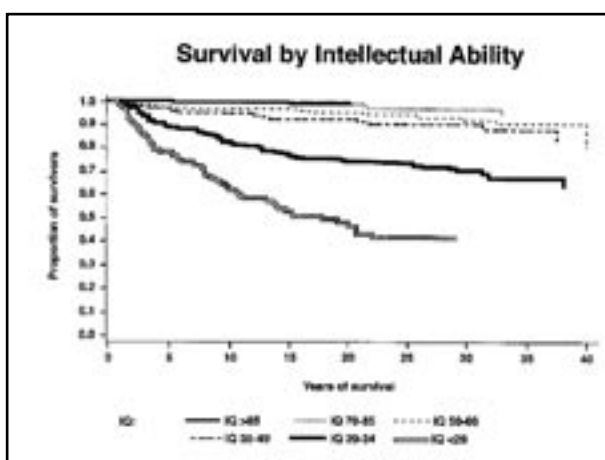


A second disturbing trend noted in data to birth year 1994 was that although there had been negligible change in frequency of cerebral palsy among term births, they exhibited increasingly severe disability. The trend did not persist in the 1995-96-born cohorts though numbers are small in this latest two-year group; we will continue to monitor this as more data become available. The figure below shows the increase in the proportion of cases born at term with scores of 11 or 12 on a 12 point scale of disability.



How long does cerebral palsy last?

As there is no cure cerebral palsy is a lifelong condition, though functional ability and quality of life may be improved by expert management. Linking the Cerebral Palsy Register to statutory Death Registers allowed us to measure the life expectancy of people with cerebral palsy. The condition itself is neither degenerative nor fatal, but it predisposes the individual to a number of potentially fatal problems, particularly respiratory infections. For a given level of disability, life expectancy has not changed since the 1950s. Of the most severely and multiply impaired, requiring lifelong assistance in all aspects of daily living, 40% will survive to adulthood - and there are now more of these very severely impaired people. The characteristic most strongly associated with mortality in persons with cerebral palsy is their intellectual ability, as shown in the Figure below.



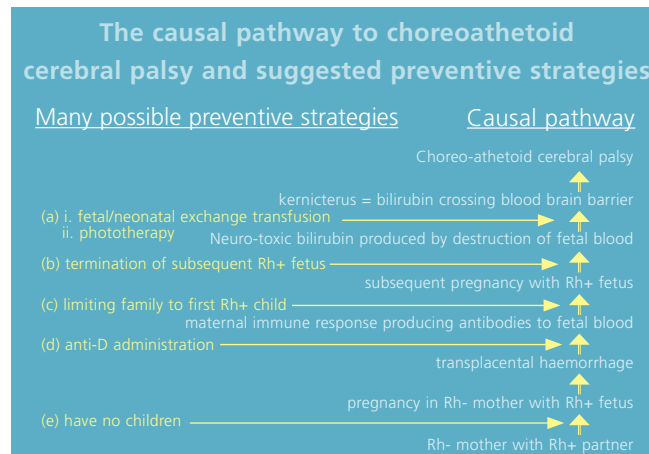
What causes cerebral palsy?

The limited dataset collected by the Register is not sufficient to answer questions about the causes of cerebral palsy and it occurs too rarely to investigate efficiently by cohort studies.

In 1981 we commenced a case control study of WA children with spastic cerebral palsy born 1975-80. While the primary hypothesis examined the association with intrauterine growth, we collected a wide variety of information. From this data set it was apparent that not only were there many aetiological routes to cerebral palsy, but that on any one route there was seldom a discrete, sufficient cause. The more risk factors recognised in any one subject, the higher their risk. Very few risk factors could plausibly represent a sufficient cause, and even those few were often preceded by predisposing factors, without which the path would not have commenced. The best known of these sufficient factors is insufficient oxygen reaching the fetus during labour and delivery. However our study was able to demonstrate that this could have accounted for only about 8% of the cases in this study.

In common with many conditions, there is a delay between the pathological event (for cerebral palsy the point of irreversible brain damage) and disease recognition. Where such a delay exists the factors most closely associated with

disease, the strongest risk factors, will be early signs of the disease, signs that appear only when it is already too late for prevention. In these circumstances the strength of association is not an indicator of causality. It is necessary to differentiate between predictive factors (which helps to prepare families, therapists and clinicians) and causal factors, the avoidance of which will prevent disease.



The figure above shows what is perhaps the only completely understood pathway to cerebral palsy. It proceeds via kernicterus and maternal Rhesus iso-immunisation to a type of motor impairment known as choreoathetosis. Appreciation of this pathway enabled prevention to be effected by blocking the production of maternal antibodies to Rhesus positive blood, by administering anti-D to the mother immediately after the birth of each Rhesus positive child. Choreoathetosis is now rare in developed countries. Considering this pathway as a model, we can surmise:

- (a) that as the length of the known causal path increases, it can suggest an increasing number of points of intervention, increasing the opportunities for prevention,
- (b) that earlier preventive strategies, implemented before the presence of actively damaging agents, are more likely to be effective than strategies implemented late on the causal path,
- (c) that early causal factors are harder to identify than later factors, because the associations will be weaker, but that they may hold the most effective keys to prevention, and finally
- (d) that the most effective forms of prevention may sometimes require strategies other than medical strategies.

In the study of 1975-1980 births, most subjects were born before the introduction of neonatal intensive care (NIC). Only 6.4% of cases were born before 30 weeks gestation, compared with 15.5% of the children with cerebral palsy born 1990-1994.

NIC has significantly changed perinatal care and markedly increased the perinatal survival of compromised neonates, so that neonates who would previously not have been at risk of being described as having cerebral palsy because they did not survive for long enough, might now do so. The

introduction of NIC quickly outdated our first case control study, which furthermore considered only spastic cerebral palsy and compared them only with normal survivors who were matched on birth weight rather than gestational age at delivery. It was time for a new study.

The new study in progress has three groups: (a) all persons with cerebral palsy born in WA 1980-1995 (b) one survivor without cerebral palsy individually matched to each case on year of birth, plurality and gestational age and (c) a random sample of intra-partum stillbirths and neonatal deaths delivered 1985-1995. An exhaustive data collection was commenced in 1996 and is now complete. Coding for automatic scanning was completed in September 2003.

During the last decade, ideas concerning the multiplicity and multi-factorial nature of cause have contributed to an increasing willingness to look beyond intrapartum asphyxia. The outlines of several possible causal pathways are taking shape, fuelled by international observations of the association of cerebral palsy with thrombotic mutations, with inflammation of the decidua and with birth defects. Some of these, such as birth defects, we have been able to investigate by combining data from both Cerebral Palsy and Birth Defects Registers. However the new case control study will provide data allowing us to determine to what extent each of the many hypothesised causal paths contribute to cerebral palsy in Western Australia.

Prevention

Obviously the interruption of any one pathway is not going to make a huge difference to the overall rate of cerebral palsy and much work remains to be done to identify which of the many hypothesised causal pathways actually do occur before rational approaches to prevention can be suggested.

However, a little progress has already been made such as this example of prevention very early in the causal path. Using data from several registers we measured the increased risk of cerebral palsy with increasing number of co-fetuses in a multiple pregnancy. Twins have a 4.5 fold increase in risk and triplets an 18 fold increase in risk of cerebral palsy. This provided evidence in support of the 1987 guideline for in vitro fertilisation, which limits the number of embryos that are transferred in any one cycle to 3. Legislation adopting these guidelines was passed in 1993 and the rising WA rate of triplet pregnancies has started to abate.

Preventive strategies implemented early on the causal path tend to be applied less selectively so it will be harder to evaluate their success. Our goal is to address a sufficient number of paths to bring about a detectable decrease in the frequency and/or severity of cerebral palsy, which we will continue to monitor with the WA Cerebral Palsy Register.

A National Cerebral Palsy Register

Australia currently has three State registers in WA, SA and Victoria, and all are limited by small numbers of cases. In 2002 establishment of a NSW

register was initiated, and with a large proportion of the total Australian live born population then covered, the logical progression was to a national collaboration. A proposal to set up an Australian National Cerebral Palsy Register was first presented in a workshop at the Australasian Academy of Cerebral Palsy and Developmental Medicine conference in September 2002 and attracted considerable interest both nationally and internationally. Nominations for representatives from each State were called for, along with expressions of interest in the site, framework and operation of the clearing house. Follow-up meetings in April 2003 resolved that the National Register would be located at the Telethon Institute for Child Health Research in Perth, operating as a Collaborating Unit of the Australian Institute of Health and Welfare. Methodological issues including the minimum data set were also agreed upon. Funding is currently being sought.

Impact of cerebral palsy studies

- Our work has helped to change the entrenched idea that cerebral palsy is primarily caused during labour and delivery, opening the door to new hypotheses about its causation.
- Our work has had a significant impact on the litigation crisis that is threatening the availability of obstetric services by challenging inconsistencies in the definition of birth asphyxia and showing that the proportion of cerebral palsy cases that could have been acquired during labour and delivery (8% in WA) is much lower than previously thought.
- By monitoring the occurrence and severity of cerebral palsy in babies who survive as a result of NIC we are able to evaluate NIC practices aimed at reducing neurological disability in very preterm infants.
- We have drawn attention to the increase in cerebral palsy acquired in infancy and early childhood from causes such as meningitis or head injury – now 15% of all cerebral palsy – and the potentially avoidable incidence in cases due to non-accidental injury.
- We contribute scientific and epidemiologic expertise to the evaluation of treatment strategies for children with cerebral palsy.

Advisory Committee:

N French (chair), P Chauvel, R Henderson, A Hockey, L Nagarajan, K Naso, T Parry, B Petterson, J Silberstein, P Silberstein, M Tait, J Valentine, I Walpole, P Walsh.

IDEA (Intellectual Disability Exploring Answers)

Leonard H, Petterson B, Bower C, J Bourke, C Philippe, R Chalmers (DSC), P Chauvel (PMH), V Morgan (UWA), R Sanders (Department of Education), J Valentine (PMH), J Wray (SCDC).

Approximately 350 children born in WA each year have an intellectual disability. For the majority of these children the cause of their intellectual disability is unknown. Down Syndrome still remains the commonest of the known causes. There are also a large number of individually rare conditions which each affect a small number of WA children.

In 2002, the Disability Services Commission (DSC) agreed to transfer the Intellectual Disability Database to the Institute and also provided funding to update it and to improve the ascertainment methods. The updating process is nearing completion and the DSC data have been combined with information from the Department of Education and Training to form the IDEA Database.

The aims of the database are to observe trends and support research into the causes and prevention of intellectual disability; to provide information to assess the health and service needs and evaluate intervention and therapy programs for children and adults with intellectual disability; and to increase community and professional knowledge about intellectual disability.

Research has already been undertaken using this database to investigate the social determinants of intellectual disability. We have found that children born to Indigenous, teenage and single mothers are at increased risk of intellectual disability. We have also found that, compared with mothers who are least disadvantaged, mothers who are most socio-economically disadvantaged are at much greater risk of having children with a mild to moderate intellectual disability.

We have also been doing some preliminary analyses of the patterns of hospitalisation in these children and found that in comparison with children who do not have an intellectual disability, they are much more likely to be admitted to hospital in their first five years of life. They are particularly likely to be admitted for infections and respiratory disease. We did note however that those children with autism as well as intellectual disability had a different pattern that was more like that of children without intellectual disability.

The next stage of our research program will involve further exploration of the factors that occur before and during pregnancy and at birth that may be associated with the subsequent onset of intellectual disability. We also plan to examine carefully the patterns of incidence of both intellectual disability and autism and work out whether the increase seen in autism worldwide is in fact real. In the meantime our current research (which has shown which groups of children are more likely to be affected by intellectual disability and the nature of their medical needs) already has important implications for service providers.

Mental health

Newborn Encephalopathy (NE) Study

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There have been few long-term studies of the outcomes following newborn encephalopathy. Of those conducted, the majority were not population based. Most concentrated on encephalopathy associated with 'birth asphyxia' while others only included infants with neonatal seizures. Few studies have been concerned with outcomes other than cerebral palsy and death. With notable

exceptions other disabilities such as cognitive impairment and developmental delay have not been considered or have only been reported for infants with hypoxic ischaemic encephalopathy. We undertook a case control study of moderate and severe newborn encephalopathy with recruitment from 1993 to 1996. This was the first population-based study of newborn encephalopathy using a broad clinical definition that investigated the possible associations between NE and a series of preconceptional, antepartum and intrapartum characteristics. Our analyses of these associations have led us to conclude that the causes of NE are heterogeneous and many of these were found to relate to the antepartum period.

We have undertaken intensive follow-up of both the cases and controls in the original case-control study in order to identify and quantify later associated morbidity and mortality and to investigate the causal pathways to poor outcome, many of which may have commenced antenatally. The cohort element of the study has several unique features compared to the only other two similar studies reported in the literature. First, the case definition for inclusion was broad and did not assume an intrapartum aetiology. Second, the study is population-based with a contemporaneously ascertained randomly selected comparison cohort. Third, we have maintained direct contact with over 80% of the survivors for both the infants with (cases) and without (controls) a history of newborn encephalopathy. Finally, in addition to direct assessment we have access to other sources of follow-up information arising from the WA Cerebral Palsy Register, the WA Birth Defects Registry and state-wide hospital discharge data, for the whole cohort. This has enabled us to maintain a more complete ascertainment of outcomes irrespective of continued direct contact.

Follow-up of both groups of children in the study has included the following. Parent completed questionnaires about development at ages 4, 8, 12, 18, 24, 36 and 48 months using the Brickers Infant Monitoring Questionnaire, and the Griffiths Mental Development Scales at age 1-2 years. A neurodevelopmental assessment was performed by a paediatrician at three and a half years. An assessment was made by a psychologist for receptive language, IQ, behaviour, temperament, academic achievement and neuro cognitive abilities at ages 5 and 8 years. When children turned 6 and 7 years, we used parent completed questionnaires designed to collect information about their child's speech and hearing, use of health services, social, emotional and physical well-being, as well as measures of family functioning, mental health of the primary caregiver, socio-economic status and education of primary caregiver and their partner, and family demographics. In June 2001 we commenced face-to-face assessments of the cohort as they turned eight years old. This stage of the assessment will be complete at the end of 2004 when the youngest children turn eight. The eight-year assessment comprises an assessment of scholastic ability, and a short form IQ derived from assessing speed of information processing, matrices, similarities and recall of digits. Additionally there is an assessment of visually guided fine motor co-ordination which will also provide performance data on handedness to complement the reported data collected at ages 18 months, 3 years, 5 years and 6 years. Neuro-cognitive efficiency is being tested using the

Symbol Digits Modalities Test in the oral or written forms as appropriate to the child's functional ability. Teacher rated assessment of competencies in English, social studies, maths and science and special educational needs are also being collected with parental consent.

The 8-year-old data collection is still on-going and due for completion at the end of 2004. Analysis of the early developmental data is also on-going with some important findings having been reported. To date 13.4% of NE cases and one control child have died. Overall 11.6% of the cases have been notified to the WA Cerebral Palsy Register as having cerebral palsy. This figure is likely to increase as the population of children age and continue to be diagnosed and notified to the Register. One control has developed cerebral palsy.

Clinical follow-up to the age of 5 years indicates that newborn encephalopathy places infants at significant risk of developmental delay or disability (see Table 1). Findings from our clinical follow-up also indicate that NE appears to increase their vulnerability to mental health problems. Thus far, as a result of routine follow up contact by clinical staff on the WA NE Study, 35 (14.6%) of the surviving case infants have been referred to primary health care providers compared to 14 (2.4%) of the surviving control infants. Of particular concern is that among the referrals for case infants, six were for psychiatric services.

Table 1. Disabilities for cases and controls at age 5 years.

DISABILITY	CASES (%)	CONTROLS (%)
Cerebral Palsy	26 (11)	1 (0.17)
Autism Spectrum Disorder	12 (5.0)	5 (0.8)
Physical Disability (excluding CP)	11 (4.6)	12 (2.1)
Multiple Disability (excluding others already listed)	14 (6.0)	1 (0.17)
Developmental Delay	7 (3.0)	1 (0.17)
Specific Learning Impairment	5 (2.0)	2 (0.35)
Hearing Impairment	2 (0.8)	0
Intellectual Disability	8 (3.3)	1 (0.17)
Sensory Impairment	5 (2.0)	1 (0.17)
Epilepsy (only occurring as a co-morbidity)	18 (7.5)	3 (0.5)
Autosomal Dominant	2 (0.8)	1 (0.17)
Chromosomal Abnormality	1 (0.4)	0
Immunological Disorder	1 (0.4)	1 (0.17)
Dyspraxia	1 (0.4)	3 (0.5)
Speech Impairment	3 (1.2)	1(0.17)
Muscular Dystrophy	0	1(0.17)
Other Neurological	0	1(0.17)
Visual Impairment	12 (5.0)	1 (0.17)
Attention Deficit Disorder	11 (4.6)	6 (1.0)

We have recently compared the characteristics of cerebral palsy among the survivors of term newborn encephalopathy with the characteristics seen in children born at term who develop cerebral palsy but without a history of encephalopathy in the newborn period. We found that a history of newborn encephalopathy was present in a quarter of the cases of term cerebral palsy. Compared to the children without a history of cerebral palsy, when present, the cerebral palsy was more severe and more likely to be accompanied by cognitive impairment, a higher disability score, epilepsy and a lack of speech. These children were also more likely to have spastic quadriplegia or dyskinetic cerebral palsy and die in the first five years of life (OR 4.5; 95%CI 1.3, 16.0). We believe our data to be unique as no other study in the literature has been able to quantify the consequences of newborn encephalopathy in a population in quite this way. As such our findings will be useful for clinicians in the counselling of parents of children with newborn encephalopathy as to their likely prognosis.

Findings during follow-up of cases with newborn encephalopathy and controls have also highlighted an association between newborn encephalopathy and a subsequent diagnosis of autism. Twelve cases and five controls have been diagnosed with an autism spectrum disorder according to DSM IV criteria. Among survivors, the incidence of autism among cases had an odds ratio of 5.9 (95% CI 2.0-16.9) when compared to the controls indicating a prevalence rate far in excess of the general population (4/1000). Additionally, six of the twelve newborn encephalopathy cases or 50% have a birth defect and one control with autism was noted to have dysmorphic features at the age of three years. The newborn encephalopathy cases have a significantly higher rate of birth defects compared with all the cases with newborn encephalopathy to whom 28% have at least one birth defect. Our population-based case-control study has highlighted a strong association between newborn encephalopathy and autism with cases being almost six times more likely to develop autism than controls. These findings are of importance to paediatricians and allied health professionals who provide long-term developmental follow-up for children after newborn encephalopathy who should be considered at increased risk of autism. Although we cannot comment on the aetiology of autism based on these findings, we hope to contribute to the growing pool of information about autistic disorders and stimulate research into the causal pathways to this increasingly diagnosed, enigmatic and disabling condition.

Our cohort has several unique features compared with similar studies reported in the literature. First the case definition for inclusion is broad and does not assume intrapartum aetiology. Second the study is population based and we have a contemporaneously ascertained randomly selected comparison cohort. Third, we have maintained direct contact with over 80% of the survivors and have access to aspects of follow-up information (eg. the cerebral palsy register, birth defects registry, state wide hospital morbidity data), which relates to the whole cohort regardless of continued direct contact.

The data being collected in each stage of this follow-up study will continue to add greatly to our ability to provide a realistic prognostic view for parents whose infant has newborn encephalopathy. The data will also enable us to explore the

factors which increase or decrease the likelihood of an adverse outcome. During this follow-up period (to the end of 2004) when the youngest child turns eight years, we aim to:

- Quantify the cumulative mortality to eight years of age;
- Determine the incidence of cerebral palsy to eight years by which time transient cases will be known and excluded from this estimate;
- Estimate the proportion of survivors who outgrow an early diagnosis of cerebral palsy and the proportion whose type of cerebral palsy changes;
- Estimate the incidence of impaired hearing, visual impairment and other sensory deficits;
- Estimate the incidence of cognitive delay;
- Estimate the incidence of attention deficit disorder and other behavioural problems;
- Describe handedness;
- Describe the pre- and peri-natal factors associated with autism;
- Obtain functional measures of socialisation and self-care skills;
- Investigate family functioning and the burden associated with having a child with a disability;
- Investigate the predictive value of our grading system for newborn encephalopathy in terms of adverse outcome. This is of particular importance in view of the fact that all other grading systems are based only on the subgroup of infants with encephalopathy which is assumed to be hypoxic ischaemic encephalopathy;
- Investigate the relationship between antepartum and intrapartum exposures and adverse long-term outcome.

The international significance of the WA newborn encephalopathy study and its findings are illustrated by the recent consensus report prepared by the American College of Obstetrics and Gynecology and the American Academy of Pediatrics. The authors pointed out that the Western Australian study is the only study that currently can provide an unbiased estimate of the full range of impairments following newborn encephalopathy since it is the only truly population-based study of newborn encephalopathy with a contemporaneous comparison cohort of infants who were not encephalopathic following delivery. Furthermore, in terms of quantifying the contribution of newborn encephalopathy to the aetiology of the cerebral palsies, when linked to the complete WA cerebral palsy register and to the Maternal and Child Health Research Database (all births since 1980), this study enables analyses both retrospectively (from cerebral palsy cases back to newborn status) and prospectively.

The Western Australian Newborn Hearing Programme

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The WA Newborn Hearing programme is funded by the Department of Health and it is affiliated with the Telethon Institute for Child Health Research. The Western Australian Hearing Screening Programme is now in its fourth year since its establishment in February 2000. The aim of the programme is the

early detection of bilateral permanent hearing loss in childhood in order to commence intervention by the time the baby is 6 months of age. The two types of technology used by the programme measure transient evoked otoacoustic emissions (TEOAEs) and automated auditory brainstem responses (AABR). These are sometimes used in combination.

Newborn hearing screening is currently offered to all babies born at one of 5 of the largest maternity hospitals in Perth i.e. King Edward Memorial Hospital, St John of God Health Care Subiaco, Joondalup Health Campus, Osborne Park Hospital and Woodside Hospital. All babies in Western Australia who are admitted to a level 2 neonatal nursery at St. John of God Health Care, Subiaco or Joondalup Health Campus or a level 2/3 neonatal nursery at King Edward Memorial and Princess Margaret Hospitals are eligible for screening.

Since the programme began in February 2000, over 39,000 babies have received a newborn hearing-screening test and about 25 babies have been diagnosed with a bilateral hearing loss at an early age. The Hearing Loss Prevalence Program (HeLP), established in 1999, also continues to identify all Western Australian children who have permanent hearing loss. This information will be used to estimate the prevalence of permanent childhood hearing loss and detect if any children who have passed the newborn hearing screen are later diagnosed with a hearing loss.

The programme is currently being evaluated by The WA Department of Health following the completion of a comprehensive research summary report in May 2003. This report collated information on all aspects of the programme from February 2000- 31st May 2003. At this stage it appears that from 1st May 2004 the programme may cease to be managed by The Institute for Child Health Research. Management of the programme may then be handed over to Women's and Children's Health Services (WCHS). Likewise, at this stage from 1st May 2004 the programme may alter and screen only babies admitted to a level 2 or level 2/3 special care nursery and babies with known risk factors for hearing loss. Both the ICHR and WCHS aim to continue to work together to develop a transitional plan to ensure that hospitals and parents of newborns will be well informed if changes are to occur to the programme in the near future.

Rett syndrome

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We continue to maintain our population-based Rett syndrome register, now known as AussieRett, using both the Australian Paediatric Surveillance Unit and the parent association (the Rett Syndrome Association of Australia) as major sources of case ascertainment. At enrolment, families and reporting clinicians complete standard questionnaires and, where possible, collection of blood samples is organised for screening for mutations in the MECP2 gene. We were particularly pleased to hear mid-year that our application to the US National Institutes of Health had been successful and that funding had been awarded to allow us to continue to follow up this Australian Rett syndrome cohort for a further five years. Funding also provided by NHMRC will allow us to include an important video component to the study. In the latter part of 2003 we undertook a pilot study to test out the process for obtaining video footage on our cases in preparation for the participation of the entire cohort in 2004. This unique data source will provide valuable material both to characterize the spectrum of this disorder and to track the course of Rett syndrome over time.

During 2003 we continued to make substantial contributions to the world literature on Rett syndrome. The report we wrote showing that left hand preference is much commoner in Rett syndrome than in the general population was published as was our article describing the mild phenotype of the R133C mutation. Additionally we have now shown that girls with the R294X mutation can also function better while those with the R270X and R255X are more severe. We have only been able to provide these important findings about the relationship between a particular genetic mutation and the clinical features because of this national population-based resource which we have developed over the last ten years.

2003 was a particularly busy year in the development of InterRett, the International Rett Syndrome phenotype database which is funded by the International Rett Syndrome Association and guided by an international reference panel. The aim of this project is to collect data on large numbers of cases from all over the world with the purpose of providing a unique web-based information resource on Rett syndrome as well as a data source for researchers. After finalising the core study materials (questionnaires based on and modified from their Australian counterparts) we carried out a pilot study to test the processes for collecting data from families and clinicians both through web-based and conventional paper-based means. This pilot, which involved 81 families returning questionnaires, was most successful and very positively evaluated by participants. As a first stage of the output to be provided to the community, an analysis of these results was undertaken and a simple graphically illustrated report was compiled and posted on the web. The study proper was launched in October with data collection in full swing by year end.

INFECTIOUS DISEASES

The Vaccine Impact Surveillance Network (VISN)

The Vaccine Impact Surveillance Network is currently undertaking enhanced surveillance of invasive pneumococcal disease (IPD) in Western Australia. IPD (primarily pneumonia and meningitis), which has been notifiable since 2001,

is an important cause of morbidity, mortality, and serious disability and thus a major burden for families and government services. Clinical and microbiological data as well as information on risk factors for IPD have been collected since 1996. The database provides an opportunity to evaluate vaccine programs and thus inform policy makers and the general public. In order to look at factors predisposing to IPD as well as the long term effects of IPD, the data will be linked to our Institute's Maternal and Child Health Research Data Base.

Impact of routine immunizations on childhood survival in Tari, Southern Highlands Province, Papua New Guinea

D Lehmann, N de Klerk, M Firth (ICHR) in collaboration with J Vail and MP Alpers

Following a report of increased risk of death associated with diphtheria tetanus pertussis (DTP) and oral polio vaccination of children living in rural areas of Guinea-Bissau, the World Health Organization Department of Vaccines and Biologicals sought proposals to determine the effects of routine infant immunization on survival in areas of high mortality. We were awarded a grant to investigate the impact of routine immunizations on childhood survival in Tari, Southern Highlands Province, Papua New Guinea. As part of other studies, continuous monthly demographic surveillance enabled us to identify births, deaths, migrations, and immunization status of all children born in Tari between 1989 and 1994. The study aimed to determine the effect of DTP, BCG and measles vaccinations on mortality in the first two years of life. We have found no deleterious effects of infant immunizations. A manuscript is currently under review.

An effectiveness study of pneumococcal polysaccharide vaccine among children in the highlands of Papua New Guinea

D Lehmann, N de Klerk, M Firth in collaboration with D Whiting, J Dyke, T Dyke, J Wilson, S Rogers, D Gehala, E Tumbiako, Michael Alpers (Papua New Guinea Institute of Medical Research).

In the 1980s pneumococcal polysaccharide vaccine was found to be efficacious in reducing mortality and severe morbidity due to acute lower respiratory infection when given from the age of 6 months onwards to young children in the highlands of Papua New Guinea. An effectiveness study of a 23-valent pneumococcal polysaccharide vaccine was subsequently undertaken between 1991 and 1995 when the vaccine was offered to all children aged 8-23 months attending rural child health clinics. The effectiveness of this vaccine in reducing mortality and hospitalisation for pneumonia is being evaluated.

Neonatal immunization with pneumococcal conjugate vaccine in Papua New Guinea

D Lehmann, (ICHR), J Reeder (Papua New Guinea Institute of Medical Research), P Holt (ICHR), P Richmond (School of Paediatrics and Child Health, UWA), W Pomat (PNGIMR/SPACH)

We have been awarded a \$2.3 million Wellcome Trust/NHMRC International Collaborative Research Grant to investigate the possibility of neonatal pneumococcal vaccination to reduce morbidity and mortality from

pneumococcal disease in young infants in Papua New Guinea. Infants in developing countries are at extremely high risk for invasive pneumococcal disease and have neonatal onset of dense respiratory tract pneumococcal colonisation, which may have long-term effects on the development of protective immunity. Approximately 300 infants will be enrolled at birth and randomised to receive pneumococcal conjugate vaccine (PCV) either at 1-2-3 months or 0-1-2 months of age with a third group receiving only routine immunizations. All children will receive a dose of pneumococcal polysaccharide vaccine at age 9 months. Serotype-specific antibodies will be assessed in blood and saliva samples and, together with ongoing morbidity surveillance, will provide data on safety and immunogenicity of neonatal immunization. To ensure immunological safety, immune responses to concomitant vaccines and viral and environmental antigens will also be examined as well as overall T-cell maturation. This study will provide proof of principle of the safety and immunological feasibility of neonatal PCV immunization, which is essential before progression to larger-scale studies in high-risk populations. It will further our understanding of the basic immunological mechanisms underlying conjugate vaccine responses during the critical neonatal period, and provide insight into the interactions between the developing T-cell system and vaccines, which occur in these infants against a background of intense microbial stimulation.

Multicentre double-blind randomised controlled trial comparing the effectiveness of topical Ciprofloxacin and Sofradex as treatments for chronic suppurative otitis media in Aboriginal children

S Couzos (National Aboriginal Community Controlled Health Organisation), I Ring, H Coates, F Lannigan, R Mueller, R Murray, D Lehmann (ICHR), S Eades (ICHR)

Chronic suppurative otitis media (runny ears) is extremely prevalent in Aboriginal babies from a young age and the management is often protracted and frustrating. Treatment may include the use of ear drops. A randomised controlled trial (the "NACCHO trial") was conducted to compare the efficacy of sofradex with ciprofloxacin ear drops. In a paper published in the Medical Journal of Australia it is recommended that ciprofloxacin be made freely available as first line treatment of this debilitating condition.

POPULATION STUDIES

The Western Australian Twin Register

Janice Hansen, Phyllis Alessandri, Kerryn Coleman, Nick de Klerk, Maxine Croft, Alan James, Paul Burton

The WA Twin Register was established in 1997 using a grant from the WA Health Promotion Foundation (Healthway), and initially comprised data on all WA multiple births between 1980 and 1992 inclusive. The main purpose for establishing the Register was to invite families to participate in the WA Twin Child Health (WATCH) study which examined the roles that genes and the environment play in the link between childhood asthma and atopy, and exposure to environmental tobacco smoke. The Register has since been extended to include 1993-1997 births, using part of a grant from the National

Health and Medical Research Council (NHMRC) for the "WATCH for Asthma" (WFA) study. This study aimed to collect detailed clinical asthma phenotype data on twins born between 1990 and 1995, and their families, and to investigate and describe the familial aggregation of childhood asthma and atopy.

A total of 11,188 multiple birth children, born in WA between 1980 and 1997 inclusive, were identified, representing 2.5% of all births during that time. They comprised 5,340 sets of twins, and 164 sets of triplets, quadruplets and quintuplets. Forty-eight families had two sets of multiples during the time period. Seven hundred and twenty nine (6.5%) of the multiple birth children were known to have died to date. Six hundred and thirty six were either stillborn or died within the first four weeks of life, giving a perinatal death rate of 56.8 per 1,000 births. Higher order multiples (triplets, quadruplets and quintuplets) had a higher rate of both total childhood deaths and perinatal deaths when compared with twins (12.0% vs. 6.3% ($p < 0.001$) for all deaths, and 11.4% vs. 5.4% ($p < 0.001$) for perinatal deaths, respectively).

The WA Twin Child Health (WATCH) study.

The aim of the WATCH study was to collect data from families of multiples who belonged to the WA Twin Register, to examine the roles that genes and the environment play in the link between childhood asthma and atopy, and exposure to environmental tobacco smoke. Over 90% of eligible families of multiples born between 1980 and 1995 have been contacted and invited to join the WATCH study. Completed questionnaires have been received from nearly 2,500 families (57%), resulting in data from over 13,000 individuals. Several factors resulted in increased response. Shorter questionnaires were more likely to be returned than the longer version (62% vs. 55%, $p < 0.001$); telephone contact of families who did not respond produced higher response rates than if they were mailed a second letter (91% vs. 78%, $p < 0.001$); and mothers who were under 20 years old at the time of their multiples' birth were less likely to reply to the introductory letter, and less likely to agree to participate than older mothers (45% vs. 79%, $p < 0.001$; 42% vs. 70%, $p < 0.001$, respectively); they were also less likely to return completed questionnaires (30% vs. 61%, $p < 0.001$). There was no difference in the overall response rates between metropolitan and non-metropolitan families (59% vs. 62%, $p = 0.11$). However, compared with families living in rural areas of WA, families who lived in the Perth metropolitan area were more likely to respond to the initial letter (80% vs. 75%, $p = 0.007$), but no more likely to participate in the study (70% vs. 69%, $p = 0.76$). But, having agreed to participate, they were then more likely to return completed questionnaires than families who lived in Perth (90% vs. 85%, $p = 0.002$).

Using the questionnaire data, we were able to examine a number of asthma and atopy endpoints, including wheezing ever, wheezing in the last 12 months, current asthma, hay fever and eczema. They all showed greater concordance in monozygotic twins compared with dizygotic twins, suggesting evidence of a genetic component. After adjusting for age, boys had a significantly higher prevalence of current asthma ($p = 0.021$), wheezing ever ($p < 0.001$) and current

wheeze ($p < 0.001$), when compared with girls, but showed no difference in the prevalence of hay fever, and eczema.

“WATCH for Asthma” Study

The “WATCH for Asthma” study commenced in 2000 using a grant from the NHMRC. Its main aim was to explore the complexity of the asthma phenotype in WA twin families by collecting detailed clinical asthma phenotype data on a sample of twins born in WA between 1990 and 1995, and their families. Families, consisting of the twins, their biological parents and any of their siblings aged 7 and over, were invited to attend one of our Clinics to undergo a series of standard breathing, allergy and blood tests. We were also offering a free zygosity test to families who are unsure of the zygosity of their twins. Our main Clinic was held at Princess Margaret Hospital in Perth, but we have been able to conduct Clinics in several country regions during school holidays. Successful clinics have been held in Busselton, Geraldton, Northam, Merredin, Bunbury and Albany.

So far, over 1,000 people from 238 families have attended one of the Clinics. Blood samples have been collected from over 95% of people, and we have completed baseline spirometry measurements on 98%, methacholine challenge tests on 93%, skin prick tests on 99%, and exhaled nitric oxide measurements on 63%. About 70 families have taken advantage of having their twins' zygosity determined from their DNA.

Skin prick tests to a panel of 9 common allergens have been performed on over 98% of those attending the clinics. The most common positive reactions were to house dust mite (*D. pteronyssinus*) (30%) rye grass (19%) and grass mix (17%). Methacholine challenge test, to measure bronchial hyperresponsiveness, was successfully completed by 93% of participants, the main reasons for non-completion being equipment failure or poor test technique. Nearly a quarter of people recorded a response to this test, as determined by a fall of at least 20% in lung function before the maximum dose of methacholine was administered.

Asthma had been diagnosed in 20% of participants (15% of mothers, 8% of fathers, 26% of twins, 24% of siblings of twins), and atopy as determined by at least one positive reaction to skin prick testing in 49% (45% of mothers, 60% of fathers, 48% of twins and 51% of siblings). Nearly 70% of asthmatics were atopic, compared with 44% of non-asthmatics ($p < 0.001$). Forty-four percent of parents were either current or former smokers, resulting in 55% of children being exposed to passive smoke at home.

RASCALS Study (Randomly Ascertained Sample of Children in Australia's Largest State)

SR Zubrick, SR Silburn, JJ Kurinczuk (University of Oxford, UK), G Dixon, DE Parsons, S Dragovic, K Moore, PR Burton (University of Leicester, UK), in collaboration with VP Dawes (formerly the Health Department of Western Australia), AJ Plant (Curtin University).

The RASCALS Study (formerly known as the Western Australian Pregnancy and Infancy Survey) was initiated in 1995 whereby a 10% random sample of all mothers in Western Australia who recently delivered a liveborn baby between 1995 and June 1997 were selected to participate in a self-completion survey. Of the 6019 mothers who were mailed a questionnaire an outstandingly high 82% returned completed forms. From this sample base a group of caregivers continue to be followed up annually at the time of the study child's birthday.

The information initially collected was used in the evaluation of health promotion and disease prevention services and centred on the mother's behaviour before, during, and after pregnancy. The survey included questions on rubella immunisation, folic acid intake, SIDS risk factors, infant feeding practices, cigarette smoking, alcohol consumption, infertility, family composition and so on. Follow-up information included childhood immunisation and passive tobacco smoking and this is to be used in the assessment of modifiable risk factors relating to the uptake of childhood immunisation and passive smoking. Other information such as stress, anxiety, depression, parental disciplinary practices, maternal and paternal employment practices, family composition and an ongoing assessment of both the study child's and primary caregiver's mental well-being, will be used to identify possible causal factors and protective factors of mental health.

The RASCALS data has been used as a baseline for the measurement of the prevalence of specific language disorders within the general childhood population of Western Australia. In doing so the RASCALS data has made an important contribution to the Looking at Language project.

Associate Professor Wendy Hall from the University of British Columbia, Canada, has analysed the data relating to behavioural sleep problems (childhood insomnias) up to the age of four years. Sleep problems are important because they have a profound effect on both the affected child and their parents. Evidence suggests these effects can lead to significant degrees of distress and effects on parenting behaviour. Sleep problems were common in the RASCALS sample with 23% of parents of one year olds reporting problems with sleeping. From ages two to four the proportions ranged from 13% to 17%. Between 28% and 62% of children with sleep problems had problems that persisted for more than a year. The prevalence and persistence of sleep problems in this group was lower than reported previously. However, this is probably because our results were from a general sample of the childhood population and not clinically derived samples of children. Nevertheless, the prevalence and persistence of sleep problems were at a level that suggests that significant numbers of parents have to contend with children who have sleep problems that persist for long periods of time. These findings are in the process of being submitted for publication.

The RASCALS study is one of a few key longitudinal studies in Australia. Data collection is now complete for the children born in 1995. We are now in the process of sending out the eight-year questionnaires to the children born in 1996. The data from these questionnaires will be enhanced, following

parental consent, by information obtained from the children's teachers. We are continuing with good response rates, these are illustrated in the table above.

Raine Study - Physical activity in adolescents

Launched in June 2003, this project is examining the circumstances and patterns of behaviour that develop from an early age that are related to levels of physical activity and the consequences of inactivity in adolescents.

Physical activity levels, body fat and obesity play a vital role in the health of adults. The consequences of inactivity include obesity, elevated blood pressure, diabetes and high-risk behaviour.

We are investigating the physical activity levels of 13-year-old children in the context of a large longitudinal cohort study.

Data is being collected for 2,000 children concurrently participating in the ongoing Western Australian Pregnancy Cohort (Raine) Study. The mothers of the children were enrolled at 18 weeks in pregnancy and the children have been followed at birth, 1, 2, 3, 5, 8, and 10 years of age.

Intensive assessment of these children at 13 years of age includes objective measurement of physical activity, physical fitness, and motor competence, as well as markers of cardiovascular health, low back pain, and mental health. Attending parents are also asked to have their height, weight and blood pressure measured.

Response rates to all elements of the 13-year follow-up by study families have been exceptionally high. In its first six months, assessments for more than 200 study children have already been completed.

Of particular note, fasting blood samples have been collected from more than 90% of study children so far assessed. Furthermore 86% of study mothers and 50% of fathers have also provided blood samples.

It is anticipated that findings from this study will assist health, education and welfare agencies to promote greater levels of physical activity in adolescence and discourage 'risky' behaviour such as smoking and the use of alcohol and drugs.

WA Mortality Study

J Freemantle, N de Klerk, A Read, E Blair, L Alessandri (deceased), in collaboration with M Divitini (Department of Public Health, University of Western Australia), and Forensic Pathology, (the PathCentre) Western Australia

The results of The Western Australian Mortality study were presented in a doctoral thesis submitted by J Freemantle in February 2004. The Doctoral Theses was funded through a Healthway PhD Scholarship. Every live birth recorded in Western Australia (WA) between 1980-1997 and Western

Australian born infant and childhood deaths (to age 19) occurring in Western Australia between 1980-1998 were included in the results. Major findings were:

- Between 1980 and 1997, births to Indigenous mothers accounted for 6% of total WA births. Approximately 46% of Indigenous births were to mothers living in a remote location compared with 9% of non-Indigenous births.
- Indigenous mothers gave birth at an earlier age (30% of births were to teenage mothers compared with 6% of non-Indigenous births), and were more likely to be single than non-Indigenous mothers (40% Indigenous, 9% non-Indigenous).
- Indigenous infants had more siblings, were born at an earlier gestation and with a lower birth weight and percentage of expected birth weight.
- The cumulative mortality rate (CMR) for Indigenous infants was 22 per 1000 live births compared with 6.7 for non-Indigenous infants, a relative risk (RR) of 3.3 (95%CI 3.0, 3.6).
- While there was a decrease in the CMR over time for both populations, the disparity between the rate of Indigenous and non-Indigenous infant mortality increased.
- The Indigenous postneonatal (>28 to 365 days) mortality rate (11.7 per 1,000 neonatal survivors) was higher than the neonatal (0 to 28 days) mortality rate (10.3 per 1,000 live births), unlike non-Aboriginal infants, where the neonatal mortality rate (4.3 per 1,000 live births) was nearly twice that of the postneonatal mortality rate (2.4 per 1,000 neonatal survivors).
- The main causes of infant mortality among Indigenous infants were infection followed by Sudden Infant Death Syndrome (SIDS) which are potentially preventable. For non-Indigenous infants the main causes were sequelae of prematurity and birth defects.
- The CMR attributable to SIDS increased over the years amongst Indigenous infants and decreased significantly over the years in the non-Indigenous population, so that the disparity in mortality between the two populations increased and, in 1995 to 1997, was over seven times higher amongst Indigenous infants.
- The CMR was highest amongst infants living in remote locations for all causes of death except for Indigenous deaths attributable to SIDS, where the risk of death was highest amongst infants living in metropolitan locations.
- With the exception of infection, there was no difference in cause-specific mortality amongst Indigenous infants according to geographical location.
- Indigenous infants living in a remote location were at a significantly increased risk of death due to infection compared with their peers living in a rural or metropolitan location.
- The risk of death for Indigenous children aged between 1 and 19 years was more than three times higher than for non-Indigenous children. This risk was significantly increased even after other perinatal maternal and infant characteristics were considered.
- Accident and injury, and infection, were the main causes of mortality amongst Indigenous and non-Indigenous children.
- The risk of accidental death for Indigenous children was nearly four times higher, and death due to infection nearly seven times higher, than for non-

Indigenous children.

- The mortality rate in Indigenous children was highest in those born in remote areas, and was highest in rural areas for non-Indigenous children.

The mortality database is currently being extended to include births to the end of 2003 and deaths to the end of 2002. The analysis of this extended database will inform the 1st Annual Report of the Advisory Council for the Prevention of Deaths in Children and Young Adults. The Advisory Council, which is chaired by Professor Fiona Stanley AC, will report to the Cabinet Standing Committee on Social Policy through the Hon Sheila McHale MLA, Minister for Community Development, Women's Interests, Seniors and Youth.

OTHER PROJECTS

Suicide Prevention

SR Silburn, J Cugley, D Robertson, K Northey, B Williams, A Brok, N Kerr, K Miller, T Barker, A Cox, M Sayers

The Institute has supported a program of translational research in suicide prevention since 1991. This research aims to ensure that new knowledge on the aetiology and epidemiology of suicidal behaviour and suicide can be applied in current policy and practice. A component of this is the accommodation of the Western Australian Ministerial Council for Suicide Prevention (MCSP) at the Institute. This Council is chaired by Professor Sven Silburn and reports to the Minister for Health, advises government and coordinates a wide range of activities throughout the State aimed at reducing the morbidity and mortality associated with suicidal behaviour. The MCSP is also active in advancing community and scientific understanding of suicide and its prevention. Corporate sponsorship from Woodside Energy Ltd, has enabled the establishment and ongoing support of the ASPIRE (Australian Suicide Prevention information and Resource Exchange) website. This is widely acknowledged as one of Australia's leading sources of publicly available information and community resources for suicide prevention. (<http://www.mcsp.org.au/about/sitemap.html>)

The MCSP has continued to support the maintenance of the WA Coroners database on Suicide, and databases on deliberate self-harm admissions to Perth teaching hospitals. In addition to monitoring epidemiological trends, the information from these databases has enabled monitoring of the implementation and impact of systematic reforms to the hospital and follow-up community care provided to people following deliberate self harm hospital admissions. During 2003 the MCSP has been involved with the Office of Mental Health in the further development of Emergency Medicine and Psychiatry case management protocols to ensure best practice and more effective links between hospital emergency departments and community based psychiatric emergency services.

National Suicide Prevention Strategy (NSPS) funding over 18 months has enabled the MCSP to research how suicidal and non-suicidal young men

aged 18 – 35 understand their experiences of personal distress, mental health disorders and suicidality as well as their attitudes to help-seeking, use of mental health and other counselling services. This study has involved the use of CATI (Computer Assisted Telephone Interviewing) methods to locate eligible participants, to screen them for current suicidal risk, and invite their participation in focus groups to discuss these issues. Very particular care has been taken to address the significant ethical and safety issues involved in research such a personally sensitive issue. The representativeness of the views expressed in the focus groups will then be assessed with a population based survey using CATI survey methods to obtain a baseline for a proposed media-based mental health promotion campaign designed to advance young men's mental health literacy and overcome negative attitudes to help-seeking.

During 2002 and 2003 the MCSP combined with the AUSEINET (Australian Early Intervention Network) at Flinders University and SPA (Suicide Prevention Australia) in Sydney to lead a successful tender for a \$700,000 NSPS national funding contract for the CommunityLife project. This involved the design, evaluation, publishing and dissemination of community development resource materials for individual communities to initiate and implement safe and effective suicide prevention programs tailored to their own particular circumstances. The materials produced are now being disseminated through the CommunityLife website which is supported by the AUSEINET website (<http://www.community-life.org.au/>). The CommunityLife resource materials are also being published by the Commonwealth Department of Health and Ageing as an additional volume in the next edition of the LIFE (Living is for Everyone) National Framework for Suicide Prevention.

Infants of teenage mothers: A High Risk Population?

K Williams, H Leonard, L Colvin, E Tursan d'Espaignet

Although it is often claimed that children born to teenage mothers are at greater risk of poor outcomes at birth and infancy, these claims are often not based on studies that have controlled for possible confounders. The project aimed to determine if children born to teenage mothers have poorer birth and infant outcomes than children born to non-teenage mothers after controlling for potential confounders.

This study used data from the Maternal and Child Health Research Data Base linking birth, death and hospital discharge data for all children born in the state of Western Australia from 1984-1997.

The results indicated that teenage mothers were more likely to be Aboriginal and socio-economically disadvantaged; and on initial analysis had poorer birth and infant outcomes than children born to non-teenage mothers, particularly among the youngest within the teenage category. After controlling for potential confounders, outcomes for children born to mothers aged 19 years were not significantly different from those of older mothers. Children born to mothers aged less than 19 years had significantly poorer birth and infant outcomes than children born to non-teenage mothers, but there was a substantial reduction in

many of the differences after controlling for potential confounders.

Monitoring trends in Sudden Infant Death Syndrome in Australia from 1980–2000

E Tursan d'Espaignet, F Stanley, R Byard (University of Adelaide)

Current public health advice to reduce the risk of sudden infant death syndrome (SIDS) stresses that newborns: (i) be placed to sleep on their back (and stressed avoiding propping on the side or placing on the stomach), (ii) have their head remain uncovered during sleep; and (iii) be kept smoke-free both in utero and after birth.

The main objectives of this project were to evaluate the impact of the advice on the incidence of SIDS in Australia; to assess whether any changes could be associated with a shift in the diagnosis of death from SIDS to other causes of infant death; and/or a postponement of death from infancy (under one year of age) to early childhood (1–4 years of age).

Changes in levels and trends in rates of SIDS, other causes of infant death and early childhood deaths were examined using Poisson regression techniques on Australian Bureau of Statistics electronic records of deaths (including age and cause of death) for each State and Territory and Australia as whole for the period preceding (1980–90) and following (1991–2000) the advice to the public. The SIDS rates were calculated for deaths in the first month, 1–5 months and 6–11 months periods. The results clearly indicated that deaths in the 1–5 month category comprised the substantial part of all SIDS deaths, and that there were substantial and significant reductions in SIDS rates particularly in that age group. There was also no indication of the reduction in SIDS being accompanied by a concomitant shift to other causes of infant death or of a postponement to death in early childhood. The paper also examined the literature for changes in the various targeted risk factors. Except for changes in sleeping position, there has been very little change in smoking prevalence. It was estimated that a further 40% reduction in SIDS rates could be achieved if women did not smoke during their pregnancy or after giving birth.

Unexplained fetal deaths (UFD)

MA Measey, E Tursan d'Espaignet, N de Klerk, FJ Stanley, P Weinstein (University of Western Australia), M Croft, A Charles (King Edward Memorial Hospital for Women)

Unexplained stillbirths or fetal deaths of at least 20 weeks gestation or 400 grams birthweight now account for the majority of perinatal deaths in Australia. Little is known about the aetiology of UFD or if affected women are more likely to experience other adverse outcomes. To date, the only Australian study to investigate the risk factors for UFDs was conducted at this Institute in the early 1990s by Louisa Alessandri and colleagues.

Preparation for a case-control study on UFD was commenced in late 2003. This study aims to (1) identify risk factors for UFD; (2) investigate the general and reproductive health of women after an UFD; (3) investigate whether other

children of women affected by UFD are at increased risk of poor health or death; and, (4) review factors associated with post-mortem examinations for fetal deaths.

This study, which is the basis for MA Measey's PhD project (recipient of an NHMRC PhD scholarship), aims to use linked data from the Maternal and Child Health Research Data and WA Hospital Morbidity Database to compare all UFD in WA between 1980 and 2002 with a random sample of live born controls drawn from the same population. Additional data will be collected from maternal medical records and post-mortem reports for a proportion of cases and controls. Ethics approvals from relevant bodies and permission for access to data from the WA Health Department's Confidentiality of Health Information Committee are currently being sought.

Student support

E Tursan d'Espaignet (coordinator)

This support project was designed and implemented for the benefit of post-graduate and honours students, and some staff at the Institute. Regular meetings were held to discuss various biostatistics and epidemiological methods and techniques.

Population health information system for the NT

E Tursan d'Espaignet

Dr Tursan d'Espaignet was contracted by the NT Department of Health and Community Services to produce a blueprint for the establishment of a population health information surveillance and monitoring system for the Northern Territory. Senior staff members of the NT Health Department are currently considering the final report.

Collaboration for Applied Research and Evaluation (CARE)

The CARE team undertakes applied research projects through contracts with government and other agencies. For the past four years, it has undertaken projects under a \$1m Department of Health contract. Other smaller projects under contracts with the Disability Services Commission and State Child Development Centre have also been undertaken.

The contracting cycle for CARE operates within the financial year, therefore the program of work for 2003 straddles two contracting years. Contracted projects for the 2002/2003 financial year were divided into three themes: evaluation, research and development, and capacity building. A total of 19 projects fell into these three research areas.

1. Evaluation

The Institute, over a number of years, has been involved in the evaluation of the Department of Health's Community Mothers Program, the Department of Community Development's Best Beginnings Program, State Child Development's Play and Learning Program and the Newborn Hearing Screening Program. The involvement of the Institute has ranged from providing evaluation advice and

support (Community Mothers, Best Beginnings) to service delivery and full program evaluation (Newborn Hearing Screening). In addition, the Institute has provided support to the Play and Learning program that has involved the writing of training manuals and the provision of training in evaluation techniques.

2. Research and development

The research and development area included a number of projects involving the provision of advice and evaluation support services specifically serving the needs of the Department of Health - Child and Community Health Services. The Institute has worked closely with a number of health services, both metropolitan and rural, and has assisted these health services in a variety of ways including strategic planning, framing research and evaluation questions and service and program development needs. CARE has also undertaken a review of effective approaches utilizing economic modelling that positively impact on community and child health policy. CARE provided an overview of economic approaches and examined conditions in which economic evaluation/analysis might be undertaken. The purpose of this exercise was to provide advice to the Department of Health (DOH) that would be directed to a process of enhancing the use of economic information in decision-making and during the policy development process.

CARE has also undertaken the provision of information system and database development and support in accordance with National Health Information frameworks for children and youth in collaboration with DOH and other government agencies. It has been involved in a feasibility assessment of a Population Research Information System (PORIS) which would provide both decision makers within the health and human services sector and researchers with access to a variety of forms of information about the health and wellbeing of populations they have an interest in (either as target groups for new or existing services or for the purposes of research).

3. Capacity Building

This project area involved the development of a video for health professionals on the science of the early years and why practitioners should be focusing on the early years and the development of a framework for placements of Department of Health Public Health Trainees to support skills training in research methods for policy practitioners. In addition, a series of presentations on a range of topics were delivered to rural health service staff as part of rural workforce development through the telehealth medium.

2003/2004

The 2003/2004 financial year sees the CARE team of 3.6 FTE responsible for the conduct or management of 21 discrete projects to a total of over one million dollars. This contract includes the continuation of the evaluation of Community Mothers and Best Beginnings Programs, the economic analysis project and the health information and databases project. The program of work for this year also includes new projects such as research into health messages for Aboriginal

people, an audit of the programs offered for Aboriginal people that focus on the Early Years, the provision of advice on in-service training materials for Community and Child Health staff on health promotion, illness prevention and early intervention as key tasks, particularly in the early years and the conduct of a literature and practice review (inter- and national) to identify the key settings for delivering health programs to school aged children. Projects such as the Vaccine Impact Surveillance Network, the Birth Defects Register, and the Family Connections project also come under this contract but as they are not undertaken directly by the CARE team are reported elsewhere in this scientific report.

Staff and Students

Head of Division and Head of Psychosocial Research

Professor Steve Zubrick

Head of Epidemiology

Clinical Professor Carol Bower

Head of Biostatistics

Profess Nick de Klerk

Staff

Please note: this list is incomplete.

Phyllis Alessandri

Kirsten Alpers

Rosemary Austin RN RM

Nadia Badawi

Alex Baptista BSc Hons

Melinda Berinson BSc Hons MPH

Sarah Beveridge

Bradley Calamel

Nichole Carlyon

Melissa Clarke

Lee Clohessy RGN RM RCHN BSc Dip Ed

Denny Craig

Piers Dawes

Glenys N Dixon BA B.Psych Mpsych (Clin)

Smilja Dragovic B.Psych

Ms Dimity Elsbury

Melanie Epstein

Ms Janine Finucane

Erika Hagemann

Stephanie Hoey

Antonietta Italiano

Dr Christine Jeffries-Stokes

Dr Garth Kendall RN BA DipSocSci MPH PhD

Jennifer Kent RN

Jennifer Kurinczuk

Marguerite Ledger

Clin Assoc Prof Deborah Lehmann

Kirsty Mackenzie

Lucy Masterson
Megan McClurg
Ms Ruth Monck
Heather Monteiro BA SocSci
Eva Muir
Virginia Muniandy
Ngio Murigu BBus
Karen Murray
Dr Fiona Nichols
Norries Pomat
Elke Scheepers
Carly Scott
Sven Silburn
Joanne Silvestri
Nick Sloan BSc Hons
Carolyn Smargiassi
Ms Annette Stokes
Michaela Stone
Kate Taylor
Jane Valentine MBBS MRCP(Edin) FRACP FAFRM

Students

Ashwini Arumugaswamy, BmedSci
Kylie Carville, Master in Applied Epidemiology
Ms Jackie Cesario UWA, Psychology (PhD candidate)
Ms Marie Deverell BSc Hons (PhD candidate)
Associate Professor David Forbes MBBS FRACP (PhD candidate)
Ms Jacqui Joseph-Bowen UWA, Paediatrics (PhD candidate)
Ms Michelle La Puma BA Hons (Clinical Psychology Masters Student)
Ms Pam Nicol UWA, Public Health (MPH student)
Nevada Pingault, PhD student
William S Pomat, PhD student
Lisha van Reyk BSc Hons (PhD candidate)

Theses Passed

Please note: this list is incomplete.

Kendall GE. Children in families in communities: a modified conceptual framework and an analytical strategy for identifying patterns of factors associated with developmental health problems in childhood [PhD thesis]. Perth: University of Western Australia (2003).

Freemantle, J. Doctor of Philosophy, Faculty of Medicine and Dentistry, Department of Paediatrics; passed May 2003. Title of thesis: "Indicators of Infant and Childhood Mortality for Indigenous and non-Indigenous Children born in Western Australia from 1980 to 1997 inclusive.

Awards

Please note: this list is incomplete.

G. Dixon (The Louisa Alessandri Memorial Award for Excellence in Research

2003)

Freemantle, J. Healthway Postdoctoral Fellowship (2004-2007), 2003
Presentations and Published Abstracts:

External Committees

Please note: this list is incomplete.

Freemantle, J. National vice-president (policy) - the Public Health Association of Australia

Freemantle, J. Member – Princess Margaret Child Death Review Committee

Freemantle, J. Member – Advisory Council for the prevention of Deaths in Children and Young Adult

Freemantle, J. Member – Scientific Advisory Council, SIDS and Kids WA.

Freemantle, J. Fellow – Guildford Grammar School Council

Freemantle, J. Member – Child and Youth Intergovernmental Partnership (CHIP)

Freemantle, J. Member – Aboriginal and Torres Strait Islander Working Party (CHIP)

D Lehmann. Member. Vaccine Impact Surveillance Network committee, WA

D Lehmann. Member. The Meningitis Centre committee, WA

D Lehmann. Member. Papua New Guinea Institute of Medical Research
Buttressing Coalition

D Lehmann. Scientific Committee of Fourth International Symposium on
Pneumococci and Pneumococcal Diseases, Helsinki 2004.

D Lehmann. Co-Director Fifth International Symposium on Pneumococci and
Pneumococcal Diseases, Alice Springs 2006.

D Lehmann. Member. Australasian Epidemiological Association

D Lehmann. Member. Medical Society of Papua New Guinea

D Lehmann. Member. Australasian Society for Infectious Diseases

D Lehmann. Member. Australian Society for Microbiology

D Lehmann. Member. Medical Association for Prevention of War.

D Lehmann. Member. John Snow Society

E Milne. Convenor, organising committee for 2003 Annual Australasian
Epidemiology Association Scientific meeting

E Milne. Member, WA Health Promotion Foundation (Healthway) Research Sub-
Committee

E Milne. Member, Confidentiality of Health Information Committee (CHIC)

E Milne. Member, Cancer Foundation of WA Skin Cancer Control Steering
Committee

E Milne. Member, Perth Epidemiology Group Committee

Presentations

Please note: this list is incomplete.

Freemantle, J. "The increasing inequality in the incidence of SIDS in Western
Australian Indigenous Infants, 1980 to 1998" - Perinatal Society of Australia and
New Zealand, National Conference , March 2003

Freemantle, J. "Trends in infant mortality in Western Australia" - Perinatal
Society of Australia and New Zealand, National Conference, March 2003

Freemantle, J. Master of Business Administration, Curtin University – Risk (and
Safety) Management from an epidemiological perspective.

Freemantle, J. Master of Public Health, University of Western Australia
 – Descriptive epidemiology – trends and patterns of mortality in Western Australian infants and children

Freemantle, J. Department of Forensic Pathology Grand Round – “The Role of Forensic Pathology in Research”

Freemantle, J. Coroner’s Ethics Committee – “Trends in infants and childhood mortality in Western Australian born children”

Freemantle, J. Postgraduate Studies, University of Western Australia – “The life of a PhD”.

Lehmann D, Tennant M, Silva D, McAullay D, Johnston J, Siwwright K, Serna P, Nannup I, Coates H, Lannigan F, Stuart J, Weeks S. Impact of swimming pools on health of Aboriginal children in two remote WA communities. Program and Abstracts. Seventh National Rural Health Conference; Hobart, Tas. 1-4 March; 2003. 161-2.

Lehmann D, Gordon C, Bowman J, Riley TV, Murphy D, Elsbury D. Kalgoorlie Otitis Media Research Project. Program and Abstracts. Annual Meeting of the Australasian Society for Infectious Diseases; Canberra, ACT. 22-26 March; 2003. 14-5. (Invited speaker)

Silva D, Lehmann D, Tennant M, Johnston J. Impact of swimming pools on health and school attendance of Aboriginal children in 2 remote W.A. communities. Program and Abstracts. Annual Scientific Meeting of the Royal Australasian College of Physicians; Hobart, Tas. 26-28 May; 2003.

Jeffries-Stokes C, Lehmann D, Johnston J, Mason A, Evans J, Elsbury D, Wood K. An Aboriginal perspective of middle ear disease in the arid zone of Western Australia. Program and Abstracts. Annual Scientific Meeting of the Royal Australasian College of Physicians; Hobart, Tas. 26-28 May; 2003.

Lehmann D, Coates H, Bowman J, Kalgoorlie Otitis Media Research Team. Otitis media in Aboriginal and non-Aboriginal children in an arid zone of Western Australia (Abstract B01). Program and Abstracts. 8th International Symposium on Recent Advances in Otitis Media; Fort Lauderdale, Fl. 3-7 June; 2003. 82.

Tennant M, Lehmann D, Silva D, Coates H, Lannigan F, Nannup I, Weeks S. Impact of swimming pools on health of Aboriginal children in 2 remote W.A. communities. ISEE Conference Abstracts. 15th Conference of the International Society for Environmental Epidemiology, Perth 23 – 26 September 2003.

M Tennant was awarded a prize for the most outstanding abstract by a new investigator.

Lehmann D, Carville K, Bowman J, Riley TV, Murphy D, Elsbury D, Stokes A, Monck R, Finucane J, Kalgoorlie Otitis Media Research Project. Epidemiology of upper respiratory tract bacterial carriage in Aboriginal and non-Aboriginal children <2 years in the Kalgoorlie-Boulder area, Western Australia. Australian Epidemiological Association Annual Meeting. “Nature, Nurture and Epidemiology”; University of Western Australia; Perth, WA. 22-23 September; 2003.

Division of Virology

Overview

Research undertaken within the Division of Virology focuses on understanding how viruses cause disease within the central nervous system (CNS). This research covers a wide range of activity, including molecular studies of viral replication, studies of the pathogenesis of viral encephalitis using animal models, the development of community surveillance for viruses causing CNS infections and the development of improved diagnostic methods. These studies overlap extensively and involve all staff within the Division in some capacity.

Reverse genetic studies on the molecular pathogenesis of Murray Valley encephalitis virus infection

Peter McMinn, Robert Hurrelbrink

Many mutations affecting the virulence of Murray Valley encephalitis virus (MVE) and related flaviviruses are located in the immunodominant envelope (E) protein. Superimposition of these mutations on the three-dimensional structure of the protein clearly identifies clusters of mutations with the potential to affect protein structure and function. Our laboratory has focused on two such regions - an Arg-Gly-Asp (RGD) motif, located on the lateral face of the putative receptor binding region of the protein, and a Ser-Ser-Ser (SSS) motif, which forms part of a hinge region believed to be involved in low-pH induced conformational change during virus fusion. Mutations in these regions markedly reduce the ability of MVE to cause encephalitis in the mouse model and in some cases perturb the fusion activity of the E protein.

Using reverse genetics we have engineered panels of virus mutants with specific amino acid substitutions to investigate the nature of this attenuation. Some mutations in the RGD motif cause a complete loss of neuroinvasiveness, but have no effect on virus binding and/or entry, despite the fact that similar motifs in other viruses (such as adenovirus and foot and mouth disease virus) have been implicated in the binding of virus particles to host-cell integrins. We believe that mutations in this region may instead affect the correct folding of the protein in the endoplasmic reticulum. Alternatively, the interaction of E with other virus proteins such as prM may be perturbed, preventing prM from fulfilling its role as a protective inhibitor of virus fusion during egress.

Like mutations in the RGD motif, mutations in the SSS motif also affect neuroinvasiveness. However, a reduction in the haemagglutination activity of these viruses further suggests that a defect in virus fusion is involved in the observed attenuation. Hydrophobic amino acid substitutions in this motif may prevent the correct reorganisation of the E protein at low pH in the endosome. Alternatively, such mutations may disrupt the receptor-ligand interaction and prevent fusion of the viral and endosomal membranes.

We are continuing our studies on virus fusion using an infectious cDNA clone of

MVE, as well as a sub-viral particle system to generate non-infectious but fusion active empty virus particles. It is hoped that such studies will shed light on the functional basis for attenuation in the encephalitogenic flaviviruses.

International collaborative study of the molecular epidemiology of enterovirus 71 in the Asia – Pacific region

Peter McMinn, Lara Herrero, Sharon Sanders, Mary Jane Cardoso (UNIMAS, Sarawak, Malaysia), Kwai Peng Chan (Singapore General Hospital), Doosung Cheon (National Institute of Health, Seoul, Korea), Eveline Irawan (Public Health Virology Laboratory, Surabaya, Indonesia), Phan Van Tu (Pasteur Institute, Ho Chi Minh City, Vietnam), Peter Siba (PNG Medical Research Institute, Papua New Guinea).

Since 1997, several large epidemics of EV71 infection have occurred in the Asia-Pacific region, the first being reported in Sarawak (Malaysian Borneo) in 1997, followed by smaller outbreaks in Peninsular Malaysia and Singapore. As with previous EV71 epidemics, numerous cases of HFMD were reported, with neurological complications arising in a small proportion of cases. In addition, many cases of brainstem encephalitis associated with pulmonary oedema and a high case-fatality rate were also described during these outbreaks. Twenty-nine fatal cases of this disease were reported in Sarawak and twelve in Peninsular Malaysia. During 1998, a large EV71 epidemic occurred in Taiwan in which 405 cases of severe neurological disease and 78 fatal cases of brainstem encephalitis and neurogenic pulmonary oedema were reported. In 1999, a large EV71 epidemic occurred in Perth, Western Australia (WA) and included fourteen cases of severe neurological disease, including three with severe neurological sequelae requiring prolonged hospitalisation and rehabilitation. EV71 epidemic activity has continued in the region during 2000-2001, with EV71 isolation from cases of HFMD and encephalitis in Sarawak, Peninsular Malaysia, Singapore and WA.

EV71 isolates are passaged on rhabdomyosarcoma (RD) cells and viral RNA extracted from cell culture supernatants. The complete VP1 gene of EV71 is amplified by reverse RT-PCR assay in two overlapping amplicons using previously published primers and assay conditions. VP1 is one of the most variable regions within the enterovirus genome and has proved to be the most valuable region for determining phylogenetic relationships, both within and between enterovirus serotypes. VP1 gene cDNA is sequenced on both strands by cycle sequencing reactions using the ABI Prism Dye Terminator Cycle Sequencing Kit. The VP1 gene nucleotide and deduced amino acid sequences are aligned and phylogenetic trees constructed by the neighbour-joining method. Previously sequenced EV71 strains (deposited in the GenBank database) are also being included in the analysis.

This study is providing valuable information on the origin of recent epidemic strains of EV71 and may also identify neurovirulent virus lineages for further genetic and phenotypic analysis. This study has recently received a major boost with the award of a five-year Wellcome Trust/NHMRC International Collaborative Research Grant. We plan to use this grant to provide technology transfer to allow surveillance of EV71 activity in developing countries within our region.

Studies on the molecular genetics of enterovirus 71 encephalitis

Peter McMinn, Chee Choy Kok, Lara Herrero, Beng Hooi Chua, Robert Hurrelbrink, Sharon Sanders, Darren Shafren (University of Newcastle)
Recent increases in the frequency and magnitude of enterovirus 71 (EV71) epidemics in Southeast Asia have provided the impetus for studies of the molecular genetics of EV71 virulence and pathogenesis with a view to developing a vaccine. This is an area in which our research group has considerable expertise. The first step in EV71 vaccine development has been the construction of an infectious cDNA clone. There are currently two Ph.D. students working on this project. The complete sequence of two local EV71 strains has been determined and a full-length infectious cDNA clone has been constructed.

We have also developed a collaboration with Associate Professor Darren Shafren, Picornavirus Research Unit, The University of Newcastle, with the aim of identifying the cellular receptor for EV71. Identification of the EV71 receptor will allow us to develop a small animal model of EV71 encephalitis by construction of a transgenic mouse incorporating the EV71 receptor gene into the mouse genome. This model will allow a detailed study of the pathogenesis of EV71 encephalitis, as we have done for MVE. It will also enable us to test the immunogenicity and efficacy of candidate live attenuated vaccine strains derived from mutagenesis of the EV71 infectious cDNA clone. This study is supported by a NHMRC Project Grant.

Staff and Students

Head of Division

Peter McMinn BMedSc (Hon) MB, BS PhD FRCPA FRCPATH DipRACOG
Clinical Associate Professor, Discipline of Microbiology, School of Biomedical and Chemical Sciences, The University of Western Australia.
Clinical Virologist and NHMRC Practitioner Fellow, Princess Margaret Hospital for Children.

Research Staff

Chee Choy Kok BSc (Hon) PhD
Robert Hurrelbrink BA BSc (Hon) PhD
Sharon Sanders BSc (Hon)

Students

Beng Hooi Chua BS. PhD candidate
Lara Herrero BSc (Hon). PhD candidate
Kristy Philippe. BSc Honours candidate
Petra Feibig. German exchange student

Visitors

Jane Cardosa, Director, Institute for Health and Community Medicine, UNIMAS, Sarawak, Malaysia

Theses passed

Sharon Sanders. BSc with First Class Honours (UWA)
Ashwenia Krishnan. BSc with Second Class Honours, Division A (UWA)

External Committees

P McMinn. Member of the W.A. State Arbovirus Control Committee

P McMinn. Member of the Health Department of WA Influenza Pandemic Planning Committee

P McMinn. Chair, Princess Margaret Hospital Infection Control Committee

P McMinn. Member, Winter Strategies Committee, Health Department of WA

P McMinn. Member, NHMRC Grant Review Panel 2B

Invited Presentations

P McMinn. The Molecular Genetics of Enterovirus 71 Virulence. 6th Asia-Pacific Congress of Medical Virology, Malaysia.

P McMinn. The Molecular Pathogenesis of Flavivirus Encephalitis. Biennial International Scientific Conference of the Pasteur Institute, Ho Chi Minh City, Vietnam.

P McMinn. The Molecular Epidemiology of Enterovirus 71 in the Asia-Pacific Region. Biennial International Scientific Conference of the Pasteur Institute, Ho Chi Minh City, Vietnam.

P McMinn. The molecular genetics of enterovirus 71 neurovirulence. Sibul General Hospital, Sibul, Sarawak, Malaysia.

P McMinn. The molecular genetics of enterovirus 71 neurovirulence. Sarawak General Hospital, Kuching, Sarawak, Malaysia.

P McMinn. Avian Influenza: could it be the source of the next pandemic? "Hot Topics in Infection Control" Seminar. Fremantle Hospital

Publications

Ambrosini GL, Mackerras D, de Klerk NH, Musk AW. Comparison of an Australian food-frequency questionnaire with diet records: implications for nutrition surveillance. *Public Health Nutrition* 2003;6:415-22.

Ambrosini GL, van Roosbroeck SAH, Mackerras D, Fritschi L, de Klerk NH, Musk AW. The reliability of ten-year dietary recall: implications for cancer research. *Journal of Nutrition* 2003;133:2663-8.

Barker RN, Burney PG, Devereux G, Hardiman M, Holt PG, O'Donnell M, Platts-Mills TA, Seaton A, Strachan DP, Weiss ST, Woodcock A. The increase in allergic disease: environment and susceptibility. Proceedings of a Symposium held at the Royal Society of Edinburgh, 4th June 2002. *Clinical and Experimental Allergy* 2003;33:394-406.

Berry G, Musk AW, de Klerk NH, Johnson A, Yates DH. Predictions of mortality from mesothelioma (letter). *Occupational and Environmental Medicine* 2003;60:458.

Binns C, Lee MK, Oddy W. Breastfeeding and the prevention of obesity. *Asia-Pacific Journal of Public Health* 2003;15:S22-6.

Blair E. Uses and misuses of the percentile (centile or quantile position). *Australasian Epidemiologist* 2003;10:26-8.

Blair E. Where does - and where should - the money come from? *disparity* 2003;2:16-20.

Bower C. Fortification of food with folic acid and the prevention of neural tube defects [editorial]. *New Zealand Medical Journal* 2003;116:U292.

Bower C, Rudy E, Ryan A, Cosgrove P. Report of the Birth Defects Registry of Western Australia 1980-2002. Perth: King Edward Hospital, Women's and Children's Health Service; 2003. Number 10.

Burton PR. Correcting for nonrandom ascertainment in generalized linear mixed models (GLMMs), fitted using Gibbs sampling. *Genetic Epidemiology* 2003;24:24-35.

Caplin NJ, O'Leary P, Bulsara M, Davis EA, Jones TW. Subcutaneous glucose sensor values closely parallel blood glucose during insulin-induced hypoglycaemia. *Diabetic Medicine* 2003;20:238-41.

Cardosa MJ, Perera D, Brown BA, Cheon D, Chan HM, Chan KP, Cho H,

McMinn P. Molecular epidemiology of human enterovirus 71 strains and recent outbreaks in the Asia-Pacific region: comparative analysis of the VP1 and VP4 genes. *Emerging Infectious Diseases* 2003;9:461-8.

Carey M, Bower C, Mylvaganam A, Rouse I. Talipes equinovarus in Western Australia. *Paediatric and Perinatal Epidemiology* 2003;17:187-94.

Collins RA, Ikegami M, Korfhagen TR, Whitsett JA, Sly PD. In vivo measurements of changes in respiratory mechanics with age in mice deficient in surfactant protein D. *Pediatric Research* 2003;53:463-7.

Collins RA, Sly PD, Turner DJ, Herbert C, Kumar RK. Site of inflammation influences site of hyperresponsiveness in experimental asthma. *Respiratory Physiology and Neurobiology* 2003;139:51-61.

Colvin L, Fyfe S, Leonard S, Schiavello T, Ellaway C, de Klerk N, Christodoulou J, Msall M, Leonard H. Describing the phenotype in Rett syndrome using a population database. *Archives of Disease in Childhood* 2003;88:38-43.

Cunningham C, Stanley F. Indigenous by definition, experience, or world view [comment]. *BMJ* 2003;327:403-4.

Dunstan JA, Mori TA, Barden A, Beilin LJ, Taylor AL, Holt PG, Prescott SL. Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: A randomized, controlled trial. *Journal of Allergy and Clinical Immunology* 2003;112:1178-84.

Dunstan JA, Mori TA, Barden A, Beilin LJ, Taylor AL, Holt PG, Prescott SL. Maternal fish oil supplementation in pregnancy reduces interleukin-13 levels in cord blood of infants at high risk of atopy. *Clinical and Experimental Allergy* 2003;33:442-8.

Fritschi L, Sim MR, Forbes A, Abramson MJ, Benke G, Musk AW, de Klerk NH. Respiratory symptoms and lung-function changes with exposure to five substances in aluminium smelters. *International Archives of Occupational and Environmental Health* 2003;76:103-10.

Fyfe S, Cream A, de Klerk N, Christodoulou J, Leonard H. InterRett and RettBASE: International Rett Syndrome Association databases for Rett syndrome. *Journal of Child Neurology* 2003;18:709-13.

Gill H, Cheadle JP, Maynard J, Fleming N, Whatley S, Cranston T, Thompson EM, Leonard H, Davis M, Christodoulou J, Skjeldal O, Hanefeld F, Kerr A, Tandy A, Ravine D, Clarke A. Mutation analysis in the MECP2 gene and genetic counselling for Rett syndrome. *Journal of Medical Genetics* 2003;40:380-4.

Gordon C, Walpole I, Zubrick S, Bower C. Population screening for cystic fibrosis: knowledge and emotional consequences 18 months later. *American*

Journal of Medical Genetics 2003;120A:199-208.

Grimbaldeston MA, Simpson A, Finlay-Jones JJ, Hart PH. The effect of ultraviolet radiation exposure on the prevalence of mast cells in human skin. *British Journal of Dermatology* 2003;148:300-6.

Grimbaldeston MA, Geczy CL, Tedla N, Finlay-Jones JJ, Hart PH. S100A8 induction in keratinocytes by ultraviolet A irradiation is dependent on reactive oxygen intermediates. *Journal of Investigative Dermatology* 2003;121:1168-74.

Grimbaldeston MA, Green A, Darlington S, Robertson BO, Marshman G, Finlay-Jones JJ, Hart PH. Susceptibility to basal cell carcinoma is associated with high dermal mast cell prevalence in non-sun-exposed skin for an Australian population. *Photochemistry and Photobiology* 2003;78:633-9.

Gunawardana DH, Basser RL, Davis ID, Cebon J, Mitchell P, Underhill C, Kilpatrick TJ, Reardon K, Green MD, Bardy P, Amor P, Crump D, Ng S, Nation RL, Begley CG. A phase I study of recombinant human leukemia inhibitory factor in patients with advanced cancer. *Clinical Cancer Research* 2003;9:2056-65.

Hall MA, Curtis DJ, Metcalf D, Elefanty AG, Sourris K, Robb L, Gothert JR, Jane SM, Begley CG. The critical regulator of embryonic hematopoiesis, SCL, is vital in the adult for megakaryopoiesis, erythropoiesis, and lineage choice in CFU-S12. *Proceedings of the National Academy of Sciences of the United States of America* 2003;100:992-7.

Hantos Z, Collins RA, Turner DJ, Janosi TZ, Sly PD. Tracking of airway and tissue mechanics during TLC maneuvers in mice. *Journal of Applied Physiology* 2003;95:1695-705.

Hartley C, Elliott S, Begley CG, McElroy P, Sutherland W, Khaja R, Heatherington AC, Graves T, Schultz H, Del Castillo J, Molineux G. Kinetics of haematopoietic recovery after dose-intensive chemo/radiotherapy in mice: optimized erythroid support with darbepoetin alpha. *British Journal of Haematology* 2003;122:623-36.

Heaton T, Mallon D, Venaille T, Holt P. Staphylococcal enterotoxin induced IL-5 stimulation as a cofactor in the pathogenesis of atopic disease: the hygiene hypothesis in reverse? *Allergy* 2003;58:252-6.

Herrero LJ, Lee CSM, Hurrelbrink RJ, Chua BH, Chua KB, McMinn PC. Molecular epidemiology of enterovirus 71 in peninsular Malaysia, 1997-2000. *Archives of Virology* 2003;148:1369-85.

Hinwood AL, Sim MR, Jolley D, de Klerk N, Bastone EB, Gerostamoulos J, Drummer OH. Hair and toenail arsenic concentrations of residents living in areas with high environmental arsenic concentrations. *Environmental Health Perspectives* 2003;111:187-94.

Holt PG. Functionally mature virus-specific CD8+ T memory cells in congenitally infected newborns: proof of principle for neonatal vaccination? *Journal of Clinical Investigation* 2003;111:1645-7.

Holt PG, Sly PD. Emerging concepts of T-cell regulation in asthma and allergy. *Allergy and Clinical Immunology International* 2003;15:255-60.

Holt PG, Rowe J, Loh R, Sly PD. Developmental factors associated with risk for atopic disease: implications for vaccine strategies in early childhood [Review]. *Vaccine* 2003;21:3432-5.

Huh JC, Strickland DH, Jahnsen FL, Turner DJ, Thomas JA, Napoli S, Tobagus I, Stumbles PA, Sly PD, Holt PG. Bidirectional interactions between antigen-bearing respiratory tract dendritic cells (DCs) and T cells precede the late phase reaction in experimental asthma: DC activation occurs in the airway mucosa but not in the lung parenchyma. *Journal of Experimental Medicine* 2003;198:19-30.

Hurrelbrink RJ, McMinn PC. Molecular determinants of virulence: the structural and functional basis for flavivirus attenuation. *Advances in Virus Research* 2003;60:1-42.

Johnston R, Cross D, Costa C, Giles-Corti B, Cordin T, Milne E, English DR. Sun safety education intervention for school and home. *Health Education* 2003;103:342-51.

Jones TW, Davis EA. Hypoglycaemia. In: Menon RK, Sperling MA, editors. *Pediatric Diabetes*. New York: Kluwer Academic Publishers; 2003. p. 369-88.

Jones TW, Davis EA. Hypoglycaemia in children with type 1 diabetes: current issues and controversies. *Pediatric Diabetes* 2003;4:143-50.

Kees UR, Ford J, Watson M, Murch A, Ringneri M, Walker RL, Meltzer P. Gene expression profiles in a panel of childhood leukemia cell lines mirror critical features of the disease. *Molecular Cancer Therapeutics* 2003;2:671-7.

Kees UR, Heerema NA, Kumar R, Watt PM, Baker DL, La MK, Uckun FM, Sather HN. Expression of HOX11 in childhood T-lineage acute lymphoblastic leukaemia can occur in the absence of cytogenetic aberration at 10q24: a study from the Children's Cancer Group (CCG). *Leukemia* 2003;17:887-93.

Kwon S, Bower C, English D. Birth defects in the offspring of non-caucasian, non-Indigenous women in Western Australia. *Birth Defects Research (Part A)* 2003;67:515-21.

Lawrence DM, Holman CDJ, Jablensky AV, Hobbs MST. Death rate from ischaemic heart disease in Western Australian psychiatric patients 1980-1998. *British Journal of Psychiatry* 2003;182:31-6.

Lee YCG, Singh B, Pang SC, de Klerk NH, Hillman DR, Musk AW. Earlier study on asbestos workers, ILO scores, and oxygenation more comprehensive - (letter in Reply). *Occupational and Environmental Medicine* 2003;60:611.

Lee YCG, Singh B, Pang SC, de Klerk NH, Hillman DR, Musk AW. Radiographic (ILO) readings predict arterial oxygen desaturation during exercise in subjects with asbestosis. *Occupational and Environmental Medicine* 2003;60:201-6.

Lehmann D, Pomat WS, Riley ID, Alpers MP. Studies of maternal immunisation with pneumococcal polysaccharide vaccine in Papua New Guinea [Review]. *Vaccine* 2003;21:3446-50.

Lehmann D, Tennant MT, Silva DT, McAullay D, Lannigan F, Coates H, Stanley FJ. Benefits of swimming pools in two remote Aboriginal communities in Western Australia: intervention study. *BMJ* 2003;327:415-9.

Leonard H, Leonard S. The ICF and Rett Syndrome. In: *ICF Australian User Guide. Version 1.0. Disability Series. Canberra: Australian Institute of Health and Welfare; 2003. p. 99-105.*

Leonard H, Petterson B, Bower C, Sanders R. Prevalence of intellectual disability in Western Australia. *Paediatric and Perinatal Epidemiology* 2003;17:58-67.

Leonard H, Colvin L, Christodoulou J, Schiavello T, Williamson S, Davis M, Ravine D, Fyfe S, de Klerk N, Matsuishi T, Kondo I, Clarke A, Hackwell S, Yamashita Y. Patients with the R133C mutation: is their phenotype different from patients with Rett syndrome with other mutations? *Journal of Medical Genetics* 2003;40:e52.

Li JH, Lavelly W. Village context, women's status, and son preference among rural Chinese women. *Rural Sociology* 2003;68:87-106.

Li JH, Morlet N, Semmens J, Gavin A, Ng J. Coding accuracy for endophthalmitis diagnosis and cataract procedures in western Australia - The Endophthalmitis Population Study of Western Australia (EPSWA): second report. *Ophthalmic Epidemiology* 2003;10:133-45.

Macaubas C, de Klerk NH, Holt BJ, Wee C, Kendall G, Firth M, Sly PD, Holt PG. Association between antenatal cytokine production and the development of atopy and asthma at age 6 years. *Lancet* 2003;362:1192-7.

Mak DB, Mackendrick A, Bulsara MK, Weeks S, Leidwinger L, Coates H, Lannigan FJ, Lehmann D. Long-term outcomes of middle-ear surgery in Aboriginal children (letter). *Medical Journal of Australia* 2003;179:324-5.

Mayne GC, Borowicz RA, Greeneklee KV, Finlay-Jones JJ, Williams KA, Hart PH. Centrifugation facilitates transduction of green fluorescent protein in human monocytes and macrophages by adenovirus at low multiplicity of infection.

Journal of Immunological Methods 2003;278:45-56.

McLennan K, Jeske Y, Cotterill A, Cowley D, Penfold J, Jones T, Howard N, Thomsett M, Choong C. Combined pituitary hormone deficiency in Australian children: clinical and genetic correlates. *Clinical Endocrinology* 2003;58:785-94.

Michel O, LeVan TD, Stern D, Dentener M, Thorn J, Gnat D, Beijer ML, Cochaux P, Holt PG, Martinez FD, Rylander R. Systemic responsiveness to lipopolysaccharide and polymorphisms in the toll-like receptor 4 gene in human beings. *Journal of Allergy and Clinical Immunology* 2003;112:923-9.

Milne E, Thompson J. The importance of timing of genotoxic exposures in the aetiology of childhood cancer: the case of acute leukemia. *Australasian Epidemiologist* 2003;10:17-20.

Morlet N, Li J, Semmens J, Ng J. The Endophthalmitis Population Study of Western Australia (EPSWA): first report. *British Journal of Ophthalmology* 2003;87:574-6.

Musk AW, de Klerk NH. Benign asbestos pleural diseases. In: Light RW, Lee YC, editors. *Textbook of Pleural Diseases*. London: Arnold; 2003. p. 428-34.

Musk AW, de Klerk NH. History of tobacco and health. *Respirology* 2003;8:286-90.

Noakes PS, Holt PG, Prescott SL. Maternal smoking in pregnancy alters neonatal cytokine responses. *Allergy* 2003;58:1053-8.

Oddy WH, Peat JK. Breastfeeding, asthma and atopic disease: an epidemiological review of the literature. *Journal of Human Lactation* 2003;19:250-61.

Oddy WH, Sherriff JL. Breastfeeding, body mass index, asthma and atopy in children. *Asia-Pacific Journal of Public Health* 2003;15:S15-7.

Oddy WH, Glenn K. Implementing the Baby Friendly Hospital initiative: the role of finger feeding. *Breastfeeding Review* 2003;11:5-9.

Oddy WH, Sly PD, de Klerk NH, Landau LI, Kendall GE, Holt PG, Stanley FJ. Breast feeding and respiratory morbidity in infancy: a birth cohort study. *Archives of Disease in Childhood* 2003;88:224-8.

Oddy WH, Kendall GE, Blair E, deKlerk NH, Stanley FJ, Landau LI, Silburn SR, Zubrick SR. Breastfeeding and cognitive development in childhood: A prospective birth cohort study. *Paediatric and Perinatal Epidemiology* 2003;17:81-90.

Oddy WH, Halonen M, Martinez FD, Lohman IC, Stern DA, Kurzius-Spencer M,

Guerra S, Wright AL. TGF- β in human milk is associated with wheeze in infancy. *Journal of Allergy and Clinical Immunology* 2003;112:723-8.

Peat JK, Allen J, Oddy WH, Webb K. Breastfeeding and asthma: appraising the controversy [commentary]. *Pediatric Pulmonology* 2003;35:331-4.

Peták F, Babik B, Asztalos T, Hall GL, Deak ZI, Sly PD, Hantos Z. Airway and tissue mechanics in anesthetized paralyzed children. *Pediatric Pulmonology* 2003;35:169-76.

Poerwanto S, Stevenson M, de Klerk N. Infant mortality and family welfare: policy implications for Indonesia. *Journal of Epidemiology and Community Health* 2003;57:493-8.

Prescott SL, King B, Strong TL, Holt PG. The value of perinatal immune responses in predicting allergic disease at 6 years of age. *Allergy* 2003;58:1187-94.

Prescott SL, Taylor A, King B, Dunstan J, Upham JW, Thornton CA, Holt PG. Neonatal interleukin-12 capacity is associated with variations in allergen-specific immune responses in the neonatal and postnatal periods. *Clinical and Experimental Allergy* 2003;33:566-72.

Ramage JG, Vallera DA, Black JH, Aplan PD, Kees UR, Frankel AE. The diphtheria toxin/urokinase fusion protein (DTAT) is selectively toxic to CD87 expressing leukemic cells. *Leukemia Research* 2003;27:79-84.

Robinson BWS, Creaney J, Lake R, Nowak A, Musk AW, de Klerk N, Winzell P, Hellstrom KE, Hellstrom I. Mesothelin-family proteins and diagnosis of mesothelioma. *Lancet* 2003;362:1612-6.

Rozovskaia T, Ravid-Amir O, Tillib S, Getz G, Feinstein E, Agrawal H, Nagler A, Rappaport EF, Issaeva I, Matsuo Y, Kees UR, Lapidot T, Lo Coco F, Foa R, Mazo A, Nakamura T, Croce CM, Cimino G, Domany E, Canaani E. Expression profiles of acute lymphoblastic and myeloblastic leukemias with ALL-1 rearrangements. *Proceedings of the National Academy of Sciences of the United States of America* 2003;100:7853-8.

Schmidt U, Begley CG. Cancer diagnosis and microarrays. *International Journal of Biochemistry and Cell Biology* 2003;35:119-24.

Semmens JB, Li J, Morlet N, Ng J. Trends in cataract surgery and postoperative endophthalmitis in Western Australia (1980-1998): the Endophthalmitis Population Study of Western Australia. *Clinical and Experimental Ophthalmology* 2003;31:213-9.

Serna P, Bower C, Payne J, Miller M, Stanley FJ. Encouraging women to participate in reproductive research. *Australasian Epidemiologist* 2003;10:26-8.

Sharp MJ, Rowe J, Kusel M, Sly PD, Holt PG. Specific patterns of responsiveness to microbial antigens staphylococcal enterotoxin B and purified protein derivative by cord blood mononuclear cells are predictive of risk for development of atopic dermatitis. *Clinical and Experimental Allergy* 2003;33:435-41.

Sly PD. Ultrastructural examination of bronchial specimens from children with moderate asthma: an associate editor's view (letter). *Thorax* 2003;58:187-8.

Sly PD, Lombardi E. Editorial: Measurement of lung function in preschool children using the interrupter technique. *Thorax* 2003;58:742-4.

Sly PD, Collins RA, Thamrin C, Turner DJ, Hantos Z. Volume dependence of airway and tissue impedances in mice. *Journal of Applied Physiology* 2003;94:1460-6.

Stanley F. Before the bough breaks: doing more for our children in the 21st century. Canberra: Academy of the Social Sciences in Australia; 2003. Occasional Paper Series 1/2003.

Stanley F. One journey, many pathways. *Internal Medicine Journal* 2003;33:59-61.

Stanley F. Promoting and maintaining good health: what role for the social sciences? In: Mann L, editor. *Social Science in Australia's National Research Priorities*. Canberra: Academy of the Social Sciences in Australia; 2003. p. 13-7.

Stanley F. The real brain drain - why putting children first is so important for Australia. *Aboriginal and Islander Health Worker Journal* 2003;27:3-8.

Stanley F. Recent trends in neurodisability: implications for research and prevention. *Developmental Medicine and Child Neurology* 2003;45:4-5.

Stick SA, Holt PG. The airway epithelium as immune modulator. The LARC ascending. *American Journal of Respiratory Cell and Molecular Biology* 2003;28:641-4.

Stumbles PA, Upham JW, Holt PG. Airway dendritic cells: co-ordinators of immunological homeostasis and immunity in the respiratory tract. *APMIS* 2003;111:741-55.

Suk WA, Ruchirawat KM, Balakrishnan K, Berger M, Carpenter D, Damstra T, de Garbino JP, Koh D, Landrigan PJ, Makalinao I, Sly PD, Xu Y, Zheng BS. Environmental threats to children's health in Southeast Asia and the Western Pacific. *Environmental Health Perspectives* 2003;111:1340-7.

Tarnow-Mordi W, Isaacs D, Gilbert G, Athayde N, Stanley F. Effect of oral clindamycin on late miscarriage and preterm delivery (letter). *Lancet*

2003;361:2162.

Thomas WR, Hales BJ, Smith W. *Blomia tropicalis*: more than just another source of mite allergens. *Clinical and Experimental Allergy* 2003;33:416-8.

Thomas WR, Chapman MD, Tovey ER, Platts-Milla TAE. World Allergy Organization - ICACI Indoor Allergen Survey and Monitoring Working Group. *ACI International* 2003;15:88-92.

Thompson PL, Bradshaw PJ, Veroni M, Wilkes ET. Cardiovascular risk among urban Aboriginal people. *Medical Journal of Australia* 2003;179:143-6.

Tursan d'Espaignet E, Measey ML, Carnegie MA, Mackerras D. Monitoring the 'Strong Women, Strong Babies, Strong Culture Program': The first eight years. *Journal of Paediatrics and Child Health* 2003;39:668-72.

Umansky R, Watson JS, Colvin L, Fyfe S, Leonard S, de Klerk N, Leonard H. Hand preference, extent of laterality, and functional hand use in Rett syndrome. *Journal of Child Neurology* 2003;18:481-7.

Upham JW. The role of dendritic cells in immune regulation and allergic airway inflammation. *Respirology* 2003;8:140-8.

Van Eekelen JAM, Bradley CK, Gothert JR, Robb L, Elefanty AG, Begley CG, Harvey AR. Expression pattern of the stem cell leukaemia gene in the CNS of the embryonic and adult mouse. *Neuroscience* 2003;122:421-36.

Verhulst FC, Achenbach TM, van der Ende J, Erol N, Lambert MC, Leung PWL, Silva M, Zilber N, Zubrick SR. Comparisons of problems reported by youths from seven countries. *American Journal of Psychiatry* 2003;160:1479-85.

Wallace MJ, Smith DW, Broom AK, Mackenzie JS, Hall RA, Shellam GR, McMinn PC. Antibody-dependent enhancement of Murray Valley encephalitis virus virulence in mice. *Journal of General Virology* 2003;84:1723-8.

Watt PM, Clements B, Devadason SG, Chaney GM. Funhaler spacer: improving adherence without compromising delivery. *Archives of Disease in Childhood* 2003;88:579-81.

Watt PM, Hoffmann K, Greene WK, Brake RL, Ford J, Kees UR. Specific alternative HOX11 transcripts are expressed in paediatric neural tumours and T-cell acute lymphoblastic leukaemia. *Gene* 2003;323:89-99.

Weaving LS, Williamson SL, Bennetts B, Davis M, Ellaway CJ, Leonard H, Thong MK, Delatycki M, Thompson EM, Laing N, Christodoulou J. Effects of MECP2 mutation type, location and X-inactivation in modulating Rett syndrome phenotype. *American Journal of Medical Genetics Part A* 2003;118A:103-14.

Weber MW, Carlin JB, Gatchalian S, Lehmann D, Muhe L, Mulholland EK. Predictors of neonatal sepsis in developing countries. *Pediatric Infectious Disease Journal* 2003;22:711-6.

Werler MM, Bower C, Payne J, Serna P. Findings on potential teratogens from a case-control study in Western Australia. *Australian and New Zealand Journal of Obstetrics and Gynaecology* 2003;43:443-7.

Westritschnig K, Sibanda E, Thomas W, Auer H, Aspöck H, Pittner G, Vrtala S, Spitzauer S, Kraft D, Valenta R. Analysis of the sensitization profile towards allergens in central Africa. *Clinical and Experimental Allergy* 2003;33:22-7.

Wilson AS, Hobbs BG, Shen WY, Speed TP, Schmidt U, Begley CG, Rakoczy PE. Argon laser photocoagulation-induced modification of gene expression in the retina. *Investigative Ophthalmology and Visual Science* 2003;44:1426-34.