TELETHON INSTITUTE FOR
Child Health Research

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Contents

Division of
Cancer Biology

Overview

Cancer is caused by dysregulated or uncontrolled growth of cells. There are two key elements to this process. One is the mechanisms that go awry within the cell itself, and the other is the result of the signals that the cell receives from its external environment. We are interested in both these processes.

In terms of the problems that can occur within a cancer cell, our studies are focused on a key molecule that is critical in the development of human T-cell leukemia. This molecule, which we discovered, is called the SCL gene and is involved
in up to 60% of cases of this devastating childhood disease. In these cases it is aberrantly 'turned-on' in cells where it should normally be silent. We are actively seeking to understand the mechanisms by which it causes leukemia in the hope that this will inform our attempts to develop new therapeutic approaches. To this end, we must also understand the normal function of this gene: we know that it is also critically important in the normal formation of all blood cells and blood stem cells in particular, but we know little about how it exerts this effect. To address this we are seeking to identify the genes through which SCL acts (so called 'target genes'). SCL also has a function in the brain about which nothing is known. Obviously we also need to understand this if we ever hope to turn the SCL gene off in cells. Our studies on SCL are closely integrated with similar work taking place within the Institute.

The role of SCL in hemopoietic development and leukemogenesis

Regulated deletion of gene function in hematopoietic stem cells

JR Goethert, S Gustin, K Becher, DJ Izon and CG Begley in collaboration with B Gottgens and A Green, Department of Haematology, University of Cambridge, UK

All blood cells are derived from the haematopoietic stem cell (HSC), which has the capacity upon transplantation to give rise to multi-lineage hematopoietic engraftment. Recently it has been described that the HSC is capable of differentiating in a couple of different cell types and tissues when located in an appropriate environment. These data suggest the HSC might be a future resource for therapeutic tissue regeneration. Existing data demonstrate that SCL plays an indispensable role in establishing the transcriptional program of the HSC. But SCL's role for the functions of the adult HSC could not be investigated because the SCL null mice die of absolute anemia in early embryonic development. Therefore we are in the process of establishing a murine system that enables us to conditionally control SCL-expression by site-specific DNA recombination.

Our approach is to use the Cre/loxP recombinase system. The Cre recombinase recognizes a 34 bp DNA sequence, denoted loxP. Where two separate loxP sites are present along a linear stretch of DNA, cre recombinase deletes the intervening DNA sequence. We have successfully generated mice that carry two loxP-sites flanking the DNA-sequence coding for the bHLH-domain of SCL (SCL-LoxP mice).

Recently a hematopoietic enhancer element located 3' of the SCL-locus has been identified which targets expression to hematopoietic progenitors in fetal liver and adult bone marrow. Now we use this SCL 3' enhancer to guide the expression of Cre-ERT in transgenic mice (CreERT-3'enhancer). Cre-ERT is a Cre-estrogen receptor fusion gene that is tamoxifen responsive. This transgenic line is not only of use for the experiments we propose but are of interest to many investigators who wish to delete gene function in HSC.

Different founder lines with this transgenic construct have been generated. Initially Cre-ERT mRNA expression in fetal liver and bone marrow has been analysed. To address whether the expressed Cre-ERT is capable of excising DNA flanked by loxP-sites we crossed them to Cre-reporter mice which express the LacZ reporter gene as a consequence of tamoxifen-induced Cre-mediated excision. This way we were able to demonstrate function of the Cre-recombinase in fetal liver and bone marrow (Figure 1). Current studies further analyse the bone marrow population expressing Cre-ERT in the adult. Having established inducible 3'enhancer transgenic lines they will be interbred with the SCL-LoxP mice.

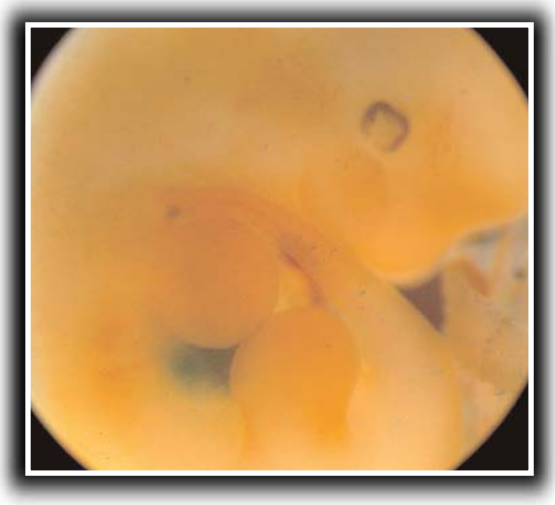

Figure 1. Murine embryo of a cross between a CreERT-3'enhancer transgenic mouse and a LacZ-Cre-reporter mouse. Tamoxifen was administered to the pregnant female with the following regimen: E9.5 0.5 mg, E10.5 1 mg, E11.5 2 mg. On day E12.5 whole-mount LacZ-staining was performed. The fetal liver is positive for LacZ-staining (turquoise/blue).

Function of SCL in endothelial development

JR Goethert, S Gustin, K Becher, DJ Izon and CG Begley in collaboration with B Gottgens and A Green, Department of Haematology, University of Cambridge, UK

It has been demonstrated that SCL plays a role in endothelial development. SCL-null mice exhibit a defect in yolk sac angiogenesis and in the zebrafish mutant cloche the endothelial and hematopoietic defect can be rescued by ectopic SCL expression. But it remains controversial if these defects are due to the lack of SCL in endothelium or if it is due to the missing support of angiogenesis by hematopoietic stem cells. If SCL plays a role in yolk sac angiogenesis it might also be crucial for the formation of new blood vessels in the adult, like tumor angiogenesis and corneal neovascularisation. In order to address these questions we are again using the Cre/loxP recombinase system. We generated mice expressing tamoxifen-inducible Cre-recombinase in endothelium of transgenic mice. We achieved this by using an endothelial enhancer which is located 5' of the SCL-locus and has no detectable capability to guide expression to hematopoietic cells (Figure 2). Further studies will address Cre-function in adult endothelium. Once validated , this transgenic line will be used to inter-cross with mice carrying a loxP flanked SCL locus and the phenotype will be analysed.

Discovery of Genes Regulating Early Blood Development by Expression Profiling of SCL-null Embryoid Bodies

CK Bradley, U Schmidt, RL Brake and DJ Izon, in collaboration with Pfizer Inc., USA and L Robb, WEHI, Australia

Early in the development of the vertebrate embryo genetic events specify the development of blood stem cells from mesoderm. Although there has been some progress in identifying molecules involved in this process, overall there is very little understanding of the inductive interactions critical for hematopoietic lineage specification and for patterning of hematopoietic organs. To identify genes involved in early embryonic blood development we have compared gene expression profiles of parental wild-type (WT) and SCL-null (SCL double knockout) embryoid bodies (EBs). Totipotent mouse embryonic stem cells, when cultured in vitro, form spherical structures known as EBs, which progress through a primitive (embryonic) and then definitive (adult) hematopoiesis over a 10-12 day period. As EB hematopoiesis closely parallels in vivo embryonic and adult blood cell development, they provide a useful model to study the complex signalling mechanisms that direct stem cells towards various hematopoietic lineages. SCL-null EBs fail to generate blood cells and demonstrate reduced transcription of hematopoietic genes.

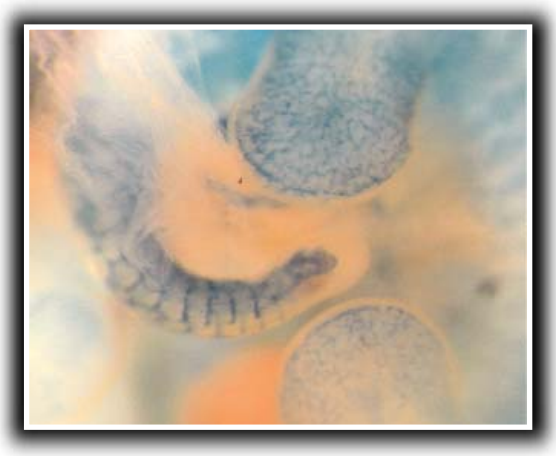

Figure 2. Murine embryo (of a cross between a SCL endothelial enhancer CreERT transgenic mouse and a LacZ-Cre-reporter mouse. Tamoxifen was administered to the pregnant female with the following regimen: E8.5 0.5 mg, E9.5 1 mg, E10.5 2 mg. On day E11.5 whole-mount LacZ-staining was performed. The embryonic blood vessels are positive for LacZ-staining (turquoise/blue). Lateral view demonstrating LacZ-staining in small capillaries of the hand-, footplate and the tail.

Affymetrix microarray technology was used to determine gene expression profiles of SCL-null and WT EBs before the onset of SCL transcription (day3), at the commencement of SCL transcription (day 4), and during SCL transcription (day 4.5 and day 5 of differentiation). Bioinformatic analysis revealed 51 differentially expressed genes (<1.5 fold change on days 3 and 4, >1.8 fold change on days 4.5 and/or 5; $p<0.07$), a remarkably small number given the heterogeneity of EBs. In comparison to WT EBs, the transcription of genes known to play a role in the regulation of blood development was significantly reduced in the SCL-null EBs, including LMO2, Lyl1, Fli1, Hhex and Flk1. Several genes have been selected for further analysis using real time RT-PCR of hematopoietic tissues and primary sorted blood cell populations. An interesting candidate gene, containing an SCL regulatory element and encoding an uncharacterised zinc finger protein, is predominantly expressed in the BM and other hematopoietic tissues. We are currently characterising this gene by multi-lineage progenitor assays to determine its contribution to various cell lineages. Greater knowledge of these key steps in embryonic development may generate new insights into the biology of hematopoietic stem cells, hematopoietic lineage specification and the genesis of hematopoietic malignancies.

The Mechanisms of T cell development – implications for leukaemogenesis

DJ Izon

T cell maturation follows an ordered programme of gene induction and cellular interactions. When this programme is perturbed leukaemogenesis can ensue. This project seeks to discover the underlying mechanisms responsible for T cell leukaemogenesis by the use of retroviral overexpression of genes in a unique in vitro system which has been perfected by only a handful of research laboratories worldwide. This approach combines the power of molecular biology with sensitive cellular techniques that will ultimately shed light on T cell leukaemogenesis and provide the basic foundation for more intelligent drug design for leukaemia treatments.

The role of SCL in Neural Development

SCL Expression & Function in the Central Nervous System

Anke van Eekelen, Cara Bradley and CG 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 (5'SCL neural promoter). 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.

To address the function of SCL in CNS, we initially performed a neuroanatomical mapping study to document the precise pattern of SCL expression in the CNS and to identify the neurochemical phenotype of these SCL-expressing cells. In this study, we utilised the SCL-LacZ knockin mouse, which has the reporter gene LacZ integrated in the SCL locus and serves as a useful model to monitor SCL expression. We demonstrated a widespread expression of SCL in the diencephalon, mesencephalon (Fig.1A) and metencephalon, including previously unrecognised brain regions. We also showed that SCL expression coincides with expression of neuronal nuclear protein NeuN, ubiquitously expressed in neurons; whereas expression of SCL did not co-localize with expression of glial fibrillary acidic protein (GFAP), a commonly used marker for glial cells. Further immuno-histochemical analysis, including co-staining

experiments for LacZ and a series of neurotransmitters and neuropeptides is required to characterise more specifically the type of neurons, which express SCL. A better understanding of the cellular phenotype of SCL expressing neurons will contribute to define the role of SCL in the brain.

In parallel to the analysis of SCL expression in CNS, we generated “conditional” transgenic mice, in which we can ablate SCL in a tissue and time specific manner. This spatio-temporal 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 is based on the cre recombinase - lox P system to delete a specific DNA sequence by recombination. Mice with loxP sites flanking the SCL gene (the floxed SCL-allele) are crossed with mice that either express cre recombinase under control of the Nestin promoter [Nestincre-mice] or under control of the 5’SCL neural promoter [BraincreER(T)-mice]. The intercross between SCL floxed mice and Nestincre-mice generates offspring, in which SCL is deleted from the onset of Nestincre-expression in the CNS onwards. The intercross between SCL floxed mice and BraincreER(T)-mice generates offspring, in which SCL is deleted only in the CNS upon tamoxifen treatment due to a fusion of cre recombinase to a mutated estrogen receptor ligand binding domain showing only affinity for tamoxifen. We have confirmed specific expression of cre recombinase under control of the 5’SCL neural promoter in SCL expressing neurons during embryogenesis and in adulthood. This was performed using morphological analysis of offspring from intercrosses between the BraincreER(T)-mice and a “reporter mouse that allows the expression of cre recombinase to be monitored (Gtrosa26-LacZ reporter mice). Thus, expression of cre recombinase is monitored by LacZ reporter gene expression. As anticipated, we observed that the degree of DNA recombination in these mice was tamoxifen dose dependent (Fig 1B, C, D).

Mouse phenotype analysis as a result the specific ablation of SCL in the brain in these two complementary conditional transgenic mouse lines will elucidate the potential role of SCL in neurogenesis and in mature functional brain circuits.

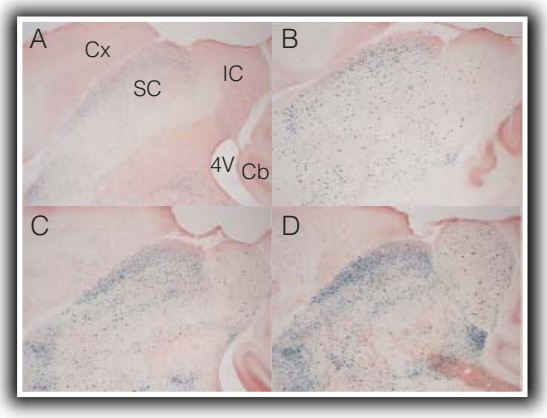

Figure 1. Adult mouse brain sections displaying LacZ gene expression in the thalamic pretectum and the midbrain tectum and tegmentum, including the superior colliculus (SC) and the inferior colliculus (IC). The cortex (Cx) and cerebellum (Cb) do not express LacZ. The observed blue ß-galactosidase staining reflects SCL expression in a section from an adult SCL-LacZ knockin mouse (A), whereas it reflects cre recombinase expression in sections from adult BraincreER(T) positive Gtrosa26 reporter mice (B,C,D). The intensity of ß-galactosidase staining, which indicates the level of LacZ expression in BraincreER(T) positive Gtrosa26 reporter mice, is tamoxifen dose dependent: 1mg/day for 5 days i.p. (B), 2 mg/day for 5 days i.p. (C), 3 mg/day for 5 days i.p. (D).

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Awards

Cara Bradley. Poster Prize, Combined Biological Sciences Meeting, Perth

Invited Presentations

JR Goethert. SCL-LacZ knock-in mice: Adding another dimension to track hematopoietic stem cells. Invited speaker at the Cottesloe Stem Cell Meeting, Perth, WA.

Division of
Cell Biology

Overview

The focus of research within the Division is on the aetiology and pathogenesis of inflammatory diseases in the respiratory tract, and in particular upon the development of novel strategies for prevention of these diseases via early diagnosis followed by “prophylactic” intervention. Our work is divided into two broad streams. The first comprises a comprehensive program targeted at elucidation of the key control mechanisms which regulate immune function(s) in childhood, and how these determine susceptibility to allergic and infectious diseases in early life. We have established important new areas of research in this area over the last 10 years, in particular related to the role of developmental factors which regulate the transition from prenatal to postnatal immune response phenotypes and how these influence the capacity of infants and young children to respond to allergens, viruses and vaccines.

Research in this human area is complemented by a parallel program of experimental animal research, targeted at the basic cellular mechanisms which control the recognition of and response to inhaled antigens, by the immune system. The key cell populations in these studies are networks of Dendritic Cells (DC) in the respiratory tract discovered in earlier work by the Division. These DC are widely distributed in conducting airway and peripheral lung tissues, and normally fulfil a “sentinel” role in alerting the immune system to incoming foreign proteins. They are additionally strongly developmentally regulated, and do not become established as adult-equivalent cellular networks in respiratory tract tissues until around the time of biological weaning. Moreover, their postnatal development is driven by inhaled environmental stimuli, which is likely to include microbial pathogens. There is a growing consensus that these DC play a central role in both induction and expression of allergic diseases, and in orchestrating anti-viral defence, and further that they constitute a prime target for drug development relevant to asthma treatment.

Immunobiology of Dendritic Cell populations in experimental models

The role of respiratory tract dendritic cells (RTDC) in T cell activation in airway tissues

D Strickland, PA Stumbles, JA Thomas, I Tobagus, M Smith, PG Holt in collaboration with JC Huh (Niigata University School of Medicine) and F Jahnsen (Institute of Pathology, Oslo), PD Sly and D Turner (Clinical Sciences, TICHR)

Our earlier studies on the regulation of T cell activation in the lung have lead to the identification of a network of specialised RTDC within the lining of the airways, which is the principal site of exposure to inhaled antigens. Resident airway mucosal DC normally perform a sentinel function, being specialised for antigen uptake and processing but lacking the capacity for effective presentation of antigen to T cells. Presentation of antigen to the T cell system normally occurs only after the subsequent migration of RTDC to the regional lymph nodes (RLN) and their functional maturation at this site. Due to their known potential for T-cell activation and their strategic location within the airway wall, RTDC are widely hypothesised to be the trigger for allergen-specific T cell activation which initiates the late phase response (LPR) in asthma. However, experimental evidence in support of this postulate has not yet been published. Our current studies utilising a rat asthma model are providing the first direct line of evidence that the response to inhaled antigen in sensitised animals is regulated via in situ interactions between antigen bearing airway mucosal DC and memory T cells. Our findings indicate that after resident RTDC acquire antigen by endocytosis they cluster with specific memory T cells and upregulate surface expression of costimulator molecules, and mobilise MHC class II and bound antigen peptide to the cell surface. At this stage they express potent T-cell stimulatory activity. These “activated/mature” RTDC then migrate to RLN over the next few hours leaving in their wake large numbers of IL-2R+ CD4+ T cells, and the animals now express airways hyperresponsiveness to inhaled methacholine. Ongoing studies in this model are focusing on the role of individual RTDC subsets in this process, genetically determined differences in response patterns, and on the nature (and fate) of the T-cells activated in this process.

Responses of rat RTDC subsets to microbial challenge

D Strickland, PA Stumbles, JA Thomas, I Tobagus, S Napoli, PG Holt in collaboration with JC Huh (Niigata University School of Medicine) and F Jahnsen (Institute of Pathology, Oslo)

The phenotypic and functional properties of RTDC subsets and their recruitment kinetics following inhalation of a bacterial antigen (heat killed Moraxella Catarrhalis), has been studied in a rat model. Confocal microscopy, flow cytometry and various functional analyses have been performed, over a series of time points post aerosol challenge, contrasting DC subsets isolated from airway wall, peripheral lung tissue and RLN.

The initial response observed in this model is characterised by a rapid shift in the proportions of RTDC subsets within the airway wall, in particular the rapid decrease in one of the major subsets (MHC class II high, SSC low), with a coordinated increase in the remaining subsets. This initial response is transient and the proportional balance among RTDC subsets is quickly normalised. This early response is associated with the rapid upregulation of costimulator CD86 on RTDC subsets that are initially high in both endocytic activity and MHC class II expression (RTDC undergo a transient decrease in endocytic activity after challenge). Rapid mobilisation of this resident subset of

RTDC and the appearance of DC with similar characteristics in the RLN suggests rapid migration from the airway wall to RLN, where these DC then undergo a transient upregulation of APC activity (within 2h of challenge). By 24h there is a large increase in the total number of CD86 positive MHC class II high DC in RLN. Additionally, a large recruitment of T cells is observed in the airway wall 24h post initial challenge, and it seems likely that this may be associated with a second wave of CD86 upregulation, which is seen in airway wall RTDC. Following the initial rapid mobilisation of RTDC subsets, we observe an influx of precursor cells at later time points that repopulate the airway wall. In contrast to the findings in the allergen-driven rat asthma model, RTDC recruitment kinetics in this system remain elevated at much later time points post challenge, suggesting a sustained alteration of the airway wall microenvironment.

Interestingly, no changes have been observed on DC from peripheral lung tissue.

Current studies are involved in further evaluation of the specific functional activities of the RT/RLN DC subsets involved in these processes.

Genetic differences in RTDC function in rats

D Strickland, PA Stumbles, JA Thomas, I Tobagus, M Smith, PG Holt in collaboration with F Jahnsen (Institute of Pathology, Oslo)

The BN and PVG rat strains are representative of the two extreme ends of the spectrum of IgE responder phenotypes, and comparison of their respective RTDC responses in both the rat asthma and Moraxella Catarrhalis models is in progress.

Interim results from these studies indicate firstly, major differences in the normal RTDC subsets between the BN and PVG rat strains, in both phenotypic characteristics and functional activities. Phenotypically, RTDC isolated from BN rats exhibit uniquely low expression of CD2, which is not observed in DC populations from RLN in the same animals. Functionally, DC isolated from BN airway wall have higher overall endocytic activity, which is a possible explanation for the high efficiency of primary immunity to inhaled antigen in this strain. Secondly, the responses observed in BN animals post aerosol challenge in both models above, are different to the response seen in PVG rats.

In the M.Catarrhalis model, many of the early characteristics that are seen in the PVG response post challenge are also mimicked in BN, with the exception that there is no coordinated upregulation of costimulator expression, in DC from either airway wall, peripheral lung or RLN. Furthermore, the large recruitment of T cells that occurs in PVG airway walls post challenge is not observed in BN animals. Work in the rat asthma model is ongoing. Further studies will focus on delineating the specific functional activities of RTDC subsets in these two strains. Future work directions include microarray studies to examine comparative gene expression on RTDC from PVG versus BN animals, and more detailed examination of the late phase asthma response in the BNs.

Distribution and functional analyses of RTDC subsets in mouse models of asthma and respiratory infectious disease

PA Stumbles, JA Thomas, I Tobagus, M Smith, R Wealthall, S Napoli, M Wikström and PG Holt in collaboration with C von Garnier (Basel University Hospital), C James (Murdoch University) and PD Sly and D Turner (Clinical Sciences, TICHR)

Studies in this new programme have focussed on the following key areas: (1) Characterisation of the complexity and distribution of RTDC subsets within mouse airways: Using cell surface

marker analysis and functional assays we have identified subsets of mouse airway mucosal and lung tissue DC with unique functional characteristics. In particular, a subset of CD11chi, CD11b-, MHC class IIhi RTDC - the dominant subset of the airway mucosa - has been identified that possesses high basal endocytic activity and naïve T cell stimulating activity, suggesting a unique functional specialisation. Other subsets have also been identified that vary in frequency depending on location within the respiratory tract and mouse strain, indicating microenvironmental and genetic regulation of RTDC subset distribution. (2) T cell activation by RTDC in vivo: These studies involve in vivo presentation of intranasally delivered antigen to CFSE-labelled, antigen-specific CD4+ transgenic T cells to monitor proliferation, surface marker expression and intracellular cytokine production in the draining lymph nodes and other sites. Analyses are performed under steady state conditions and conditions of inflammatory insult (bacterial endotoxin) to visualise the parameters regulating the induction of tolerance and immunity respectively. (3) RTDC function following allergen and infectious challenge: The above studies have been integrated into mouse models of allergic airways hyperresponsivenss and influenza infection to analyse the role that RTDC play in either resistance or susceptibility to these immune-mediated respiratory disorders. In particular, it is hoped that a greater understanding of the role of RTDC in the pathogenesis of these diseases will identify some key check points for drug targeting and enable development of more effective vaccine strategies.

The role of regulatory T cells in maintaining peripheral tolerance to inhaled antigens

PA Stumbles and PG Holt

Regulatory T cells (T reg) are a subset of CD4+ T cells that can prevent or suppress T cell activation. Studies in a number of systems have identified an important role for T reg in controlling immune responses to a variety of antigens including self antigens, tumour antigens, infectious agents and foreign proteins. However, despite the broad-range suppressive activities of T reg, the factors governing their generation in the periphery are not well understood. DC have been proposed to play a key role in the generation and function of T reg in peripheral tissues and studies will be initiated to examine the role of RTDC in this process. A mouse model is being developed for this purpose. This work will focus on the role of RTDC in the generation of T reg to inhaled antigens and how the enhanced generation of these cells may be harnessed for prevention or treatment of airway allergic and infectious disorders.

Regulation of T-cell immunity to aeroallergens in early life

Antenatal cytokine production as an aetiologic factor in allergy and asthma

C Macaubas (now at Stanford University, California), BJ Holt and PG Holt in collaboration with PD Sly (Clinical Sciences, TICHR) and N de Klerk (Biostatistics, TICHR)

Earlier studies in our group identified a strong association between attenuated production of both Th1 and Th2 cytokines during infancy, and genetic risk for atopy. We have recently completed a prospective study which has extended this observation into the foetal microenvironment, demonstrating an inverse correlation between IL-4, IFNg and TNFa levels in cord blood, and allergy and asthma outcomes at age 6 years. This suggests that the mechanism(s) responsible for reduced immune capacity in infants at high risk of allergy, are already operative before birth. Moreover, as the principal source of cord blood cytokines are placental trophoblasts, this infers that the operation of these genetic mechanism(s) is not restricted to cells of the immune system. An additional important observation was that

maternal smoking was associated with increased risk for asthma manifestations at age 6 and also with reduced cytokine levels in cord blood. This finding has significant theoretical implications, as it suggests that genetically determined immunological mechanism(s) linked to increased risk for allergy/asthma in infants and children are susceptible to maternal-associated environmental exposures which occur prenatally, of which smoking may be just one example.

IL-12p40 promoter polymorphism and severity of asthma

PG Holt, GP White, BJ Holt in collaboration with G Morahan (Walter & Eliza Hall Institute, Melbourne), PD Sly (Clinical Sciences, TICHR) and GE Kendall (Epidemiology, TICHR)

As part of our ongoing research on regulation of Th1 function in atopy and asthma, we have collaborated with colleagues at the Walter & Eliza Hall Institute of Medical Research in a study on IL-12p40 regulation in a large cohort of 6 yr olds. The principal findings from this study, published recently in Lancet, indicate linkage between a polymorphism in the promoter region of the IL-12p40 gene and severity of both atopic and nonatopic asthma. An unexplained finding from this study is that the affected subjects are heterozygous for the polymorphism: these subjects display more intense asthma symptomatology and attenuated IL-12p40 gene expression. From an immunological perspective, this provides a plausible common pathway to disease severity in both allergen-induced (atopic) and viral-associated (nonatopic) asthma, as IL-12 driven Th1 immunity is central to anti-viral defence and to antagonism of Th2-polarised immunity to allergens. However, the molecular mechanism for this heterozygous effect remains unknown. It will be important to ascertain whether similar associations are demonstrable in other cohorts, and this issue is being addressed by a number of our colleagues internationally.

Dendritic cell function and allergic sensitisation in early life

JW Upham, A Rate, S Ehteshami, PG Holt in collaboration with PD Sly and M Kusel (Clinical Sciences, TICHR) and SL Prescott (Paediatrics, UWA)

Dendritic cells (DC) are the principal professional antigen presenting cells of the body and are fundamental to regulation of the immune response. Experimental animal studies suggest that the functional capacity of DC is attenuated at birth and develops progressively during the preweaning period, but relatively little detailed data is available on humans, and our studies in this area seek to elucidate this important issue. Our major focus is on the way in which DC function changes with age over the preschool years, how this is related to resistance to infectious and allergic diseases.

We have examined peripheral blood DC subsets during infancy, and have shown that the numbers of these DC subsets, and their maturity, correlate with variations in Th1 function. DC are relatively immature at birth, and only slowly develop the capacity to produce IL-12, the key Th1 polarising cytokine. Surprisingly, we have found that full development of the capacity to produce IL-12 does not occur until adolescence. Important additional components of this work include cross-sectional studies to assess the potential contribution of variations in DC maturation kinetics to genetic risk for atopy/asthma, and parallel prospective studies to track postnatal development of DC functions in children who do/do not develop atopy. With the enthusiastic participation of Samira Ehteshami, a visiting medical student from Arizona, we have also examined ways in which the maturation of IL-12 producing capacity can be accelerated in vitro, and in ongoing studies are focusing on the role of microbial-derived stimuli as potential “drives” of this process.

DC function is also a key regulator of immune

memory development. Studies in progress suggest that the capacity to detect memory T-cell responses to both allergens and vaccine antigens in the peripheral blood is limited by the functional maturity of circulating DC, which are responsible for presentation of antigen in these cultures. In particular, our recent studies suggest that initial preactivation/maturation of blood DC prior to introduction of antigen into the culture system can unmask covert T-cell memory to antigens that would not be recognised in standard cell cultures. Thus it appears that the repertoire of antigen specific T-cells in peripheral blood is much broader than previously thought, and this finding has significant implications in studies on the pathogenesis of immunologically mediated diseases. Moreover, initial findings suggest that in vitro expression of Th-cell memory to vaccine antigens in infants may also be severely limited by the functional capacity of circulating DC, and this possibility is being actively explored in ongoing studies.

Regulation of IFNg gene expression in nonatopic and atopic children

E Hollams, M Bassami, GP White, BJ Holt, PG Holt in collaboration with PD Sly and M Kusel (Clinical Sciences, TICHR) and SL Prescott (Paediatrics, UWA)

We have recently published novel findings indicating an important role for promoter CpG methylation in negative regulation of IFNg gene expression in T-cells in early life, and follow-up studies are in progress to further probe the underlying mechanism(s). Of particular interest to us is the possibility that variations in the levels of IFNg promoter CpG methylation may be one of the factors responsible for the differences we have previously identified in IFNg response capacity between infants at high versus low genetic risk for allergy. Our approach to this issue involves firstly cross-sectional studies, contrasting IFNg promoter methylation levels in CD4+ and CD8+ T-cells from atopics and nonatopics at different ages, and parallel prospective studies to determine whether methylation levels in cord blood T-cells are predictive of atopic disease expression patterns in subsequent childhood. We are additionally contrasting the patterns of change in IFNg gene methylation during cytokine-driven in vitro Th1- and Th2-polarised differentiation of adult-derived versus neonatal CD4+ CD45RO- T-cells. These studies are approaching completion and will soon be submitted for review and publication.

Predictive markers of atopy in cord blood

J Rowe, D Suriyaarachchi, MJ Sharp, BJ Holt and PG Holt in collaboration with M Kusel and PD Sly (Clinical Sciences, TICHR)

This study seeks to determine if immunological response capacity at birth predicts the future development of atopy. Cord blood mononuclear cells (CBMC) were collected from a prospective cohort of 230 subjects at high genetic risk of developing atopy. Cytokine responses to a wide range of stimuli including allergen (house dust mite, ovalbumin, cat), microbial stimuli (purified protein derivative [PPD], staphylococcal enterotoxin B [SEB], and lipopolysaccharide) and the polyclonal T-cell stimulant PHA, were determined. Data analyses were undertaken to determine whether these responses were predictive of risk for subsequent development of atopic dermatitis (AD) or positive skin prick tests (SPT) up to the age of 2 years. SEB-induced IL-5 production by CBMC was elevated in children who developed AD at 6 months, while production of all the cytokines analysed was elevated in those who became SPT positive at 2 years. IL-5 and IL-10 responses to PPD were also elevated in those who developed persistent AD up to 2 years of age.

A key new finding was increased IFNg production in response to the polyclonal stimulus PHA

in those who became SPT positive at 2 years of age. Our earlier studies, since confirmed in numerous independent laboratories, indicate that at the population level, IFNg response capacity is significantly attenuated in neonates and infants at high risk (HR) for atopy. However, the present findings suggest that within the HR group, this relationship is reversed i.e. IFNg may itself become a pathogenic factor in relation to ongoing atopic disease expression, once HR subjects have progressed beyond the stage of initial disease expression. This suggestion is consistent with other published findings from our group and others, which indicate very high IFNg expression levels in a subset of atopics. Studies with this cohort are ongoing and will track immunological and clinical phenotypes up to age 5 years, and an important focus will be changes in IFNg gene expression.

Microarray studies in allergic disease

A Bosco, K Williams, C Devitt, A Rate, BJ Holt, PG Holt in collaboration with PD Sly (Clinical Sciences, TICHR), WR Thomas (Molecular Biology, TICHR), N de Klerk (Biostatistics, TICHR), R Loh (Clinical Immunology, PMH), C Suphioglu (Monash University, Melbourne) and H Sampson (Mt Sinai Hospital, New York)

The Division has initiated a series of wide-ranging studies on differential gene expression in allergic disease, focusing initially upon peanut allergy and allergic rhinitis. The aim of these studies is to identify key genes activated during in vitro allergen challenge of CD4+ and CD8+ T-cells from affected subjects, which are associated with disease severity. We have opted for exclusive use of the Affymetrix system for these studies, and have devoted the first year of the project to establishment of rigorous methodology for the overall program, in particular cell separation and stimulation techniques, and subsequent RNA preparation, and to addressing logistical issues related to bioinformatics/biostatistics. Initial microarray experiments involving kinetic studies on Th1/Th2-associated gene activation, and rigorous analysis of the implication of RNA sample pooling in relation to efficiency of detection of differentially expressed genes, are in progress.

Studies on responses to Cat allergens

T Heaton, J Rowe, D Suriyaarachchi, M Serralha, PG Holt in collaboration with R Aalberse (CLB, Amsterdam)

Recent studies suggest that individuals with severe cat allergy may display different patterns of immune responses to cat allergens when compared with their house dust mite (HDM) allergic counterparts. Increased exposure to HDM is associated with an increase in serum levels of HDM-specific IgE antibody, which is in turn associated with production of type-2 cytokines including IL-4 and IL-13. However, it has been shown that in some subjects, increasing exposure to cat allergens reduces the cat-specific IgE levels with a concomitant increase in IgG4 antibody, production of which paradoxically is also driven by type-2 cytokines. Many of these subjects become cat-IgE-negative but remain strongly cat-IgG4-positive. This unusual phenotype has been termed a “modified” Th2 response. However, little direct information is available regarding T-cell cytokine production patterns in these subjects.

Ongoing studies in our group are focusing on a cohort of 12 year old children from which we have identified a number of individuals who display this pattern of cat-specific antibody production. Using these cells we are studying the in vitro T cell responses to the major cat allergen Fel d1, in order to determine whether there are specific feature(s) of their cytokine production patterns following stimulation with Fel d1, which may explain their unique antibody response phenotype. Results to date indicate that individuals who are SPT+ to HDM show a very clear type-2 cytokine response following stimulation with HDM in vitro, and exper-

iments are in progress to contrast these with responses to Fel d1 in the same individuals.

Interactions between T-cell immunity to environmental allergens and environmental microbial stimuli in the aetiology of atopy and asthma

Role of responses to Staphylococcal Enterotoxin B in the pathogenesis of Atopic Dermatitis

T Heaton, HS Lehmann, PG Holt in collaboration with D Mallon (Clinical Immunology, PMH and Fremantle Hospital)

The pathogenesis of atopic disease is complex, involving contributions from a range of genetic, immunological and environmental factors. In Atopic Dermatitis (AD) it is becoming increasingly clear that toxins released from skin-dwelling bacteria – bacterial superantigens – play a significant role in disease pathogenesis. As part of our continuing investigation into the mechanism of action of these superantigens, we have studied the in vitro cellular responses to Staphylococcus Enterotoxin B (SEB) of peripheral blood mononuclear cells (PBMC) from children with active disease and compared these with responses from control groups of children without atopic disease. In addition, swab samples were taken from the children from both lesional and non-lesional skin, with the aim of determining the association between immune responses to SEB, disease severity, and carriage rate of bacteria on the skin.

Consistent with data from recent studies on adult AD in our lab, we have observed that the type-2 cytokines IL-5 and IL-13 are increased in the PBMC responses to SEB in young children with active disease. Additionally, there was a striking correlation between the SEB-induced levels of the type-1 cytokine IFN-_ and the severity of disease, although the patient numbers were small, and this may indicate a role for this inflammatory cytokine in chronic disease. Analysis of the swab samples confirmed results by other groups that the carriage rates of Staph. aureus were higher among the patient group. In other ongoing studies (see "Predictive markers of atopy in cord blood") we have shown that high level production of IL-5 in cord blood in response to SEB stimulation was predictive of susceptibility to later AD development. Taken together, these results suggest that superantigens may have an important role in AD. We plan follow-up studies to elucidate more clearly the role of AD, and to investigate the mechanisms involved.

Prospective cohort study on interactions between susceptibility to atopy, viral infection and asthma

T Heaton, J Rowe, D Suriyaarachchi, M Serralha, BJ Holt, M Wikström, JW Upham, PG Holt in collaboration with PD Sly and M Kusel (Clinical Sciences, TICHR) N de Klerk (Biostatistics, TICHR) and S Johnston (Imperial College, London)

This prospective study has tracked a birth cohort of 230 children up to age 5 years (5 year follow-up currently in progress), and has included collection of complete respiratory infection history over the period, including post-nasal aspirates or nasal swab samples at each infectious episode for PCR identification of the relevant pathogens. Analysis of Year 1 samples for RSV and picornavirus has been completed and PCR studies on a further 12 organisms are in progress. The study has several foci, including attempts to identify predictive immunological markers of disease susceptibility in cord blood (see interim report "Predictive markers of atopy in cord blood"), determination of the nature of associations between susceptibility to allergic sensitisation versus viral infection in infancy, the relationship between factors associated with postnatal maturation of immune function and atopy/infection risk, and ultimately,

how these factors interact in the context of asthma development. New initiatives within the project include analysis of the contribution of recent thymic emigrants to initial T-cell responses in vitro, a case/control study on blood DC functions in children who do/do not develop atopy, substudies on T-cell immunity to RSV and vaccine antigens and also to peanut allergen, and the introduction of flow cytometric methodology for cytokine studies at the single cell level. An important additional component of the study will be assessment of the contribution of a wide range of candidate genes to disease susceptibility and expression of different immunophenotypes, and this work will commence during 2003.

Vaccine immunity in early childhood

Studies on the persistence of long term memory against DTPa vaccination in 5 year old children

J Rowe, D Suriyaarachchi, and PG Holt in collaboration with PD Sly (Clinical Sciences, TICHR), P Richmond and R Loh (Vaccine Trials Group, PMH)

In a previous study, we examined immunological responses to the Tetanus component of the Diphtheria, Tetanus, Acellular Pertussis vaccine (DTPa) in a cohort of 130 healthy infants. We demonstrated an important link between the kinetics of postnatal maturation of systemic T cell cytokine production capacity (i.e. "global" T cell function), and the capacity to generate stable immunological memory against the vaccine antigens. Furthermore, those with a positive family history of atopy (AFH+) displayed consistently more Th2 skewed responses to the vaccine antigen. However by 18 months of age, this disparity had disappeared and the Th1 component of the vaccine response "rebounded" in the AFH+ children and exceeded that of their AFH- counterparts. As a follow-up, we have collected blood samples from a subset of this original cohort, both before and after their preschool DTPa booster. Using this group, we will examine whether T cell memory is preserved at age 5 years, and whether early cellular immune responses are predictive of subsequent T cell memory to vaccines. This cohort will also enable us to confirm that the initial disparity in vaccine responses in those with or without a family history of atopy has disappeared after the completion of the standard boosting regime.

Effects of vaccination on immune system maturation in healthy toddlers

J Rowe, S Yerkovich, D Suriyaarachchi, and PG Holt in collaboration with PD Sly (Clinical Sciences, TICHR) and P Richmond and R Loh (Vaccine Trials Group, PMH)

At birth, the immune system is immature, with the Th1 arm of the immune system not reaching adult functional capacity for a number of years. In previous studies, we have identified the period from 12 to 18 months as a key break point in this immune maturation process in children. We were thus interested in identifying factors which may be involved in triggering T cell maturation during this 12-18 month period. It is widely speculated that exposure to microbial stimuli after birth plays a major role in driving this postnatal maturation. It is feasible that microbial stimuli in this context may not be restricted to live commensals and pathogens, but may also include vaccines. Of particular interest in this regard is the measles, mumps, rubella (MMR) vaccine which is first given at 12 months of age. It has been speculated by some researchers that this vaccine may have covert immunomodulatory properties, but no systemic studies have been reported which either confirm or refute this suggestion. In order to address this potentially important issue, we initially performed a pilot study involving a group of 48 children, examining the response to a wide range of stimuli before and after the administration of the 12 month MMR

vaccine. At the population level, there were no significant differences when comparing pre- and post-vaccination peripheral blood mononuclear cell responses to allergen (house dust mite), vaccine antigen (tetanus), or polyclonal stimuli (PHA). However, these data also showed that response patterns are highly heterogeneous within the population, with a subset of children showing increased polyclonal T cell responses six weeks following vaccination. From these studies, it is unclear whether these increased responses represent a significant effect of the vaccine or simply reflect variations in the postnatal maturation process which occurs in all children, but at varying rates. To address this issue, we have now initiated recruitment of a larger cohort study of 150 children, including control groups who do not receive any vaccines over the study period. In this study, we will be able to directly compare pre- and post-vaccine responses to those occurring naturally in the absence of vaccine stimulation, and thus obtain a definitive answer to this important question.

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Invited Presentations

PG Holt. Symposium Speaker: Environment and susceptibility to allergic diseases: the role of antenatal influences - Royal Society of Edinburgh, International Symposium on Environmental Factors in Allergy, Edinburgh.
PG Holt. Workshop Participant: T-cell immunity to vaccine antigens in human infants - NIAID International Workshop on RSV, Washington.
PG Holt. Plenary Speaker/Chair: Allergen-specific Th1-polarised immunity in asthma - European Respiratory Society Annual Congress, Stockholm.
PG Holt. Symposium Speaker: Development of T-cell immunity to inhalant allergens in childhood - Keystone Symposium on the Origins of Asthma, Santa Fe.

PG Holt. Symposium Speaker: The role of genetic and environmental factors in the development of T-cell mediated allergic disease in early life - 5th International Congress on Pediatric Pulmonology, Nice.
PG Holt. Symposium Speaker/Chair: The role of Dendritic Cells in the late phase response in asthma - World Congress of Immunopathology, Singapore.
PG Holt. Symposium Speaker/Chair: Sensitisation to airborne allergens in early childhood - World Congress of Immunopathology, Singapore.
PG Holt. Symposium Speaker: Developmental factors associated with risk for atopic disease: implications for vaccine strategies in early childhood - International Symposium on Protection of Newborns through Maternal Immunisation, Foundation Merieux, Annecy.
PA Stumbles. Symposium Speaker: Functional subsets of murine respiratory tract dendritic cells - 11th International Congress of Mucosal Immunology, Orlando, Florida.
PA Stumbles. Symposium Speaker/Chair: Dendritic cells and mucosal infections - W1 III 11th International Congress of Mucosal Immunology, Orlando, Florida.
PA Stumbles. Speaker: Regulation of airway immunity by subsets of airway dendritic cells - Asthma and Allergy Research Institute Annual Meeting, Margaret River.
PA Stumbles. Speaker: Characterisation and Functional Analysis of Mouse Airway Dendritic Cell Subsets - Flow Cytometry Users Group, Perth.
JW Upham. Symposium Speaker: Fundamentals of the immune response to allergens - Thoracic Society of Australia and New Zealand, Annual Scientific Meeting, Cairns.

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.

Division of Children's Leukaemia and Cancer Research

Overview

Without medical treatment, most paediatric 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 is involving epithelial cells. Hence, 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 member 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. 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 commis-

sioning 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 discriminating 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'. In the validation of the yeast system, the interacting oncoprotein pairs corresponding to our target oncoprotein complexes (see below) were shown to activate transcription of the counterselectible reporter genes in our selection system and to cause the death of the host yeast strain under the restrictive selection condition.

Genetic alterations in paediatric leukaemia

Gene expression profiles in a panel of childhood leukaemia cell lines mirror critical features of the disease

UR Kees and J Ford in collaboration with M Watson and A Murch, Women's and Children's Pathology, King Edward Memorial and Princess Margaret Hospitals, Perth, Western Australia, and M Ringnér, RL Walker and P Meltzer, National Human Genome Research Institute, NIH, Bethesda, MD, USA.

The development of new drugs against cancer requires established cell lines. They are needed for in vitro studies to identify candidate drugs, and in xenograft models to measure drug efficacy in vivo. Specific criteria need to be fulfilled by cell lines used in the evaluation of potential novel therapeutic agents. It is imperative that they display the features of the particular cancer under investigation. Given the documented heterogeneity of cancers, relevant subtypes need to be represented. In this study we have examined these aspects for paediatric acute lymphoblastic leukaemia (ALL). A panel of thirteen leukaemia cell lines recently established in our laboratory was analysed. We used cDNA microarrays to define the gene expression profiles and compared the data with immunophenotyping and cytogenetic analyses. The expression profiles obtained showed excellent concordance with corresponding protein levels. Importantly, the panel of lines displayed the critical genetic features identified in clinically important ALL subtypes in childhood leukaemia patients.

Gene expression levels detected in oligonucleotide arrays using small specimens from patients

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

Large scale gene expression profiling using microarray technology is often limited by the amount of tissue or cells available. A number of RNA amplification protocols have been published to overcome this problem. However, additional amplification steps can result in a 3' bias and/or poor reproducibility for low abundance transcripts. We performed microarray experiments using GeneChip, arrays to ascertain whether less than the recommended amount of RNA can be used, thus avoiding additional amplification steps. In a titration experiment, 2-10 µg of total RNA from the same specimen was used to prepare biotinylated cRNA, and the recommended standard amount of 15 µg of each preparation was used for hybridisation. Statistical analysis using boxplots, correlation coefficients, MvA plots and concordance percentages revealed almost identical levels of gene expression, independent of the amount of RNA used for target preparation. Most importantly, there was no statistically significant difference when the concordance percentages for low abundance genes were compared, thus demonstrating that 2 µg of total RNA is sufficient to perform GeneChip, analysis.

Detection of hemizygous deletions in genomic DNA from tumour specimens is relevant for the diagnosis of patients

UR Kees, PA Terry and J Ford in collaboration with NH de Klerk, Biostatistics and Genetic Epidemiology, Telethon Institute for Child Health Research, J Everett and A Murch, Department of Pathology, Women's and Children's Health Service, Perth, Western Australia, and DL Baker, Department of

Haematology-Oncology, Princess Margaret Hospital, Perth, Western Australia

Hemizygous deletions in genomic DNA appear to play a more important role in tumorigenesis than previously assumed. The loss or inactivation of tumour suppressor genes (TSGs) is of critical importance in most malignancies. Importantly, the loss of tumour suppressor proteins has been reported to affect the response to chemotherapeutic drugs. These recent data lead to the prediction that the presence or absence of particular TSG in tumour cells will determine the response to chemotherapy. The detection of gene deletion in primary patient specimens is confounded by the presence of normal cells. In this study we designed a quantitative real-time polymerase chain reaction (Q-PCR) suitable to detect two TSGs at the INK4A/ARF locus, p16INK4A and p14ARF. The method is suitable for detection of hemizygous loss in primary patient material and the quality of the method was verified by three independent approaches.

Deletion of one copy of the p16INK4A tumour suppressor gene is implicated as a predisposing factor in paediatric leukaemia

PM Watt, TL Carter, PA Terry, NG Gottardo and UR Kees in collaboration with DL Baker, Department of Haematology-Oncology, Princess Margaret Hospital, Perth, Western Australia

The p16INK4A tumour suppressor gene is frequently disrupted by mutation or deletion in a wide range of cancer types, ranging from leukaemia to cancers of the bladder, skin, lung, liver and spleen. We have previously shown that deletion of at least one copy of the p16INK4A gene is associated with an increased risk of relapse in paediatric leukaemia. Our data from this study suggest that hemizygous p16INK4A deletion may be inherited, conferring susceptibility to leukaemia. Data from primary leukaemia specimens is also presented here which examined the possibility that the remaining allele of the gene was inactivated by another mechanism such as mutation or was silenced by methylation. These possibilities were formally excluded in a case of hemizygous loss of the p16INK4A gene in leukaemia, establishing that in this case the p16INK4A deletion was either semidominant or fully haploinsufficient for relapse susceptibility in this disease. Implementation of high throughput methods such as those used here for detecting hemizygous loss of tumour suppressors genes, will become increasingly important for molecular diagnosis of cancer. This is particularly true for the emerging class of tumour suppressor genes where deletion of one allele is sufficient to confer cancer susceptibility or poor prognosis with standard treatment.

The role of HOX11 in T-cell acute lymphoblastic leukaemia

Specific alternative HOX11 transcripts are expressed in paediatric neural tumours and T-cell acute lymphoblastic leukaemia

PM Watt, K Hoffmann, WK Greene, R Brake, J Ford and UR Kees

HOX11 is a proto-oncogene which is silent in normal mature T-cells while being aberrantly activated in T-cell acute lymphoblastic leukaemia (T-ALL) by translocation. Many oncogenes are expressed in several forms in cancer, however to date only one form of the human HOX11 transcript has been described. In this study we describe the identification and extensive characterization of a range of alternative transcripts of the HOX11 protooncogene which are expressed in primary T-ALL specimens. These alternative transcripts are concurrently produced with the known HOX11 transcript as a result of translocations. Bioinformatic analysis of the DNA sequence at

the HOX11 locus revealed multiple expressed sequence tag (EST) clones which were demonstrated to form novel exons of the gene. Surprisingly, portions of the HOX11 gene are transcribed in a range of normal adult tissues, including aorta and stomach. Using rapid amplification of cDNA ends (RACE) and targeted RT-PCR we have cloned and sequenced more than 45 individual cDNA clones corresponding to these novel transcripts and mapped a range of transcriptional start sites. Based on sequence details from novel exons, specific probes were designed to survey their expression profiles across a panel of tissues. Significantly, particular novel exons were identified which are expressed in T-ALL, while not expressed in normal T-cells. These transcripts include sequences from within intron 1 and intron 2 of the previously characterized HOX11 transcript. To date, aberrant expression of HOX11 has only been associated with leukaemia. Strikingly, we also demonstrate expression of these novel transcripts in a range of neural tumour cells lines (neuroblastomas and primitive neuroectodermal tumours, PNET) but did not detect expression in a variety of normal brain tissues. In these tumours of neural origin, the dominant transcript is more than 1 kilobase larger than the dominant transcript in T-ALL. These observations combined with sequence data from several EST clones derived from medulloblastoma cDNA libraries, supports a new hypothesis that HOX11 may also function as a neural oncogene or brain tumour marker.

The search for genes regulated by HOX11

DN Dixon, K Hoffman, A Boudville, J Ford, UR Kees and WK Greene in collaboration with MJ Callow, Lawrence Berkeley National Laboratory, Berkeley CA, USA

Abundant evidence suggests that the immortalising ability of HOX11 is not restricted to T-cells. We therefore examined the effect of its enforced expression on gene transcription in multiple cell lineages including T-cell, erythroid and fibroblast. This was achieved by a combination of representational difference analysis, cDNA array and high density oligonucleotide array (Affymetrix GeneChip) technology. A number of candidate target genes have been identified and confirmed to be differentially expressed in the presence of HOX11 by semi-quantitative RT-PCR. Intriguingly, these genes encode proteins involved in cell growth, chromatin remodelling and cell fate. Luciferase reporter assays are currently in progress to assess whether HOX11 can transactivate gene transcription from the promoter regions of these genes. Future studies will seek to determine whether these genes are oncogenically relevant in an attempt to elucidate the mechanism by which this homeoprotein promotes tumorigenesis.

Regulation of ALDH1A1 gene expression by HOX11

KL Rice, J Ford, UR Kees and WK Greene in collaboration with I Kng, Division of Veterinary and Biomedical Sciences, Murdoch University, Perth, Western Australia

In childhood T-ALL aberrant expression of the homeodomain 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 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). A 2 kilobase region of the human ALDH1A1 promoter was cloned into the pGL3-Basic vector to allow luciferase reporter experiments to be conducted. In a formal demonstration of the status of ALDH1A1 as a HOX11 target gene, we have assessed the ability of HOX11 to transactivate reporter gene

expression from the ALDH1A1 promoter. In the presence of HOX11, the ALDH1A1 promoter was significantly activated in erythroid cells and repressed in a T-cell line.

The available evidence suggests that HOX11 binds to a core sequence consisting of TAAGTG. This site, however, was identified by employing short random oligonucleotides in vitro and using purified HOX11. It is well known that homeodomain proteins exhibit a remarkably low specificity in vitro when binding DNA in the absence of appropriate cofactors. The availability of a target gene promoter capable of being transactivated by HOX11 will now enable its cognate recognition sequence to be determined with greater confidence. In order to map the HOX11 responsive site on the ALDH1A1 promoter, various deletions of the promoter were generated by PCR and cloned into pGL3-Basic. A potential HOX11 responsive element was then identified in the proximal ALDH1A1 promoter between –91 and –50 relative to the transcriptional start site. The effect of this element is abrogated upon deletion of a CCAAT-box located within this region (-74 to -70). EMSAs will now be used to assess the nature of bound factors to this element.

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 fact that each of the three components of this pathway have been implicated in causing malignancies in their own right serves to strengthen this argument. As a first step towards establishing the presence of a HOX11-ALDH1A1-RA pathway in T-ALL, luciferase reporter gene assays were performed using a retinoic acid response element (RARE) introduced into pGL3-Basic. Strikingly, a significant difference in transcription from the RARE was found between HOX11 and non-HOX11 expressing T-cells, consistent with our hypothesis that alteration in ALDH1A1 expression by HOX11 subsequently leads to a change in retinoic acid levels.

HOX11 associates with pericentromeric heterochromatin in leukaemic T-cells

M Heidari, UR Kees and WK Greene in collaboration with JK Phillips, Division of Veterinary and Biomedical Sciences, Murdoch University, Perth, Western Australia

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 approach, we analysed human genomic DNA for high-affinity HOX11 binding sites. Surprisingly, almost all clones isolated contained satellite 2 DNA sequences. To confirm that HOX11 associates with such pericentromeric heterochromatin in vivo, we characterised HOX11 in terms of its nuclear localisation during interphase in unsynchronised HOX11-positive leukaemic T-cells (ALL-SIL). Using indirect immunofluorescence and confocal microscopy, anti-HOX11 antibody produced a punctate pattern of staining in the nucleus with approximately 20-30 discrete areas of dense staining superimposed on a diffuse distribution of HOX11 protein. By dual staining, the bright HOX11 foci correlated with centromeres

since they overlapped with signals detected by an antibody specific for the centromeric protein CENP-B. Further evidence for a direct interaction of HOX11 with satellite 2 DNA was provided by bandshift and ChIP assays. In the presence of an anti-HOX11 antibody, DNA fragments containing satellite 2 sequences were immunoprecipitated from ALL-SIL chromatin. 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 tumour suppressor genes involved in the growth and development of primitive neuroectodermal tumours

PB Dallas, PA Terry, D Holthouse and UR Kees

In children, the incidence of brain tumours is second only to leukaemia, and yet mortality and morbidity associated with brain tumours are significantly greater. This situation has arisen largely because the molecular biology of brain tumours, and in particular, the most common type of brain tumour affecting children, the primitive neuroectodermal tumour (PNET), is poorly understood. This lack of knowledge has severely hampered the development of improved treatment strategies that are urgently required.

Complete or partial chromosome loss is a common feature of PNET cells suggesting that loss of multiple tumour suppressor genes (TSGs) is a fundamental aspect of PNET pathogenesis. To investigate this further, we have determined the locations of genomic deletions in our PNET cell lines by both representational difference analysis (RDA), and by microsatellite analysis employing 400 microsatellite markers spread across the entire human genome and approximately 10cM intervals. This work has been undertaken in collaboration with the Cancer Genome Project at the Sanger Centre, Cambridge, UK. To build on these data and identify specific genes of interest, we have recently completed a series of microarray expression experiments on our 5 cell lines and panel of 21 primary PNET specimens using state of the art Affymetrix technology. This novel correlative approach, combining genomic deletion analysis, microarray expression studies, and extensive use of bioinformatics will lead to the identification of genes of interest representing putative PNET TSGs. We are currently verifying the expression levels of sets of genes of interest identified using this approach by real-time PCR.

We anticipate that our studies will lead to a clearer understanding of the molecular pathways involved in PNET growth and development, 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. We have generated a new peptide library consisting of over 2 million bio-active peptides derived from a bio-diverse set of 19 compact genomes. These ‘phylomer’ peptides exploit evolution’s structural information to enhance the number and affinity of blocking peptides obtained. Unlike aptamer libraries, these natural peptides range in size from small oligopeptides to polypeptides of approximately 150 residues and encode both natural open reading frames

and random peptide structures. The Phylomer library which we are currently screening is highly diverse containing genomes from 19 distinct species drawn from both archaebacteria and eubacteria.

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 Bogoyovich, Department Biochemistry, University of Western Australia

Our system has confirmed the interaction of a peptide (named TI-JIP) which interacts with JNK, inhibiting its interaction with JUN, its phosphorylation substrate. The work of our collaborator, Dr Bogoyovich 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 actions 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 point mutants to address the importance of these regions and better define the TI-JIP-JNK binding interface. This should help clarify the mechanism by which TI-JIP inhibits JNK and might highlight a novel region of JNK to target for drug design.

Targeting complexes involving the SCL oncoprotein in T-ALL

R Hopkins and PM Watt

The model target of this screen is interaction of the SCL (or TAL-1) bHLH (basic helix-loop-helix) protein with the ubiquitously expressed bHLH E2A protein, E47. While SCL is important for the regulation of haematopoeisis, its aberrant activation has been implicated in a high percentage of paediatric T-cell acute lymphoblastic leukaemias (T-ALL).

Using E47 as a "bait" protein and SCL as the "prey" in a modified reverse yeast two-hybrid screen, we have isolated peptide blockers that disrupt this interaction. To test the selection procedure and set death thresholds, the Id1 protein was used. This binds E47 and prevents dimerisation with HLH factors such as SCL. For the purposes of stringently validating the discriminating blocker trap, a large panel of yeast strains has been assembled containing various bait/prey combinations. This panel contains both SCL-specific interactions and E47-specific interactions to determine specificity of Phylomer blocking. The SCL-specific interaction pairs are SCL/E47, SCL/E12, SCL/E2-2, SCL/LMO2, SCL/p44, SCL/DRG, SCL/HEB, while the E47-specific interaction pairs are E47/Id2, E47/MyoD.

Preliminary data suggest that our screening platform can successfully select for peptide blockers of protein-protein interactions. The flexibility of titrations unique to the discriminating blocker trap, appears to allow for modulation of the screening stringency and the isolation of specific blockers. From a sample screen of less than 1/10 of our 2 x 106 phylomer library, we have isolated 6 specific peptide blockers of the SCL/E47 interaction. While these blockers have yet to be validated further, the results are very encouraging. Published standard aptamer screens have isolated between 2 to14 interacting peptides from

libraries ranging in size from 107 to 109. Phylomer peptides therefore appear to yield a 10 to 1000-fold better hit-rate than random aptamers. The peptides isolated from such screens could be used for target validation, specific pathway inhibition and to provide structural information for designing chemotherapeutic leads.

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 system being used is novel, and is currently being developed and optimised. New yeast strains have been generated as well as a number of vectors expressing hTERT and hTR, and these are being tested for expression and interactions. Once the interactions have been confirmed, a new peptide library will be used to block interactions between hTERT and hTR, and the peptides isolated may ultimately be used for structural modelling of new drugs to target telomerase in tumour cells. The optimisation of this system will also provide a new way to analyse protein-RNA interactions and to isolate specific blockers of these interactions. This technology will have applications in both basic scientific research and pharmaceutical development.

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

Given the changes to the reverse yeast two-hybrid system, full-length cDNA clones of the interacting oncoproteins PLZF and ETO were made in the next generation of both bait (pDD) and prey (pJFK) vectors. The high level of auto-activation observed for these full-length constructs when expressed in yeast, resulted in the cloning of the interacting domains of the PLZF and ETO proteins. More specifically, the PLZF RD2 domain which interacts with ETO containing the PLZF binding site at 220-230aa, were cloned into bait and prey vectors. These interacting interfaces of both PLZF and ETO have also been cloned into the new library expression vector (pYTB3) for positive controls in the blocker interaction trap. Further, the relevant PLZF and ETO domains have been cloned for interaction with N-CoR, SMRT and Sin3A proteins. These second domains will be used for specificity of interaction of Phylomers in dual bait yeast two-hybrid experiments. New thresholds for the above-mentioned interacting pairs are currently being set-up in preparation for a primary screen using the phylomers in the new library vector.

In addition, the identification of specific blockers of protein/protein interactions also provides a useful source of dominant negative probes for dissecting mammalian gene pathways and validating candidate drug targets. These studies represent the establishment of a practical, high-throughput screening system for novel peptide-based anticancer drugs which has elicited interest from leading biotechnology companies in the US and Europe. An institute

spin-off company Phylogica Ltd (http://www.Phylogica.com) has been created for the commercialization of this drug discovery platform technology and a full-time project management assistant appointed to assist the directors and the research team in this venture.

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Invited Presentations
PB Dallas. Gene loss in paediatric brain tumours. Third WA State Cancer Conference, 2002, Perth, Western Australia.

UR Kees. Gene expression profiling in paediatric leukaemia. Annual Scientific Meeting. Haematology Society of Australia and NZ Australasian Society of Blood Transfusion, 2002 Adelaide, South 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.
PM Watt. Committee Member of The Combined Biological Sciences Association of Western Australia

Patents arising from this work
PM Watt and UR Kees (1998). Named inventor on patent entitled "Peptide detection method" filed internationally under the PCT (PCT/AU99/00018) and in the USA (09/227,652) as a full patent application. Priority date January 9, 1998 claimed from US provisional application number 60/070989. Proceeded to National Phase Examination in Europe, USA, Japan, New Zealand and UK. Applicant: TVW Telethon Institute for Child Health Research. This patent is now granted in Australia and also allowed in the US.

PM Watt (1998). Named inventor on patent entitled "An improved drug delivery device and methods therefore" filed internationally under the PCT (PCT/AU99/00290), in Malaysia (PI 9901507) and in the US (09/294, 759). Priority date April 20, 1998 claimed from Australian provisional applications PP3054, PP4001 and PP6601.
Applicant: InfaMed Ltd.

PM Watt and WR Thomas (1999). Named

inventor on patent entitled: “Isolating biological inhibitors from natural domain libraries” filed under the PCT (PCT/AU00/00414) and as a full application in the US on May 5, 1999 (60/132711).
Applicant: TVW Telethon Institute for Child Health Research

UR Kees and PM Watt (1999). Named inventor on patent entitled: “Method of detecting the presence or absence of specific genes”, filed internationally under the PCT (PCT/AU00/01334). Priority dated: November 3, 1999 claimed from US provisional application (No. 60/163252).
Applicant: TVW Telethon Institute for Child Health Research

PM Watt (2000). Inventor on provisional patent application entitled: “Improved high throughput reverse two hybrid/reverse three hybrid screening method” filed provisionally in Australia (PQ6830).
Applicant: TVW Telethon Institute for Child Health Research.

PM Watt and R Hopkins (2000). Inventor on provisional patent application entitled: “Dual fluorescent reporter construct for two hybrid and reverse two hybrid screening applications” filed as an Australian provisional application (PR1256) on 6/11/00.
Applicant: TVW Telethon Institute for Child Health Research.

PM Watt, R Hopkins, I Serebriiskii and E Golemis (2001). Inventor on patent application filed under PCT entitled: “Improved high throughput reverse N-hybrid screening method”. Applicant: TVW Telethon Institute for Child Health Research.

PM Watt, R Hopkins and WR Thomas (2003). “Methods of constructing and screening diverse expression libraries” (Continuation-in-part application of US Serial No. 09/568,229 filed May 5, 2000 which claims the benefit of priority under 35 USC &19(e) from US Provisional Application No 60/132,711 filed May 5, 1999)

PM Watt and R Hopkins (2003). “Method of regulating gene expression” filed as an Australian provisional application on February 21, 2003. Applicant: Telethon Institute for Child Health Research.

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Division of Clinical Sciences

Overview

2002 has been a good year for the Division of Clinical Sciences. The first year of the program grant titled "Developmental aspects of respiratory inflammation, allergy and asthma", with Chief Investigators, Peter Sly (Clinical Sciences), Patrick Holt (Cell Biology), Wayne Thomas (Molecular Biology), Peter LeSouef (Department of Paediatrics, UWA), Stephen Stick (Clinical Sciences & Respiratory Medicine, Princess Margaret Hospital for Children), John Upham (Cell Biology & Department of Medicine, UWA) and Philip Stumbles (Cell Biology) has been very successful. This grant has allowed us to develop collaborative relationships between the various groups involved. The main achievements from the Program grant for 2003 can be summarised as follows:

1. Genetic studies
Achievements in the genetic studies included: the identification of the IL-12b promoter polymorphism associated with asthma severity at 6 years of age; and establishing the first age-dependent gene by environment relationships in childhood asthma (the CD14 promoter polymorphism has significant associations with allergy and asthma-related phenotypes but only between 8 and 14 years of age, whereas others such the one in the IL-10 gene demonstrate consistent non-age dependent relationships);

2. Physiological factors measured at birth that predict respiratory outcome in childhood
In 2002, we published the first of a series of papers on one of our birth cohorts with longitudinal data to 12 years of age. We have established the physiological factors that can be measured at birth and predict respiratory outcome at 12 years of age. The importance of these factors is that they demonstrate that the early physiological status is in itself an important risk factor for the development of asthma and that it remains important throughout this period of life. The next follow-up will be undertaken through the program grant commencing next year.

3. Development of methods for measuring airway tone in intact mice in vivo
By developing techniques for "tracking" respiratory input impedance during a slow deep inflation and deflation manoeuvre that can be performed in intact mice under

general anaesthesia, we have been able to measure airway tone. From the impedance spectra we derive respiratory mechanics parameters and can plot these against lung volume or trans-respiratory pressure. The area contained within the airway resistance- volume (or pressure) loops represents airway tone. The basic methods paper has been accepted for publication in the Journal of Applied Physiology.

4. Allergen studies

During 2002 we have made significant advances in understanding how aeroallergens interact with the immune system, including:

a) obtaining serological evidence that people inhale sufficient quantities of the inflammatory mediator EN-RAGE from the S100 calgranulin family to induce immune responses. This has led to an hypothesis that the inhalation of inflammatory mediators released by animals (and humans) produce a dose-dependent modulation of allergic responses;

b) studies with the major mite allergen Der p 1 showed that the intranasal (mucosal) administration of peptides does not induce tolerance as generally accepted but rather induces a compartmentalisation of immunoregulation where responses to respiratory sensitisation are increased while responses to systemic of antigens are decreased. The demonstrates the need for correct models for testing new types of immunotherapy and counter indications for the use of mucosal routes for desensitisation of a respiratory allergy; cDNA cloning and characterisation of a major cat allergen p20 in addition to the allergen Fel d 1. The allergen which reacts with sera from allergic subject at a higher frequency than Fel d 1 may be responsible for many unresolved observations in cat allergy which was previously accepted as being predominantly directed to Fel d 1;

c) the first comparative study of a IgE and T-cell cytokine responses to a panel recombinant mite allergens has shown the dominance of the Der p 1 and Der p 2 allergens;

d) first comparative study of allelic variants of an allergen showed that the most commonly found polymorphism induce similar T-cell cytokine responses but less common polymorphisms, even with only minor amino acid sequence changes, induced deviant responses for a significant number of allergic people. This study with the highly polymorphic major mite allergen Der p 2 demonstrated that genetic variations have the potential to affect immunotherapeutic procedures and that the defined major polymorphism (WRT previous studies) would be most effective.

Infant Lung Function

Comparison of different techniques to measure exhaled nitric oxide in infants (eNO).

SM Stick, P Franklin, A Moeller.

Work has continued comparing tidal breathing techniques with a single breath technique developed by our group. The tidal breathing technique in non-sedated infants appears more variable and less repeatable than the equivalent technique during sedation. The single breath technique can detect increased NO production in a group wheezy infants whereas the tidal breathing technique fails to discriminate wheezy and healthy groups. Manuscript submitted. Funding: NHMRC Project Grant.

Effects of inhaled glucocorticoid on lung function and exhaled nitric oxide in infants.

SM Stick, P Franklin.

A double blind, randomised-controlled study of inhaled Fluticasone Dipropionate (FDP) has been completed. Outcome measures were lung function using the RVRTC method, exhaled nitric oxide (FeNO) and symptoms. A unique feature of this study is an attempt to recruit infants most likely to have airway inflammation indicated by raised levels of eNO. We studied 40 children. The results indicate a significant effect of FDP on FeNO even in the absence of significant changes in lung function or symptoms in this relatively mildly affected group of infants. Manuscript in preparation. Funding: Glaxo-Smith-Kline

Epithelial Nitric Oxide Synthase expression in asthma and atopy.

SM Stick, J Legg, A Moeller, S Burgess, C Lane, R Searles, D Knight.

Epithelial cells have been collected from over 100 children and mRNA extracted. Cells have also been successfully cultured. A preliminary analysis using gene array technology supported by real time RTPCR has confirmed increased NOS mRNA expression in atopic epithelium. Furthermore FeNO is positively correlated with epithelial iNOS expression. This strategy has been used as proof of concept for further analyses focussing on genes involved in inflammation and airway remodelling. Interesting observations to date include a relative down-regulation of EGFR and EGF in children with asthma. EGF and EGFR are factors that are important in epithelial responses to injury and are up-regulated in adults. These data suggest that airway remodelling might not play the same role in childhood asthma as in adults. Data presented at TSANZ (Adelaide) and ATS (Seattle) meetings. Manuscript in preparation. NHMRC Program Grant.

Epithelial Nitric Oxide Synthase expression in cystic fibrosis.

S Stick, A Moeller, S Burgess, C Lane, R Searles, D Knight.

Epithelial cells are being collected from infants with cystic fibrosis at diagnosis and each year for 3 years in order to determine whether the decreased epithelial nitric oxide production observed in CF is a primary or secondary problem. Funding: Australian CF Association

The epithelial response to indoor environmental pollutants.

S Stick, P Franklin, A Fonseca, R Searles.

Cultured epithelial cells from an unselected population of children will be exposed to a range of pollutants including formaldehyde, volatile organic compounds and particulates. Responses will be determined using microarray technology and real time RTPCR. The effects observed in vitro will be tested in vivo using a mouse model. Funding: NHMRC Program Grant.

Breath condensate analysis in infants and children.

A Moeller, G Hall, P Franklin, SM Stick.

Exhaled nitric oxide is thought to be a marker of airway inflammation in asthma. Recently it has become apparent that nitrite/nitrate in breath condensates might be a better indicator of inflammation. Other potential advantages of analysing condensates include the ability to detect proteins ie., leukotrienes and cytokines and the fact that nitrite/nitrate concentrations are not flow dependant making measurements in infants feasible. We are examining some methodological issues related to collection of condensates from infants and determining nitrate/nitrate concentrations in infants with a variety of respiratory conditions. Data presented at ERS (Stockholm), ATS (Atlanta), TSANZ (Melbourne) annual meetings.

Personal exposure of infants to environmental pollutants.

P Franklin, J Jones, SM Stick.

Environmental exposures are thought to play a role in the genesis of asthma. However, there needs to be valid, accurate information regarding personal exposure since many assumptions based on data from adult studies are unlikely to apply to infants. Methods have been developed that can be used to assess the exposure of infants to formaldehyde, volatile organic compounds and particulates using combinations of time activity data, environmental monitoring and personal monitoring. Data presented at ATS (Seattle). Funding: Asthma WA

Arousal responses during hypoglycaemia.

S Stick, A O'Donnell, T Jones.

Counter-regulatory hormone responses to hypoglycaemia are damped during sleep. Diabetics therefore depend upon spontaneous or serendipitious arousal during hypoglycaemia as a protective mechanism. We are investigating whether the arousal threshold is raised during hypoglycaemia a situation that would increase the risk for serious hypoglycaemia during sleep. Pilot studies have indicated that hypoglycaemia affects latency of auditory brainstem responses but not the threshold of response. Therefore we are proceeding with an auditory threshold as the arousal stimulus. An appropriate device has been designed and constructed in collaboration with Andrew Turpin (Curtin University, Computer Science) and a local specialist electronics company (Nautronix). Funding: NHMRC/JDF Program Grant.

Respiratory Physiology

Inhaled Glucocorticoids: Effect on lung structure and function during the early postnatal period

J Kovar, PD Sly

The use of inhaled steroids in children under 2yrs of age has posed concern given that the human lung continues to develop during this time period. Previous work in the rat has shown that high systemic exposure to glucocorticoids during the period of septation (alveolar formation) causes adverse changes in lung development. This study examined the effects

of chronic exposure to inhaled glucocorticoids on lung structure and function during the early postnatal period using the rabbit as a model. Results from this study show that neonatal rabbits exposed to inhaled steroids had smaller lung size, thinner peripheral airway wall dimensions and fewer alveolar attachments. However, there was no difference in body weight or lung function between the groups. This study reached its conclusion this year, culminating in Jana Kovar submitting the work for her PhD.

The role of neutrophil proteinases in susceptibility to bleomycin-induced fibrosis

RA Collins, DJ Turner, PD Sly, S Dunsmorea, G Laurenta
aUniversity College, London.

This project is a collaborative study with Dr. Sarah Dunsmore and Prof. Geoff Laurent, University College, London. The project is funded by a Wellcome Trust Biomedical Research Collaboration Grant.

Pulmonary fibrosis (scarring of the lungs) and emphysema are the end result of an abnormal injury repair process and represents the end-stage of many lung diseases. As such, pulmonary fibrosis is a common clinical problem, yet little is known about the underlying mechanisms. Bleomycin is an anticancer drug which causes lung injury and fibrosis in up to 5% of patients and induces lung fibrosis in animal models. This study examines the underlying susceptibility to bleomycin-induced pulmonary fibrosis in three types of transgenic mice with the gene deleted for neutrophil elastase (NE), cathepsin G (CG) or both NE and CG. These knock-out mice are expected to alter the susceptibility to bleomycin-induced lung fibrosis and to bacteria-induced emphysema. Understanding how and why fibrosis occurs will potentially allow new treatment to be developed which prevents this scarring. To date we have collected baseline physiological data on each genotype, and examined the effects of bleomycin on the wild type (WT) controls. WT mice show altered parenchymal lung mechanics 30 days following belomycin. The effects of bleomycin on the remaining 3 genotypes is currently being examined.

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

RA Collins, DJ Turner, Z Hantos, PD 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. This is a three year project which forms the basis of Rachel Collins PhD research. To date, preliminary work has concentrated on developing the appropriate methodology for assessing airway tone in these animals. In 2003 we will characterize the acute phase of RSV infection in adult, neonates and juvenile Balb/c mice. Further work will concentrate on the effect of age of infection (neonates vs juveniles vs adults) and the long term effects of virus exposure, ie whether or not lung function is/remains altered at 4, 8 and 24 weeks post infection.

Mechanisms of respiratory disease following influenza virus infection

DJ Turner, EM Bozanich, PD Sly, PA 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 above. 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. This project was approved and commenced late in 2002 and to date we have been characterizing the dose of flu virus needed to induce clinical symptoms in adult mice, which forms the basis of ongoing work in 2003.

Bacterial modification of the allergic response in the sensitized lung

DJ Turner, J Burchell, PD 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. It will not only lead to a better understanding of the interaction between allergens and bacterial products in the lung, but may ultimately lead to new approaches for treatment and prevention of allergic disorders. The aim of this project is to investigate the modifying role of LPS on allergic responses in a murine model of allergic inflammation. In the first year of this project we have used naïve mice to characterise the inflammatory response to both single and multiple LPS exposures. Results to date show that a single LPS aerosol induced an immediate inflammatory response as early as 2 hrs that returned to baseline by 24hrs, accompanied by an altered cytokine profile but no alteration to lung mechanics. In contrast, multiple LPS exposures enhanced responsiveness to inhaled methacholine at 6hrs, returning to baseline by 24hrs. In 2003 these experiments will be conducted in sensitised mice to examine the interaction of bacterial LPS exposure and allergen responses. This project formed the basis of Jennifer's honours project and she was awarded a BSc Hons (first class) from Murdoch University.

Determining airway tone and tissue properties in mice

DJ Turner, RA Collins, C Thamrin, Z Hantos, PD 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 loud speaker 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). 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

C Thamrin, Z Hantos, PD 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. It enables the partitioning of respiratory impedance into airway and tissue parameters, which is not possible in cruder methods such as plethysmography. This project aims to expand the current FOT by examining lung mechanics over a broader band of frequencies than conventionally used. 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 application of the oscillatory signal and the data acquisition process to be superimposed 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 determine the optimum range of frequencies with which FOT measurements should be made, while simultaneously providing a better understanding of the mechanical behaviour of the lungs. The initial work is being undertaken in rats and mice, with an aim towards applying this knowledge to improve the FOT in infants and children.

Mechanisms of persistent airway inflammation and airway remodelling

DJ Turner, N Carrolla, A Jamesa, R Kumarb
aPulmonary Physiology, Sir Charles Gairdner Hospital, bSchool 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 (remodelling) 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 is seen in asthma. This project involves assessment of inflammatory cells and LAs in an established

ovalbumin-sensitised mouse model1,2 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.

Cystic Fibrosis

Early detection of inflammation in cystic fibrosis

S Brennan, K Winfield, PD Sly

In 2002 this research group continued investigations in the area of early development of inflammation and infection in cystic fibrosis through a project funded by the 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 investigate the nature of the relationship between markers of lung disease and the breakdown products of lung tissue excreted by the kidneys.

3. To investigate a mouse model of CF inflammatory lung disease stimulated by bacterial products or infection.

4. To correlate the inflammatory response in the mouse model with lung function measured using a new adaptation of the low frequency forced oscillation technique.

Our findings to date are outlined below:-

∑ Over one hundred and fifty broncho-alveolar lavage fluid samples have been collected from 65 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 Balding, K Winfield

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, and enrolment of subjects into the study has been finalized and we expect results from this trial to be forthcoming in the next 12 months.

Inflammation in cystic fibrosis: Friend or Foe?

S Brennan, K Winfield, K Burchell, PD Sly.

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 and adults with CF, in both stable and clinically unwell states from CF clinics in Perth and in other centers nationally. We aim to investigate whether the breakdown products of elastin and collagen fibres found in urine and measured by high performance liquid chromatography (HPLC) correlate with the inflammation measured from sputum in patients at times of stable clinical health and at times 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 project formed the basis of Katherine's honours project, for which she was awarded a BsC Hons (first class) from UWA.

We have now collected samples from many patients through the BAL program and through the CF clinic at Princess Margaret Hospital. This study received funding from the National Cystic Fibrosis Association for 2002.

Two international collaborations have also resulted form 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 Ryan[a], P Robinson[b]
aSir Charles Gairdner Hospital, bRoyal Children's Hospital in 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. There are now

over 60 subjects recruited through the US arm of the study and Australia. Clarithromycin is being tested for it's ability to reduce inflammation and improve lung function when used in conjunction with current antibiotic therapies. Recruitment of subjects into this study is almost complete and we expect that the results of this study will be available in 2004.

Clinical Asthma Research Group

Factors Influencing the Expression of Atopy in the Lungs of Children Aged 5 Years With Positive Skin Prick Tests

T Douglas, PD Sly, R Loha, PG Holt
aDepartment of Immunology, Princess Margaret Hospital for Children

This study aims to investigate the factors occurring early in life that influence the development of atopy in the airways of children with positive skin prick tests. Children, selected from a prospectively followed birth cohort, at age 5 with positive skin prick tests to house dust mite are being invited to undergo bronchial allergen challenge with house dust mite extract. The responses of these children will be analysed with demographic, environmental and medical history data prospectively collected from birth. The second phase of the study involves investigation of in vitro immune responses to allergens and non-specific stimuli on blood samples taken from the children.

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 Loha
aDepartment of Immunology, Princess Margaret Hospital for Children

This prospective cohort study involving a total of 263 families commenced in 1996. Over 6,000 'infectious' & control mucous specimens have been collected over the past 5 years. The specimens collected in the children's first year of life have been analysed for Respiratory Syncytial Virus (RSV) and Rhinovirus. Analysis of these results is currently underway.

Three quarters of the cohort have completed the 5 year followup visit with the children undergoing their third skin prick test, further blood test and lung function tests as they turn 5 years of age. The first phase of this study will be completed in 2003 when all the children turn 5 years of age.

The study team would like to acknowledge the ongoing commitment and tremendous contribution the study children and their families have made to this unique and important study of atopy and asthma.

The role of viral lower respiratory infections in allergy and asthma

S Young, B Hesselmar, T Fukushima, J Tizard, B Holt, PG Holt, PD Sly

This study aims to: determine the mechanisms by which RSV/PF alters lung function in both the recovery period after the acute wheezing LRI, and in the longer term; assess the relationship between immunologic status, severity of infection, and subsequent atopic outcome. Infants, under one-year-of-age, admitted to PMH for RSV-bronchiolitis or PF, will be recruited. Infants are prospectively assessed at several intervals during the first 12 months post infection. Assessment includes: per nasal aspirate (on admission) to determine the cytokine types and levels present; infant lung function; symptom history questionnaire; and blood samples to determine response to common allergens and the genetic predisposition to atopy. Reassessment of the infant cohort will occur at regular intervals throughout childhood.

Vaccine Trials Group

The Vaccine Trials Group (VTG) was established in 1999 as a collaborative venture involving the TVW 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. Our group is also collaborating with Professor Pat Holt's Cell Biology Laboratory to examine the cell mediated response to vaccines and the effect of vaccines on the developing immune system. In the coming year we hope to focus further attention on the impact of pneumococcal vaccine on mucosal immunity in children with recurrent otitis media and broadening immunisation services.

In 2002 we conducted or commenced several international studies. These included:

• A phase 3, single-blind, randomized, multicentre study to assess the immunogenicity and safety of two lots of SmithKline Beecham Biologicals' live attenuated measles-mumps-rubella-varicella vaccine (MeMuRu-OKA), at two different titres, given as a single injection to healthy children in their second year of life with SB Bio's measles-mumps-rubella vaccine (Priorix') as a control group.

• A Phase 3 double-blind randomized multicentre primary vaccination study to bridge the DTPa-HepB-IPV vaccine manufactured according to the large scale manufacturing process with the DTPa-HepB-IPV vaccine manufactured by the small scale manufacturing process when administered intramuscularly to infants at 2, 4 and 6 months of age, co-administered with Merck's Hib conjugate vaccine (LiquidPedvaxHIB®) in a separate injection at 2 and 4 months of age.

• A Randomized, Placebo-Controlled Trial to Assess the Safety, Tolerability and Immunogenicity of Influenza Virus Vaccine, Trivalent, Types A & B, Live Cold-Adapted (FluMist) and Measles, Mumps, Rubella (MMRII®) and Varicella (VARIVAX®) Vaccines Administered Concurrently to Healthy Children.

• Open, randomised phase 3b, clinical trial to compare the immunogenicity and reactogenicity of GSK Biologicals' DTPa-IPV vaccine (Infanrix™-IPV), with GSK Biologicals' DTPa (Infanrix™) and Aventis Pasteur MSD's IPV vaccine (IPOL®) administered separately to healthy children 4 to 6 years of age, previously vaccinated with 4doses of DTPa, and polio vaccine, and co-administered with GSK Biologicals' MMR Vaccine (Priorix™).

• The role of biofilm in the aetiology of chronic ear disease in children.

• Effects of Measles Mumps Rubella vaccine and Varicella vaccine on immune system maturation in healthy toddlers.

• Impact of pneumococcal vaccine on mucosal immunity in children with recurrent otitis media.

• A study to evaluate the immune response and safety of 3 doses of human Papillomavirus (HPV) vaccine in 18-23 year olds and to determine the minimum dose of the vaccine that produces a protective immune response

Staff and students

Head of Division

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Honours students

Kathryn R Burchell BSc Dip Ed
Jennifer T Burchell BSc

Research Support

Lauren Bamberger BA (Hons)
David Sly

Theses passed

PD Sly MBBS, MD, FRACP, DSc University of Western Australia 2002
Asthma in infants and children.
MMH Kusel MBBS PhD University of Western Australia 2002
The influence of viral respiratory tract illnesses in early childhood on the development of atopy
JT Burchell BSc Hons (first class), Murdoch University 2002
Characterisation of LPS-induced lung inflammation in the mouse.
K Burchell BSc Hons (first class), University of Westarn Australia 2002
Do inflammatory markers reflect clinical status during pulmonary exacerbations of cystic fibrosis.

Awards

RA Collins. Boehringer Ingelheim best oral presentation, TSANZ WA branch meeting, 2002
RA Collins. Dora Lush PhD scholarship. NHMRC, 2002 –2005.

External Committees

International

PD Sly. Joint American Thoracic Society – European Respiratory Society Task Force on Standards for Infant Respiratory Function Tests (1997-)
PD Sly. European Respiratory Society Task Force on Forced Oscillation (2000-2)
PD Sly. World Health Organisation advisor on asthma and lung diseases in children (2001-)
PD Sly. World Health Organisation Working Party on Management of Asthma in Children under 5 years the Developing World, (2000-)
PD Sly. Long Range Planning Committee, Pediatric Assembly, American Thoracic Society (2000-)
PD Sly. International Task Force, Pediatric Assembly, American Thoracic Society (2001-)

National

PD Sly. NH&MRC Discipline Panel (2002)

Local

PD Sly. Asthma Foundation of Western Australia Medical Advisory Committee (1991-)
PD Sly. Human Ethics Committee, Princess Margaret Hospital for Children (1991-)
PD Sly. Scientific Advisory Subcommittee, Human Ethics Committee, Princess Margaret Hospital for Children, Chairman (1993-)
PD Sly. Research Committee, Arthritis Foundation WA (2001-2)
DJ Turner. Treasurer of the annual Combined Biological Sciences Meeting (Western Australia) (1999-)
DJ Turner. Member of the MBBS selection committee, Faculty of Medicine, UWA (1999-)
RA Collins. TSANZ Associates Subcomittee (WA branch).

Invited Presentations

PD Sly. Measurement of Airway Tone in vivo to Assess Post-Viral Wheeze Mechanisms. Keystone Symposia, Santa Fe, February 2002.
PD Sly. Overview: Asthma – Mini Symposium. Thoracic Society of Australia and New Zealand, Annual Scientific Meeting 2002.
PD Sly. PEF Monitoring in essential to good asthma control – Against. Asthma Begins in Childhood IV. AstraZeneca, Copenhagen, April 2002.
PD Sly. Workshop Participant: T-cell immunity to vaccine antigens in human infants - NIAID International Workshop on RSV, Washington, June 2002.
PD Sly. Summary/lessons from measuring peripheral lung mechanics in mice. 4th International Congress of Pathophysiology, Budapest, July 2002.
PD Sly. Emerging Trends in Paediatric Asthma. Indian Academy of Paediatrics, Bangalore, January 2002.
PD Sly. Environmental Threats to the Health of Children. World Health Organisation, Bangkok, March 2002.
PD Sly. Bronchoalveolar lavage: cells and cytokines. American Thoracic Society, Annual Scientific Meeting, Atlanta, May 2002.
PD Sly. Assessment of Pulmonary Function in the Mouse. American Thoracic Society Postgraduate Course, Atlanta, May 2002.
PD Sly. Structure/relationships in the developing lung: is lung volume a measure of alveolar surface area in infants? (Postgraduate Course) European Respiratory Society, Stockholm, September 2002.
PD Sly. Difficult to treat asthma and bronchiectasis (Grand Round). European Respiratory Society, Stockholm, September 2002.
SM Stick. George Polgar Visiting Professor. Children's Hospital of Philadelphia, 2002
SM Stick. Asthma Australia Conference, 2002
SM Stick. Role of environmental pollutants in primary prevention of allergic disease. World Allergy Conference, Vancouver, 2002.

Division of
Molecular Biotechnology

Overview

The Division of Molecular Biotechnology conducts molecular analyses into the development of childhood disease and the use of recombinant DNA technology for experimental investigation, and the development of diagnostic and therapeutic application. The principal research theme has been the causes, treatment and prevention of allergic disease and childhood asthma, especially the development of allergen vaccines and improved immunotherapy. The projects listed below are currently being studied.

• The molecular characterisation of house dust mite allergens and the determination of their importance in allergic disease. Recent investigations have concentrated on high molecular allergens and genetic polymorphisms.

• The production of recombinant allergens and allergen formulations and arrays for the treatment and diagnosis of allergic disease.

• Molecular cloning of cat allergens and immune responses to recombinant cat allergens during peptide and allergen-specific immunotherapy.

• The interaction of allergic responses to mixtures of allergenic and non-allergenic molecules.

• Experimental desensitisation models to test the mechanism and efficacy of novel immunotherapy especially with molecular constructs of isolated B and T-cell epitopes.

• Peptide mimetics for the representation of complex allergens and allergen epitopes and for improved agonists and antagonists of ligand-receptor interactions.

A major undertaking in 2002 was to improve the production of a panel recombinant mite allergens. A collaboration with Professor Valenta in Vienna showed that IgE binding to microarrays of the allergens was able to identify cross-reactivities and a distinct class of multi-sensitive patients. Microarray diagnosis could thus have a critical

application. The panel of mite allergens was also used to measure IgG subclass antibodies which, as shown for cat allergy, can identify the presence of modifying effects on allergic sensitisation. Allied studies on T-cell cytokines are being conducted to explore the interaction of responses to different allergens.

Comprehensive molecular cloning of cat allergens has been initiated so that more complete information can be obtained from the clinical trials with peptides of the major cat allergen Fel d 1 being conducted (elsewhere). Recent reports show a similar analysis of the interaction of responses to allergens can be made for the modulation of allergic responses induced by high dose exposure to cats. As part of the study of cat allergy it was shown that allergic subjects produce IgE antibody to a cat protein with potent inter-species inflammatory activity, The protein called EN-RAGE is inhaled in sufficient quantities to induce antibody so it should also mediate its pharmacological effects which include the recruitment of immune cells, the activation of endothelial cells and the induction of cytokines. The concept that the inhalation of EN-RAGE can induce allergic or protective responses is being investigated.

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

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

Allergic responses are regulated by cellular interactions and cytokines but the direct measurement of these functions is not fully informative for the accumulative effects of chronic exposure. A major initiative has therefore been to produce purified recombinant antigens and allergens with high serological activity and to develop IgG subclass antibody assays. Human IgG1 antibodies result from Th1 type immunoregulatory mechanisms induced by infections and IgG4 antibodies result from the Th2 type responses found in asthma. There are however different types of Th1 and Th2 responses and, relevant to the studies here, the Th2-dependent IgG4 and IgE antibodies are often produced independently. A solid phase microtitre plate immunoassay was used to measure antibodies with time resolved immunofluorescence assays (DELFIAs). Approximately 50 % of mite-allergic subjects produced IgG4 antibodies to the allergens Der p 1, 2, 5 and 7 with few responses to the less allergenic Der p 8. The most frequent responses were to the major Der p 1 and Der p 2 allergens. Non-allergic subjects did not have the Th1-type IgG1 antibodies and only occasionally had IgG4 antibodies, usually to Der p 5 or 7 and not the major allergens. In the allergic population, the IgG4 antibodies to a particular allergen were primarily only found when the subject also had IgE antibody. Antibodies to the non-allergenic mite ferritin heavy chain were frequently produced by both allergic and non-allergic subjects and were of the IgG1 isotype in both populations. High titres of IgG1 antibodies to the OMP6 outer membrane protein of the bacteria Haemophilus influenzae were found in the sera of allergic and non-allergic subjects. IgG4 antibodies were found in fewer patients but interestingly were more frequent in sera from allergic subjects.

In conclusion the results show that non-allergic subjects did not have Th1-type of IgG responses and that less important allergens did not induce Th1 responses. Modified Th2 responses were not frequent in non-allergic subjects but could be present in a small number of individuals, and interestingly to the less allergenic allergens. The increased IgG4 antibodies in allergic subjects to bacterial antigen OMP6 provide the first evidence of cross or co-regulation of responses to allergens on other mucosal antigens.

Regulatory and effector specificities in T-cell responses to house dust mite allergens

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 by their propensity to elicit large IgE and Th2-cytokine responses there is still conjecture about the relative importance of the many other lesser IgE binding proteins. A particular aspect is the potential of effector or regulatory interactions of responses between allergens. The positive potential interactions of responses to allergens has been evident from repeated observations that T-cell responses to house dust mite extracts are usually higher than responses to major mite allergens, even though the individual constituents of the extracts are present in sub-optimal doses. The possibility that regulatory interactions might occur was indicated by observations that the less allergenic proteins can induce larger Th1-type protective responses than major allergens, particularly in non-allergic individuals. This is now being investigated in in vitro culture systems with particular emphasis on the use of highly purified, endotoxin -free, non-fused recombinant house dust mite allergens. The results to date have confirmed that the less-major allergen Der p 7 induces higher Th1

cytokine responses (interferon-g) than the major allergens. In addition the combined response to Der p 1 and Der p 7 can result in a synergistic production of interferon -g and a decreased production of the Th2 cytokine IL-5. The experiments will target potential immunomodulatory effects of the regulatory cytokine IL-10 although, unlike studies with bee venom allergy, the data does not show it is over produced in the responses of non-allergic subjects.

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 (from mice immunised with chromatographically purified natural Der p 3). 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 of house dust mite allergy

WR Thomas, KL Mills, LA Hazell with K Westritschnig, G Pittner, S Vrtala and R Valenta, Dept Pathophysiology, University of Vienna

The diagnosis of house dust mite allergy is performed with extracts of dried house dust mites. These contain a complex mixture of different IgE-binding and non-allergenic proteins with different pathophysiological significance. Studies of microarrays of allergens from varying sources (tree and grass pollen, mites, insects, mammals) have demonstrated how the knowledge of IgE binding pattern can differentiate between cross reactivity and pinpoint the major specificities for allergic disease. This procedure of component-resolved diagnosis has the potential to provide a cost-effective advance in allergy diagnosis. To investigate the responses to mite allergens in more detail the laboratory has concentrated on improving the production, purification and IgE-binding quality of an array of purified and recombinant house dust mite allergens (Der p 1, 2, 5, 7, 8 and 10) and the non-allergenic polypeptide ferritin heavy chain. Two important conclusions have been obtained from the measurement of IgE-binding to the arrays of house dust mite allergens. Firstly a comparison of sera from Europe and Zimbabwe has shown how the tropomyosin allergen (Der p 10), with its evolutionary conserved amino acid sequence, reacts with IgE at high frequency in the African population with little reactivity in the European. It is probable that this allergen detects a cross reactivity with sensitisation to other sources. A second larger study of mite-allergic patients in Europe 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 almost predominantly directed to Der p 1 and Der p 2 and a population with IgE-binding to a broad spectrum of allergens. 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.

Recombinant high molecular weight allergens of the house dust mite Dermatophagoides pteronyssinus

KL Mills, M van de Reijden, C Gangell, NNR Chu, LA Hazell, N Malainual, BJ Hales and WR Thomas

Many of the lower and intermediate sized house dust mite allergens have been characterised but the study of higher molecular weight components has been incomplete. This is partially due to greater technological difficulties for both immunochemical and recombinant studies especially if the proteins are post-translationally processed or modified. High molecular allergens which are being studied are the 95 kDa paramyosin allergen (group 11) described by others in other mite species and the 177 kDa vitellogenin-like allergen (group 14) previously reported from the laboratory. Partial cDNA has been obtained for the paramyosin from D. pteronyssinus and this will be used for further cloning from a newly-constructed long-length cDNA libraries. Further analysis has been made on the 177 kDa allergen which is known to be processed into several small proteins in the mite egg, and based on the molecular biology of vitellogenins, will be lipidated and complexed with other proteins. A recombinant N-terminal domain constructed on the basis of structural data of lamprey vitellogenin has been shown to have IgE binding in 70% of mite allergic subjects. This confirms the data reported for the homologous protein of D. farinae in a Japanese population and points to the potential high importance of the allergen.

Cat EN-RAGE. A new allergen and a new concept

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

Most studies with cat allergy have been based on the premise that a single protein called Fel d 1 accounts for most of the allergen produced by this organism. The instigation of clinical trials for immunotherapy of cat allergy with peptides representing only Fel d 1 has however prompted the conduct of a more comprehensive study of cat allergens. This has been particularly directed at studying allergens produced from oral secretions. It is now revealed that not only do cat-allergic subjects respond to a number of previously unidentified cat allergens but also that people inhale a pharmacologically active inflammatory peptide from the cat. Screening a library constructed from mRNA from the cat tongue (to include von Ebner glands) resulted in the isolation of cDNA clones encoding a 9 kDa polypeptide homologous to human and bovine EN-RAGE showing, 74 and 90% amino acid identity respectively. EN-RAGE (Extracellular Newly identified ligand for the Receptor for Advanced Glycation Endproducts) is a inflammatory mediator which is produce d by many types of leucocyte and which has many inflammatory effects including chemotaxis of inflammatory cells, the induction of adhesion molecules on the endothelial and inflammatory cells and the induction of inflammatory cytokines by the activation of the NF-kB pathway. As shown for bovine EN-RAGE it has potent activity across species barriers. Since EN-RAGE is produced in sufficient quantity to induce IgE in 40% of cat allergic patients it is hypothesised that it also can be pharmacological active and can be a factor in modifying immune responses to high dose exposure to cats.

Production of recombinant peanut allergens

KL Mills, LA Hazell and WR Thomas in collaboration with A Bosco and PG Holt, Cell Biology

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 responses of T-cells from peanut-allergic subjects to the major peanut allergen Ara h 2 are now being studied by gene expression analysis. It is important for the food industry and the severe nature of the response may help uncover basic molecular mechanisms of sensitisation. In order to expedite the high reproducibility required for microarray analysis a highly purified major allergen is being produced in the quantities required for repeated investigations. The known sequence of Ara h 2 has been used for PCR cloning of cDNA encoding the polypeptide sequence reported in the literature and a new isoform with a 12 amino acid insertion. The expression and purification of these polypeptides in Escherichia coli and Pichia pastoris is being studied.

Model allergen mimotopes and B-cell immunomodulation

SR Gunn, JC Lenzo, KL Mills, LA Hazell and WR Thomas with PM Watt, Children's Leukaemia and Cancer Research

Mimotopes are peptides which mimic the shape of an epitope on an antigen. Typically they are selected from phage-display peptide libraries by monoclonal antibodies to the target antigens. The studies here have used monoclonal antibodies to a model allergen and to the FLAG peptide (YTKDDDK) to isolate mimotopes which can be used to test the therapeutic efficacy of modulating responses to allergens via B-cell epitopes. IgG1 monoclonal antibodies have been prepared against a cysteine protease protein allergen and used to test their effect on the allergic sensitisation mice. A pool of 4 monoclonal antibodies given in high concentration was unable to inhibit the development of IgE antibodies and eosinophilic inflammatory responses, or when given only before lung challenge the effector phase of the eosinophilic inflammation. Mimotopes isolated by phage display from combinatorial random peptide libraries are being tested for their ability to modify allergic responses and monoclonal antibodies of different isotypes are being isolated. Given that there is no information on allergen immunotherapy directed to B-cell epitopes, and that immunisation protocols for mimotopes need to be developed, studies on the defined FLAG epitope have been instigated. An anti-FLAG monoclonal antibody has been used to isolate phage expressing mimotopes (or epitopes), most of which have an amino acid sequence similar to the canonical FLAG sequence. Experiments have been conducted to induce antibodies to FLAG peptide in mice to optimise B-cell responses to a determinant which is known to be effective as a small peptide and can be readily studied by peptide ELISA and peptide inhibition. IgG antibody responses were elicited after repeated injection of phage into mice but the titres clearly indicated that more efficient protocols were required. Studies are continuing to improve the immunisation and to ascertain the effect of the immunisation on allergic responses to the recombinant allergen Der p 2 produced with a FLAG tag.

Phylomers: a diverse source of peptides for page display

TK Heinrich and WR Thomas with PM Watt and R Hopkins, Children's Leukaemia and Cancer Research

A new strategy for producing agonist and antagonist peptides is being tested. Here random DNA fragments from a collection of 18 genomes from a diverse range eubacteria and archaebacteria have been prepared and

cloned stochastically for the expression of peptides in a phage display system. The strategy is designed to test the ability of a diverse but a non-random and biologically-selected library of peptides to provide stable ligands. Incorporated into the strategy is use of genomes from organisms which a have been sequenced so that the binding (or non-binding) of peptides with homologous sequences can be compared. Peptides from the same sequences of homologous proteins from a range of organism can also be examined in follow-up experiments and now that the genomes of over 60 bacteria have been sequenced the system has increasing bioinformatic potential. The DNA fragments were generated by a combination of random PCR and restriction enzyme digestion in strategy designed to produce overlapping 100-200 bp fragments which have the potential to encode peptides of 30-60 amino acids. A key technical hurdle was to develop the amplification procedures to produce an unbiased array of fragments with desired size. This required considerable modification of whole genome amplification techniques. The protocols developed can however now be applied to the full range bacterial genomes even when only small amounts of DNA are available. The fragments were cloned into the T-select T7 bacteriophage phage display vector for expression in both orientations and all reading frames. A library of the expected number of fragments 2 x 107 has been constructed and clones have been sequenced to verify the size of the inserts and the random representation. Panning experiments with anti-FLAG monoclonal antibodies have been used to establish and authenticate an affinity purification process. The system is now being used to isolate allergen mimetics.

Experimental sensitisation and immunotherapy

JC Lenzo, AG Jarnicki and W. R. Thomas with PG Holt, Cell Biology

Chronic inhalation of antigens has repeatedly been shown to induce transient IgE responses followed by a tolerance to further sensitisation. In the course of a systematic investigation of possible conditions for inducing sensitisation by mucosal exposure it was found that intranasal administration of a cysteine protease enzyme homologous to the mite allergen Der p 1, induced prolonged and boostable IgE responses. Further study has now shown that the mice sensitised with this allergen produce a lymphocytic and eosinophilic lung infiltrate on intranasal challenge and Th2 cytokine. Enzymatic activity was not absolutely required for the sensitisation but could under some circumstances be stimulatory or inhibitory. Other allergens do not induce these responses even when administered at the same time as the cysteine protease. The T-cell epitopes for the response to this allergen have been defined and the model has been used to study immunotherapy. Successful prophylactic immunotherapy has been achieved with a standard protocol which reduces IgE and eosinophilia.

Staff and Students

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Nat Malainual BSc MSc PhD candidate
Kristina L Mills BSc Hons PhD candidate
Nora N.R. Chu BSc contract student

Theses Passed

Nat Malainual. Human immune responses to house dust mite allergens. PhD University of Western Australia.

External Committees

WR Thomas. Chairman NHMRC Grant Review Panel of Inflammation, Allergy and Haematology
WR Thomas. Chairman International Union of Immunological Societies/WHO Allergen Nomenclature Committee
WR Thomas. Member, Asthma Foundation of Western Australia Executive

Invited Presentations

WR Thomas. Recombinant allergens. New diagnostic and therapeutic products. American Academy of Allergy Asthma and Immunology. New York, USA
WR Thomas. Allergology from past to future developments. International Symposium, Vienna, Austria

Division of
Population Sciences

Overview

The Division of Population Science comprises 144 staff and students engaged in a diverse range of scientific activities. The research program for the Division is focussed principally in three broad areas: documenting the burden of disease in children and young people, assessing the causal pathways that lead to disease or health, and assessing 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, 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, developmental disorders and innovative methodologies (Figure 1).

	Burden of disease	Causal pathways	Science of prevention	Policy and practice
Asthma and atopy	*	*	*	
Cancer				
acute lymphoblastic leukaemia		*		
Infectious diseases				
otitis media	*	*	*	
meningitis	*	*	*	*
Developmental disorders				
autism	*	*		
birth defects	*	*	*	*
cerebral palsy	*	*		*
intellectual disability	*	*		
mental health disorders	*	*	*	*
newborn encephalopathy	*	*		
newborn hearing screening	*			*
Rett syndrome	*	*		
specific language impairment	*	*		
Innovative methodologies				
Aboriginal health research	*	*	*	*
genetic epidemiology	*	*		
geocoding/mapping	*	*	*	*
population databases	*	*	*	*
population surveys	*	*	*	*

During 2002 several achievements should be noted.

• Senior researchers from within the Division, in collaboration with the School of Population Health, University of Western Australia, were successful in winning the first NHMRC Public Health Capacity Building grant. The aim of this grant is to provide salary support to grow the research capacity of more junior post-doctoral researchers and establish them in their chosen fields, under the guidance and mentorship of senior colleagues with strong track records.

• The Curtin University of Technology Centre for Development Health (CDH), located at the Institute, was awarded a $US5 million grant from the United States National Institutes of Health to study the genetics of Specific Language Impairment (SLI) in children. This is a collaborative grant lead by chief investigators Professor Mabel Rice (University of Kansas) and Associate Professor Kate Taylor and Professor Stephen Zubrick at CDH.

• In the New England Journal of Medicine we reported on the high level of birth defects in babies born to mothers treated for infertility using intracytoplasmic sperm injection (ICSI) and in vitro fertilisation (IVF) compared to naturally conceived infants. This created considerable international interest.

• A proposal to set up an Australian National Cerebral Palsy Register was 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. This will be followed up with meetings of State and Territory representatives in 2003.

• The field work for the Western Australian Aboriginal Child Health Survey concluded and data analyses commenced in earnest. This project has involved 11,300 family members with intensive interviews with the carers of over 5,000 Aboriginal children under the age of 18. This is the first large scale random sample of families with Aboriginal children that seeks comprehensive psychosocial and health information on Aboriginal children and carers. Planned over several years, the survey has achieved an 83% participation rate of eligible families.

• We are setting up an international web-based Rett syndrome database to provide important information about the syndrome worldwide. This will serve as a model for data collection in rare disorders.

• The Cancer Epidemiology section was successful in winning a 5-year NHMRC project grant to undertake a national case-control study into the causes of acute lymphoblastic leukaemia in children. This study will involve using epidemiological methods to link genetic, dietary and environmental data to investigate possible causal pathways for the most common childhood malignancy. The research team is multidisciplinary and includes expert cancer and molecular biologists, epidemiologists, biostatisticians and paediatric oncologists from around the country.

• We commenced work on a major national suicide prevention initiative. Strategies include those aiming to build people's resilience through to protecting people at high risk.

More detailed reports of these and other Division activities may be found on the following pages.

Aboriginal Health

Kulunga Research Network

Daniel McAullay, Jacinta Johnson, Kate Butler and Heather D'Antoine

The Network was established in 1999 as a joint initiative between the Western Australian Aboriginal Community Controlled Health Organisations (WAACCHO) and the Institute. The primary philosophy of the Network is to act as an advocate for Indigenous children and families in Western Australia.

The Network encompasses all of the Aboriginal health projects conducted by the Institute and collaborative partners. Some of the projects include the Western Australian Aboriginal Child Health Survey, the Bibbulung Gnarneep 'Telling our Stories', the Kalgoorlie Otitis Media Study, Swimming Pool Study and the survey of health professionals about Fetal Alcohol Syndrome.

The WAACHS survey and the Fetal Alcohol Syndrome (FAS) survey are in the process of analysing data. The first report for the WAACHS survey will be available within the next six to eight months and the report for the FAS survey by the end of the year. Data collection continues for the Kalgoorlie Otitis Media Study, the Swimming Pool Study and the Bibbulung Gnarneep 'Telling our Stories'.

The staff at Kulunga have participated in the development of 'The NHMRC Roadmap: A strategic framework for improving Aboriginal and Torres Strait Islander Health through Research' and have played a key role in developing the Guidelines for ethical conduct in Aboriginal and Torres Strait Islander Health Research which have now been accepted by the NHRMRC.

The Network hosted a successful Information Forum on 'Research and Reconciliation'. This forum is held in honour of Dr. Joan Winch. The keynote speakers debated the issue on how there is a place for reconciliation in Aboriginal research.

The Rio Tinto Initiative is a major initiative involving the Institute, the Kulunga Research Network, Rio Tinto, the Commonwealth government through the Alcohol Education and Rehabilitation Foundation, and the State governments for Western Australia, Northern Territory and Queensland. The key stakeholders have now signed off to the Initiative.

A key focus for Kulunga for the next twelve months is to ensure information about Aboriginal research is taken back to the Aboriginal community and to other key stakeholders including government and non-government organisations. Kulunga and the Institute are currently negotiating with the Western Australian and Commonwealth government for funding for the Communication Strategy for the WAACHS. Funding for this strategy will ensure that the Aboriginal community, Western Australian and Commonwealth government are informed about the WAACHS survey.

Impact on health of children and adolescents of introduction of swimming pools into remote Aboriginal communities

D Lehmann, M Tennant, D Silva, D McAullay, J Johnston, K Butler in collaboration with I Nannup (Derbarl Yerrigan Health Service), P Richmond (Department of Paediatrics, University of Western Australia), H Coates and F Lannigan (Princess Margaret Hospital), J Stuart (Department of Paediatrics, John Hunter Hospital, Newcastle, NSW), B Currie (Menzies School of Health Research, Darwin, Northern Territory), S Weeks (Disability Services Commission).

Swimming pools were opened in the remote Aboriginal communities of Jigalong,

Burringurrah and Mugarinya in Western Australia in September 2000. The pools were jointly funded by the Western Australian Department of Housing and Works, the Department of Sport and Recreation and the Lotteries Commission. The Royal Life Saving Association is managing three of the pools. The Institute for Child Health Research was asked to evaluate the impact the pools may have on the health of children in two of these communities (Jigalong and Burringurrah).

Children have been assessed to see whether there have been any changes in the burden and severity of ear and skin disease. The communities adopted a policy of "no school no pool" or "school means pool" and so we have been monitoring school attendance. Investigators also interviewed members of the communities to obtain opinions on any social changes that may have occurred as a result of the pools.

Children in these communities were examined by a paediatrician before the pools were opened (July-August 2000) and at 6 monthly intervals until March 2002. A hearing test and tympanometry was done on the first and fourth visit. Pictures of eardrums have been taken using a video-otoscope and stored on computer file. These are now being examined independently by three specialists.

Swabs of some skin sores have also been collected to obtain information on the strains of Group A streptococcus circulating in the communities and to determine whether there is any change in strain distribution after children have swum in the pool. We have recorded information on health clinic visits for all children in the study throughout the study, beginning one year prior to construction of the pool. These data will assist in assessing whether there has been any change in the number of courses of antibiotics that have been prescribed.

Preliminary results are encouraging. There has been a decline in the number of children with perforated ear drums and a marked reduction in the number of children with skin sores. In one community there are reports of reduced crime rates. Residents were very happy with the added sporting and social venue for their children. Ongoing funding from the WA Department of Health and the WA Department of Housing and Works will enable us to monitor the sustainability of this innovative intervention.

Western Australian Aboriginal Child Health Survey

SR Zubrick, S Eades, SR Silburn, D Lawrence, F Mitrou, H D'Antoine, T Wilkes, K Butler, J Johnston, and D McAullay

In August 2002 we completed all of the field work for the Western Australian Aboriginal Child Health Survey. This is the first attempt to gather a fully representative random sample of families with Aboriginal children and to seek information on their health and well-being. The project is being conducted under the auspices of the Kulunga Research and Training Network with funding from Healthway, the WA Lotteries Commission, Rio Tinto Aboriginal Foundation and several State and Commonwealth Departments. The Australian Bureau of Statistics has been a major partner providing consultancy services as well as out-posted staff and support for all aspects of the survey development and execution of the field work.

Over 130 screeners and interviewers (60% of whom have been Aboriginal) enumerated a selection of 761 census districts from across Western Australia, listing 166,287 dwellings and randomly sampling 2,288 families with Aboriginal children under the age of 18 years. A total of 1,999 (83%) of these families have consented to participate in the survey. Intensive interviews have been conducted gathering information on 5,289 children with separate interviews on 1,070 young people

aged 12-17, and additional interviews being now complete on 3,157 carers of these children. At present we have school data on 2,452 of these children.

During 2002 intensive data screening, cleaning, editing and validation has been complete. With consent of the carers, the survey data have been linked to population registers maintained by the Linked Data Unit of the Western Australian Department of Health. Over 96% of families consented to data linkage and high rates of linkage (97%) were achieved. This will allow careful study of the reliability and validity of many of the survey items and adds a longitudinal perspective to the data in the form of enriched maternal and child health information. The survey results will first be communicated to participating Aboriginal communities in a culturally appropriate form with the assistance of the project's Aboriginal Steering Committee and the Kulunga Research and Training Network. To this end a pilot study, funded by the Health Promotion Foundation of Western Australia, was undertaken during 2002 in the Fitzroy Valley Aboriginal communities to assess community recall of the survey, establish interests and needs for survey findings, and explore presentation formats and opportunities. Collaboration with the National Key Centre for Social Applications of Geographic Information Systems (GISCA) in South Australia commenced in order to develop an index of remoteness and accessibility to services appropriate for use in analyzing survey data. Reports on non-response characteristics of the sample, data reliability and quality, and cost analysis are being prepared for release in 2003. The first report on survey findings is being planned for November 2003.

Bibbulung Gnarneep

H D'Antoine, K Hunt, H Lette, R Martin, J Nannup, L Slack-Smith, E Tursan d'Espaignet

The Bibbulung Gnarneep project started in the mid-1990s and can be divided into three phases. The first phase involved two sub-projects – the conduct of a quantitative longitudinal survey of infant care practices on 280 Aboriginal women following them from pregnancy to 18 months post delivery; and, the conduct of a qualitative study of services provided to the mothers of young Aboriginal children. This first phase has led to two PhD theses (one completed and submitted for examination, and the second thesis almost completed and expected to be submitted for examination in 2003).

The early results from the first phase led to an action-research project about a Home Visiting Program aimed at supporting Aboriginal families achieve the health and well-being potential of their children. An important focus of the project in 2002 was the skills and support that Aboriginal Health Workers need to enable them to provide adequate and effective support to the families. Two Aboriginal women were employed to visit and provide support to the families of about 40-50 Aboriginal infants.

An informal review of the Home Visiting Project was undertaken in August 2002. This review indicated that changes beyond the control of the research team had adversely affected the study. At design stage, it was envisaged that the project would focus on the Midland/Swan Valley area of the Perth Metropolitan Area. Arrangements had been made for the local Aboriginal Medical Service (Derbarl Yerrigan) and local GPs to refer pregnant women to the project. With the unexpected closure of Derbarl Yerrigan (the major source of referral), the project relied more and more on referrals from other sources including word of mouth, and from social workers based at the King Edward Memorial Hospital. These changes from the original protocol resulted in the families being located over the whole of the Perth Metropolitan Area substantially increasing costs of the research project.
As part of the review, a series of semi-structured interviews were conducted with the two

visiting health workers to assess the skills and support that they felt were necessary for a home visiting program. These have been identified as clarity of purpose, time needed to develop meaningful relationships, support for workers, and training for workers in areas such as communication skills, assertiveness training, and advocacy skills.

The second lesson from the review was that for a home visiting program to be effective, activities needed to focus on the aspirations, needs and strengths of the main care givers rather than on the children. This lesson together with the diffusion of the project across the whole of the Perth Metropolitan Area led to a change in the project towards the design of a qualitative study to capture the stories of the mothers on their aspirations and strengths. Much of the second half of 2002 was taken up with training of the Aboriginal Health Workers in qualitative study designs, and in data collection techniques. This new phase of the Bibbulung Gnarneep Project known as the 'Sharing Stories Phase' aims to work together with mothers and carers of Aboriginal children to present a more complete view of life in Aboriginal communities.

An important component of the project involved Institute support for professional and personal development of the two Aboriginal staff members on the research project. The first, an Aboriginal Health Worker with substantial mental health service delivery experience was assisted in undertaking courses in preparation for tertiary university studies. Substantial resources were also made available for the second worker to undertake the Aboriginal Health Worker Primary Health Care Level IV Certificate.

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, K Carville, C Jeffries-Stokes in collaboration with Bega Garnbirringu Health Services Aboriginal Corporation, Ngunytju 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, J Kyd (Faculty of Applied Science, University of Canberra), J Bowman, D Smith (Pathcentre), D Murphy (Public Health Bacteriology Laboratory, Brisbane), A Leach, B Harrington (Menzies School of Health Research, Darwin).

We have completed recruitment of 180 non-Aboriginal and 100 Aboriginal children into a study investigating the most potent factors predisposing children to otitis media (OM). 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. Data on demographic, socioeconomic, environmental risk factors are being collected. Nasopharyngeal aspirates (NPAs) are collected to investigate upper respiratory tract bacterial and viral carriage. In addition to serotyping, molecular typing of pneumococci by PFGE is being done. Haemophilus influenzae isolates are being ribotyped at Menzies School of Health Research, Darwin. Mucosal immune status is being investigated by collection of saliva at each follow-up and a breast milk sample at first visit. Ear health is assessed by tympanometry at each follow-up visit from age 4 months onwards, by an ENT specialist on three occasions and an audiologist assesses hearing once in the second year of life. All Aboriginal babies are now offered a pneumococcal conjugate vaccine (Prevenar ™) as part of the routine immunization schedule and through a donation from Wyeth we were also able to offer this vaccine to non-Aboriginal babies participating in this project. We will thus be able to investigate the impact of this vaccine on upper

respiratory tract carriage in this population.

Fifty Aboriginal and 75 non-Aboriginal children have reached the age of 2 years. 53% and 24% of Aboriginal and non-Aboriginal children, respectively, have had OM before age 4 months; 20% of Aboriginal children have had a perforated tympanic membrane. Overall isolation rates from 164 NPAs collected throughout the first 2 years of life from Aboriginal babies were: 47% Streptococcus pneumoniae (Pnc), 45% Moraxella catarrhalis (Mc), 36% Haemophilus influenzae (Hi) and 38% Staphylococcus aureus (Sa); equivalent figures for 264 NPAs from non-Aboriginal babies were: 22% Pnc, 23% Mc, 11% Hi and Sa 43%. By age 2 months, Pnc had been isolated from 41% and 12% of Aboriginal and non-Aboriginal children, respectively. Preliminary analysis by BMedSci student, Claire Gordon, suggests that passive smoking, maternal unemployment and cold weather put children at increased risk of OM irrespective of ethnicity. Findings from this study will be used to develop appropriate interventions for prevention of OM, which can seriously affect childhood development, school performance and subsequent social and economic well-being.

Investigation of smoking and child feeding practices among the Aboriginal population in the Goldfields

D Lehmann, C Jeffries-Stokes, F Nichols, D McAullay, in collaboration with HC Coates, S Weeks, Bega Garnbirringu Health Services Aboriginal Corporation, Ngunytju Tjitji Pirni Inc.

In a recent study among Aboriginal people in the Kalgoorlie-Boulder area we have found that (1) there is a limited understanding of the disease process (which is not surprising given the frequently asymptomatic nature of the disease until the ear drum has perforated and there is a visible ear discharge) (2) maternal tobacco smoking is associated with OM at a young age, (3) there is a lack of awareness that tobacco smoking puts children at risk of OM and (4) exclusive breastfeeding is of short duration. With a grant from Healthway we are addressing these issues in collaboration with the Aboriginal community through workshops as well as in-depth 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. Thus we aim to develop an awareness program at the same time as describing tobacco use, the exposure of babies to passive smoking and infant feeding practices in more detail. This information will assist in developing appropriate intervention programs for the Goldfields.

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

D Mak (Kimberley Public Health Unit), K Sivwright (data coordinator, Institute for Child Health Research), D Lehmann (associate investigator, Institute for Child Health Research).

The project aims 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. Pre- and post-operative clinical information from Aboriginal children undergoing tympanoplasty are documented. One component of the study includes follow-up of Aboriginal patients aged 15 years or less who have undergone tympanoplasty since 2000 and another component consists of the long-term follow-up of Aboriginal children aged less than 15 years old who had tympanoplasty done in the Kimberley Region 5 years earlier. K Sivwright has been collating the data at the Institute for Child Health Research. The study will provide information to assist clinicians in deciding when and on whom to operate and should also assist in health services' planning regarding ear health.

Cancer

Cancer epidemiology

E Milne, C Bower, N de Klerk, U Kees, K Peirce 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).

The Childhood Cancer Epidemiology program commenced in 2001. Researchers in this section were successful in winning a 5-year (2003-2007) NHMRC funded national case-control study into the causes of childhood acute lymphoblastic leukaemia (ALL) 2003-2007, and most of the developmental activities were completed in 2002. The study is funded from January 2003. Its primary hypothesis 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 over the first 3.5 years of the study. They will be identified through all the paediatric oncology centres in Australia. Three controls will be selected per case, frequency matched to cases by age (in years), gender and State of residence, a total of 1,050. Three controls per case are required to ensure that the study is sufficiently powered. A contracted survey research organization (SRC) will identify control families by random digit dialling (RDD). This will involve 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 will then be linked to a name and address wherever possible (ie, where the phone number is directory-listed), and a letter outlining the study will be pre-mailed to those addresses. The phone numbers will then be dialled, and parents expressing interest in participating in the control group will be mailed information sheets and consent forms, describing the purpose and nature of the study and informing them what would be involved should they choose to participate.

Data will be collected from participants by parent questionnaires and molecular biological measurements in blood and buccal swabs. A computer-assisted telephone interview will record birthplace and ethnic origin of parents, use of nutritional supplements, and exposure to environmental chemicals. Interviewers will be blinded to subject status. A food frequency questionnaire will be mailed to participants and used to assess dietary folate intake of the parents preconception, the mother during pregnancy, and the child since birth, taking into account foods fortified with folate as they

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

Other research activities:
Causal pathways in childhood cancer will be modelled through the collection and analysis of genetic, environmental and social data on children and their families.

Linkages between the Cancer Registry and the Maternal and Child Health Database will also be used to examine medical, social and familial factors in the aetiology of childhood cancer.

Developmental Disorders

WA Register for Autism Spectrum Disorders

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

Over the last decade, the prevalence of autism and the related spectrum of autism disorders has increased worldwide. To address the issue in WA, a prospective register for all newly diagnosed cases was initiated in 1997, led by Associate Professor Joachim Hallmayer, and began to formally collect data in 1999. It was established through the UWA Department of Psychiatry and Behavioural Science and was relocated to this Institute in March 2002. It is the only prospective statewide register for autism in Australia and is governed by an Advisory Committee that consists of representatives from the major autism diagnostic and service delivery bodies in WA. It has attracted national and international interest and has been approached by media, service providers, researchers, families, and therapy groups for information. Our current knowledge of the aetiology and general nature of autism is limited, and it is hoped that this resource will assist in understanding some of the unknown aspects of the disorder.

Birth Defects

Neural tube defects

C Bower, M Miller, J Payne, P Serna.

The major analysis of our Healthway-funded case control study has been completed. Only a third of women reported taking folic acid supplements in the periconceptional period. Folate from fortified foods was found to make an important contribution to the prevention of neural tube defects. Further analyses of the factors related to knowledge of folate and its association with birth defects, and to use of folic acid supplements, are now being undertaken.

Birth defects in children conceived by assisted reproduction

M Hansen, C Bower, E Milne, N de Klerk, S Webb (WA Department of Health), J Kurinczuk (University of Leicester).

Following on from our study showing a two-fold increase in major birth defects diagnosed up to one year of age in infants born after IVF and ICSI (intracytoplasmic sperm injection), we are extending our research to include birth defects diagnosed up to six years of age, cerebral palsy, intellectual disability, and admissions to hospital for children born after assisted con-

ception, compared with naturally conceived children.

We are also undertaking a systematic review of published studies of birth defects in infants born after IVF. A comprehensive search of the literature (using Embase, Medline, and Current Contents) was conducted to identify studies published since 1978. Studies were restricted to those examining the relationship between ICSI/IVF and birth defects for which there was a comparison group. A meta-analysis of the results of these studies was performed. A variety of methodological limitations was found in many of the published studies, including inappropriate comparison groups and small sample size. Many studies showed an increase in birth defects in ICSI and IVF infants.

Fetal alcohol syndrome (FAS)

C Bower, J Payne, Heather D'Antoine, D McAullay, E Elliott and A Morris (Australian Paediatric Surveillance Unit - APSU), E Haan (South Australian Clinical Genetic Services), S Leivers and C O'Leary (WA Department of Health), G Ridley (APSU), I Bucens (Royal Darwin Hospital), A Leversha (Community Paediatrics New Zealand), J Kurinczuk (University of Leicester).

FAS was first identified in the 1970s and has been described as a preventable tragedy. 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 utero. 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 from the USA and Canada range from 0.3-7.2 per 1000 live births. There are no national data for FAS in Australia. Data from the WA Birth Defects Registry show a rate of 0.18 per 1000 births, but this is considered to be an underestimate of the true prevalence.

With a research grant from Healthway, we are conducting a study on FAS in Australia, which has three objectives:
1. To ascertain the incidence of FAS diagnosed by child health specialists in Australia, using the well-established and highly successful Australian Paediatric Surveillance Unit;
2. To collect information from health professionals to determine knowledge, beliefs, practices and needs in relation to FAS and alcohol in pregnancy; and
3. To collate contemporary Australian data on alcohol consumption in pregnancy.

The APSU surveillance has been operating for 2 years, and has another year of case ascertainment to go. We have conducted surveys of obstetricians, general practitioners, Aboriginal health workers, child and community nurses, and allied health professionals in WA. We had a 79% response to the surveys. Data analysis of the surveys and data collection for the third objective will be undertaken in 2003.

Trends in perinatal, infant and childhood mortality associated with birth defects

J Bourke, C Bower.

Studies on trends in perinatal, infant and childhood mortality have previously been undertaken in WA using data from the Maternal and Child Health Research Database (MCHRDB), however there has not been a study done specifically on mortality associated with birth defects. The objectives of this study were to analyse the data on the Western Australian Birth Defects Registry in order to identify trends in mortality associated with birth defects and the relative contribution to overall perinatal, postneonatal and childhood mortality over the period 1980-1998. Mortality by diagnostic category of birth defect was examined as well as factors affecting mortality associated with birth

defects, such as sex, race and geographical location. The proportion of cases where the birth defect diagnosis was made or improved at a postmortem examination was calculated. Deaths with birth defects were analysed by the diagnostic category of the birth defect and trends in mortality within individual birth defect categories over 1980-98 were investigated. The effects of terminations for birth defects on the mortality associated with birth defects as well as on the total population mortality were estimated.

Birth defects contribute heavily to perinatal , postneonatal and childhood mortality and the proportion of stillbirths with birth defects is increasing. The 2-fold increased risk of death associated with birth defects for Aboriginal children compared with Caucasian children is maintained in both rural and remote regions, indicating that factors other than access to health care may be important. The observed decrease in perinatal and postneonatal mortality associated with mostly multiple and nervous system defects is significantly reduced when the terminations of pregnancies with these defects are considered. Perinatal plus postneonatal mortality with chromosomal defects increased by 7.9% over 1980-98, however after including the estimated mortality associated with terminations of pregnancies with chromosomal defects, the increase was 104%. Although mortality associated with birth defects has decreased over 1980-98, probably due to improved medical and surgical care, part of this reduction is also due to increased prenatal diagnosis and subsequent termination as well as a possible reduction in nervous system defects due to increased uptake of periconceptional folate. Terminations with birth defects have an important effect on total population perinatal and postneonatal mortality and should be considered when looking at trends in perinatal and postneonatal mortality over time.

Birth defects in the offspring of non-Caucasian, non-Indigenous women in Western Australia

S Kwon, C Bower, D English (Cancer Council of Victoria)

Birth defects in infants born to non-Caucasian, non-Indigenous mothers in Australia have not been described in detail previously. Using data from the Western Australian Maternal and Child Health Research Data Base, an index cohort of all non-Caucasian, non-Indigenous mothers giving birth in Western Australia between 1985 and 1995 (n = 17,706 births) was compared with a 10% random sample of births to Caucasian mothers (n = 24,180) over the same period. Index mothers overall had 21% less risk of having a baby with birth defects (prevalence ratio 0.79, 95% confidence interval 0.72-0.86). The six major countries of birth of index mothers were Vietnam, The Philippines, Singapore, Malaysia, India and New Zealand. Mothers from Vietnam and The Philippines were significantly less likely to have a baby with a birth defect. The prevalences for the remaining four maternal countries of birth were not significantly different from that in the comparison cohort. The difference in risk of birth defects is unlikely to be due to confounding by maternal age, plurality, parity, socio-economic status, marital status or place of residence, but may result from ascertainment bias, or be real.

Speech and Language Development

K Taylor, S Zubrick, S Beveridge, P Dawes, M Epstein, M Stone, S Hoey, Mabel Rice (Kansas University), E Hagemann

Language impairment is a serious developmental health problem that has long-term consequences for academic, social and behavioural success, and adult employment opportunities. Specific Language Impairment (SLI) affects approximately seven per cent of children with otherwise normal development – normal hearing, normal intellectual abilities and normal physical development. But these children have difficulty with language - they are late in start-

ing to talk and continue to lag behind their peers into adulthood.

We do not know what causes SLI but recent twin and family studies suggest it may be strongly genetically determined.

The Looking at Language study will help us understand more about possible factors that influence language abilities in the preschool and school years. This study will collect valuable evidence from a population sample of Western Australian twins and single-born children aged between two and eight years, and their family members.

We are investigating possible genetic and environmental causes of language impairment using new and precise measures (markers) of young children's grammatical development and measures of environmental risk.

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 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 (figure 1). Figure 2 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

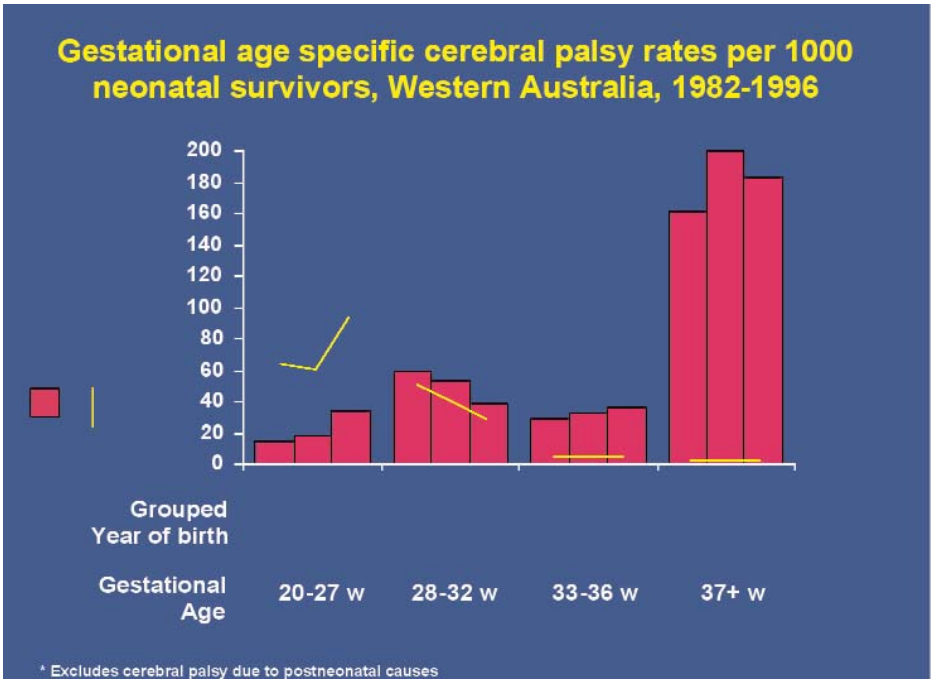

Figure 1

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

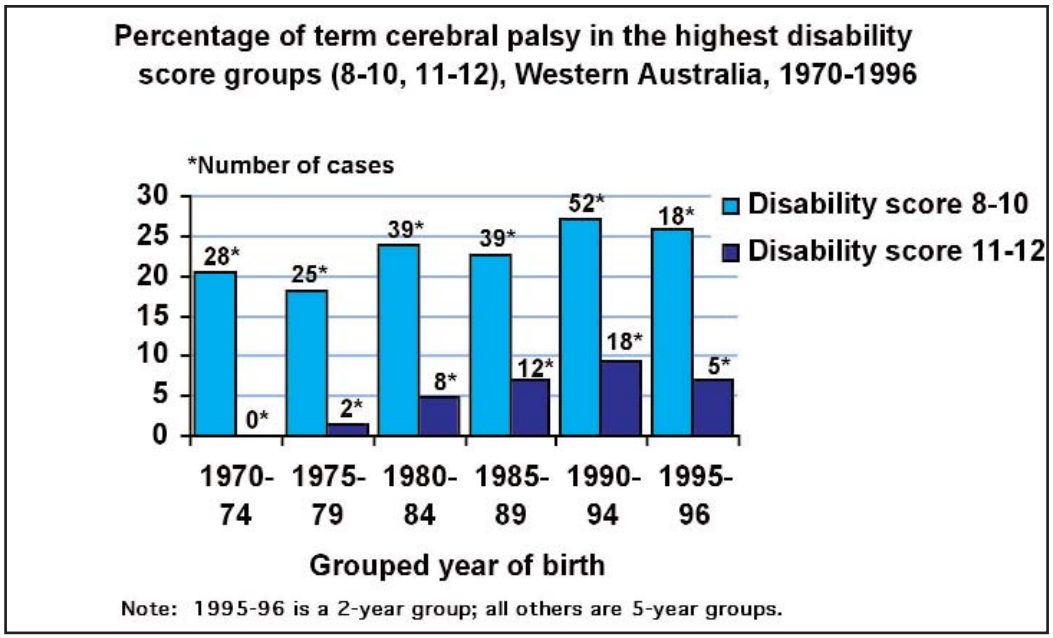

Figure 2

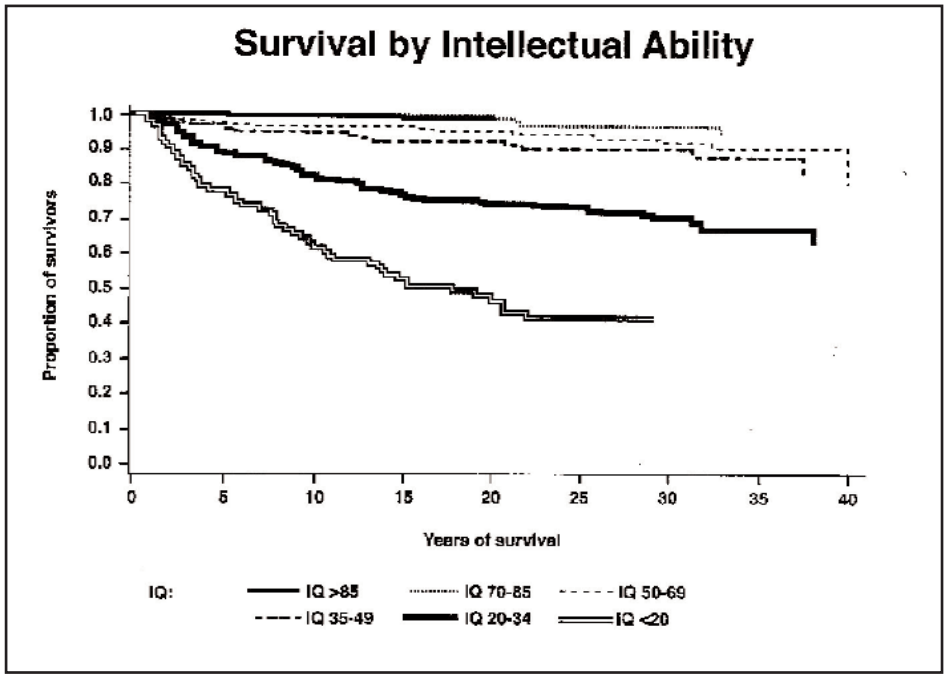

Figure 3

persons with cerebral palsy is their intellectual ability, as shown in figure 3.

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.

Figure 4 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

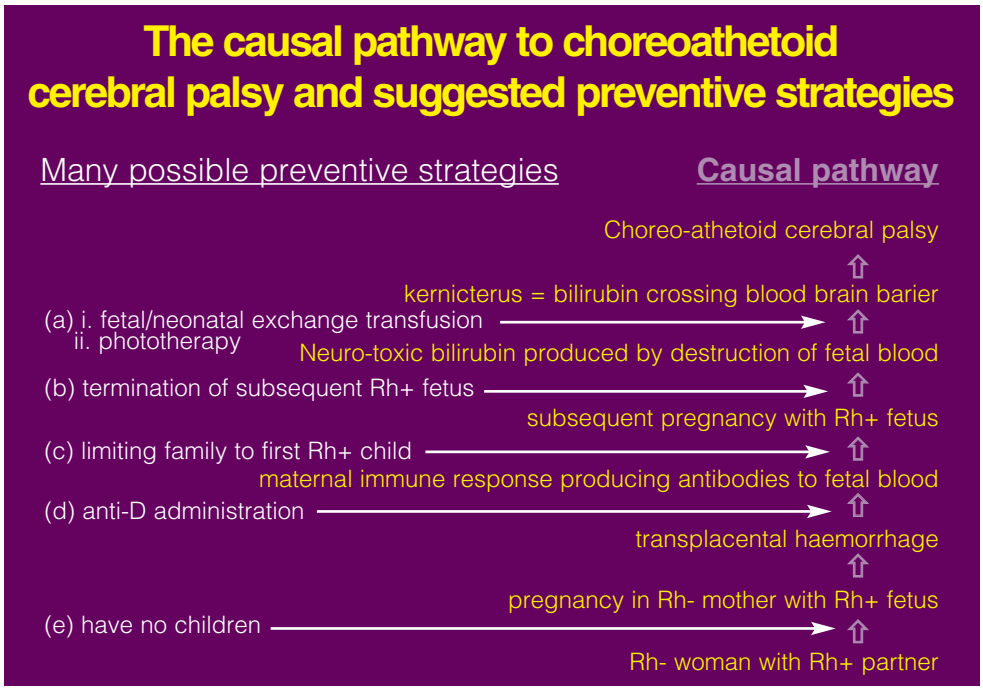

Figure 4

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
(d) finally 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 is expected to be complete by 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 and 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 cofetuses 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. These will be considered at follow-up meetings in 2003.

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 a flood of 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, J Crowhurst, R Henderson, A Hockey, L Nagarajan, K Naso, T Parry, J Silberstein, P Silberstein, M Tait, J Valentine, I Walpole, P Walsh.

Intellectual disability

H Leonard, C Bower, B Petterson, J Bourke, R Sanders (Department of Education), X Wen (Australian Institute of Health and Welfare)

In the international arena we contributed to a review of the epidemiology of neurodevelopmental disorders. Specifically we identified some of the problems and challenges associated with the epidemiological study of intellectual disability. These relate particularly to how intellectual disability is defined and classified and how definitions may change over time. We explained how important it was to take these changes into account as well as other differences in ascertainment and methods when comparing intellectual disability prevalence over time and place. Whilst we identified problems with existing etiological classification systems we also highlighted how advances in technology can provide new and efficient methods of data collection both by data linkage and by use of web-based methods to study rare diseases. We reviewed currently known risk factors for intellectual disability but hypothesised that a population strategy aimed at improving the social milieu surrounding the occurrence of individual risk factors might be the best preventive approach. We discussed the impact of biotechnological advances such as antenatal and neonatal screening and assisted reproduction on intellectual disability. However we also raised the issue of how inequalities in access to technology may impact on case identification and even have the potential to further widen inequalities. Finally we emphasised the importance of extending the use of epidemiological tools to study not only the prevalence and aetiology of intellectual disability but also its social, health and economic burden.

Locally in Western Australia we have been able to use Disability Services Commission data and educational sources with linkage to the Maternal Child Health Research Database to define a cohort of children born between 1983 and 1992 with an intellectual disability. We have shown that the prevalence was 14.3 per 1000, 10.6 per 1000 for children with mild or moderate and 1.4 per 1000 for those with a severe level of intellectual disability. Prevalence was greater in males (prevalence ratio 1.6) and in children of Aboriginal mothers (prevalence ratio 2.3). During 2002, we also continued to study the sociodemographic, antenatal and perinatal risk factors associated with the subsequent onset of intellectual disability. We are examining a wide range of variables including maternal age, marital status, ethnicity, pregnancy and delivery complications, baby's birthweight and condition at birth. Our analysis will provide important information on the determinants of intellectual disability-information not generally possible to gather elsewhere in the world.

An important landmark for the Institute in 2002 was the decision that as from mid 2003 the Intellectual Disability Database would be housed and managed from the Institute. This means that computerised data held at DSC have to be updated and made as complete as possible prior to this occurrence. At the same time a new system for ascertaining intellectual disability in Western Australia has to be set up. This work has been in process for the latter half of 2002 and has involved a comprehensive review of those cases not previously assigned

a medical diagnosis. The new system which will operate from July 2003 will provide another important addition to the network of linked population-based childhood databases coordinated from the Institute.

Mental Health Disorders

Foundations of Social and Emotional Wellbeing

A Williams

In April 2002 the Telethon Institute for Child Health Research was commissioned by the Department of Education to progress the initial workplan of the social emotional developmental continuum research project in partnership with the Department of Health, and develop user friendly educational resources to support and facilitate positive social outcomes of schooling.

Background

In August 1999 the Telethon Institute for Child Health Research was commissioned to research and develop a social emotional developmental continuum. It was envisaged that the final product would serve as a tool to assist schools in the promotion, assessment, and monitoring of academic, social and emotional wellbeing of students, and inform schools on evidence based interventions shown to be effective at preventing mental health problems.

Phase 1

The first phase of the research project articulated a social and emotional developmental continuum from conception to seventeen years of age. The product identified and described the influences of environmental resources that contribute to learning, health and wellbeing and provided the evidence base to guide the selection of promotion, prevention and early intervention strategies in home, school and community settings.

Phase 2

Phase 2 objectives progressed the workplan by reviewing the international and national evidence base on promotion and prevention interventions and programs, and refined the product in order to facilitate understanding and uptake into school communities

A mechanism was established internally within the Department of Education to support the consultancy and product development. A consultation working group comprising representation from Student Support Services, Monitoring Outcomes, Standards and Effectiveness in Education, and Curriculum Learning Areas informed the process, and consultants from the Centre for Excellence in Teaching were subcontracted to translate the research findings into user friendly teacher resources for use in pre primary, primary and secondary schools.

Phase 3

The Department of Education has recently refined the product through workshopping the material with Departmental staff and stakeholders and prepared the Social and Emotional Developmental continuum for publication and statewide dissemination in July 2003

Policy and practice implications

This research has contributed to informing the Health and Wellbeing Plan in Western Australian schools, the National Early Childhood Agenda, the Third National Mental Health Plan, as well as local community initiatives such as Smart Start in the Upper Great Southern Health Region, and the Smith Family's Learning for Life initiative in disadvantaged communities.

Newborn Encephalopathy (NE) Study

N Badawi (The Children's Hospital at Westmead, N.S.W.), JJ Kurinczuk (University of Leicester, UK), PA Alessandri, GN Dixon, S. Dragovic, K Dixon, FJ Stanley, S Silburn, SR

Zubrick, JM Keogh (Hornsby Ku-Ring-Gai Hospital, NSW), PR Burton (University of Leicester, UK), J Valentine (Princess Margaret Hospital).

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 subsequently followed the cases and controls longitudinally to ascertain developmental status of the children in their second year of life, and their later neurological, cognitive and behavioural outcomes. To date 14% 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. No controls have cerebral palsy.

A Griffiths Mental Development Scales assessment was performed on 190 cases and 443 controls at a mean age of 16 months. Four cases and two control children received alternative developmental assessments and one case was too disabled to be assessed with formal instruments. The developmental follow-up fraction was 81% of eligible cases and 79% of eligible controls.

Statistically significant differences were found between cases and controls for General Quotient (GQ) and all developmental subscales. Overall 39% of cases had a poor outcome as defined by death, cerebral palsy or a significant degree of developmental delay, compared with 2.7% of controls. Furthermore, 62% of those with severe encephalopathy had a poor outcome compared with 25% of those with moderate. Cases with a history of seizures were three times more likely to develop cerebral palsy than cases without.

The findings from our population-based study indicate that newborn encephalopathy places infants at significant risk of developmental delay by the second year of life. We found differences, in all areas of development as assessed by the Griffiths Mental Development Scales, which were both statistically and clinically significant. Of note the largest deficits were seen in speech and hearing which are crucial areas for all aspects of development and learning.

As part of the research protocol, when the children reach three years of age they receive a full neurological assessment performed by a paediatrician or a medical assessment performed by an allied health professional. Currently 77.7% of the cases and controls have undergone an assessment by a doctor or paediatrician and the follow-up continues to be arranged for those 'hard to contact', rural, interstate and overseas participants. Psychological assessment at age 5 years is almost complete with a follow-up fraction of 68.9% of eligible children to date. These assessments include receptive language, verbal and visual reason-

ing, verbal short-term memory and retrieval and application of knowledge. Parents also complete a questionnaire on their child's temperament, behaviour and current medications. Comprehensive questionnaires on the demographics, social and psychological functioning of each family are being collected as the children turn six and then seven years.

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 of 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.

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 future 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. Seven cases and two controls have been diagnosed with autism by an accredited diagnostic panel according to DSM IV criteria and registered with the Disability Services Commission of Western Australia. Among survivors, the incidence of core autism among cases had an odds ratio of 8.5 (95% CI 1.7, 41.0) when compared to the controls indicating a prevalence rate far in excess of the general population (4/1000). Additionally, five of the seven newborn encephalopathy cases or 71% 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 over eight times more likely to develop autism than controls. These findings 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 for 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 to 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:

- Estimate the cumulative mortality to eight years of age;
- Estimate 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;
- To describe handedness;
- To describe the pre- and peri-natal factors associated with autism;
- To obtain functional measures of socialisation and self-care skills;
- To investigate family functioning and the burden associated with having a child with a disability;
- To 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;
- To investigate the relationship between antepartum and intrapartum exposures and adverse long-term outcome.

The Western Australian Newborn Hearing Screening Program

H Bailey, C Bower, K Gifkins, J MacLean, F Smith, J Zach, S Kendrick, M Biggs, R Jagnathan, M Gualda-Barr, L Hollywood, C White, L Hooper, M D'Ortona, R Silburn, H Coates, J Krishnaswamy, N French (PMH/KEMH),P Howe (Australian Hearing Services), P Higginbotham (The Speech and Hearing Centre), J Richards (WA Institute for Deaf Education), S Weeks (Disability Services Commission), V Verma (State Child Development Centre).

The Western Australian Newborn Hearing Screening Program is funded by the Department of Health Western Australia and it is affiliated with the Telethon Institute for Child Health Research. The aim of the programme is the early detection of hearing loss in babies in order to commence intervention by the time the baby with a hearing loss is six months of age. Speech and language development in children with permanent bilateral hearing loss is thought to be better if intervention is commenced before six months than if started later. A related project, which is funded by the Garnett-Passe and Rodney Williams Foundation looked at the characteristics of children with a permanent hearing loss who were enrolled in educational intervention programs.

Newborn hearing screening involves a set of simple screens that are done prior to a baby's discharge from the maternity hospital. In 2000

the programme commenced at the five largest maternity hospitals in the Perth metropolitan area and was later expanded to the neonatal unit at Princess Margaret Hospital. Hearing screening is offered to all well babies on the second day of life. Babies who are admitted to a special care nursery are screened when at least 34 weeks gestation. If a good response is not obtained from the screening tests, the babies are re-tested in about two weeks. If there are not good responses in both ears at that stage, the baby is referred for diagnostic evaluation by a paediatric audiologist.

Since the start of the program over 30,000 babies have received hearing screening and about 20 babies have been diagnosed with a permanent bilateral hearing loss. As part of the evaluation of the program, surveys were conducted to see how newborn hearing screening is viewed by mothers, midwives and screeners. Another part of the evaluation has been the continuation of the Hearing Loss Prevalence Program (HeLP). The HeLP program aims to identify all Western Australian children who have a permanent hearing loss. This information will be used to estimate the prevalence of permanent childhood hearing loss and to detect if any children who have passed the newborn hearing screen later are diagnosed with a hearing loss. Based on our findings, recommendations will be made about which is the best model of newborn hearing screening for Western Australia.

Rett syndrome

H Leonard, L Colvin, S Fyfe, H Moore, A Cream, S Leonard, N De Klerk, C Bower, P Serna, C Philippe, N Leonard, L Nagarajan (Princess Margaret Hospital), in collaboration with J Christodoulou, C Ellaway, L Weaving, B Bennetts, S Williamson (Children's Hospital at Westmead, Sydney), M Davis (Royal Perth Hospital), M Msall, M Tremont (Brown University, New Jersey, USA), R Umansky (Child Development Centre, Children's Hospital, Oakland, California, USA), J Watson (Department of Psychology, University of California, Berkeley, USA), Y Yamashita, T Matsuishi (Kurume University), A Clarke, D Ravine (University of Wales)

We continue to maintain our population-based Rett syndrome register 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. During 2002 follow up information was obtained on the existing cohort of cases through the administration of a questionnaire replicating some of the same type of information collected in 2000 relating to the health and functional ability of girls with Rett syndrome. Additional information was also collected which will allow us both to assess how families are coping and to start quantifying the burden of this disorder on families and the community. Simultaneously with the collection of data from cases (as part of an Honours project carried out by H Moore) data on health episodes and health service utilisation were collected using the Internet from a comparison group of schoolchildren to supplement similar data collected in 2001.

During 2002 we made a number of contributions to the medical literature. In relation to the phenotypic spectrum of Rett syndrome we provided data on 152 cases involved in the 2000 follow up. We categorised cases according to four different scales and were able to show the range in clinical characteristics as well as how some of these characteristics changed with age. In collaboration with colleagues from Southern California we have now shown that the prevalence of left hand preference in Rett syndrome is much higher than in the general population. We also found that the extent of

laterality in hand use was greater for externally-directed than internally-directed activities. In terms of molecular genetics we contributed the phenotypic data to a publication which examined the spectrum of pathogenetic mutations in Australian cases as well as the influence of X inactivation in a proportion of cases. In conjunction with colleagues from Japan and the UK we were the first to characterise the mild phenotype associated with the R133C mutation, a missense mutation in the methyl binding domain of the MECP2 gene. This type of work now continues in relation to the other common MECP2 mutations.

Finally in mid 2002 we received funding to set up an international web-based Rett syndrome database. This database is operating under the guidance of an international reference panel and the data collection pilot phase commenced at the end of 2002. This enterprise has substantial potential to provide important information about Rett syndrome on a world-wide basis and to serve as a model for data collection in rare disorders.

Infectious Diseases

Vaccine Impact Surveillance Network

The Vaccine Impact Surveillance Network was established under the umbrella of the Meningitis Centre in 1996 to assess the impact of vaccines on burden of infectious diseases. In recent years, in collaboration with WA Department of Health, we have been focusing on collecting clinical, demographic and epidemiological data for all cases of invasive pneumococcal disease (IPD, now a notifiable disease) and doing serotyping and antimicrobial susceptibility on pneumococcal as well as meningococcal isolates. Serotyping of pneumococci is done at the Pneumococcal Reference Laboratory in Brisbane. On average, there are approximately 180 cases of IPD reported annually. Recently a new vaccine (Prevenar‰) has been licensed for use in children in Australia but is currently only available free for children at high risk of IPD, which includes Indigenous children. We now aim to collate all data on IPD from 1996 onwards, link the data to the Maternal and Child Health Research Database in order to describe the epidemiology of IPD in WA. Our database will help evaluate the impact of Prevenar‰ on burden of disease. In addition to providing information to the general public, the information generated from this database will assist policy makers in establishing appropriate prevention programs and ensuring treatment schedules are appropriate. Since Prevenar‰ may alter serotype distribution of disease, ongoing surveillance is needed to monitor the types of pneumococci causing serious pneumococcal disease in WA. Ultimately we aim to establish a database of invasive disease caused by all potential bacterial pathogens.

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

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

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 immunisation on survival in areas of high mortality. We were awarded a grant to investigate the impact of routine immunisations on childhood survival in Tari, Southern Highlands Province, Papua New Guinea. The study is being done in collaboration with the Papua New Guinea Institute of Medical Research. As part of other studies, continuous monthly demographic surveillance enabled us to identify births, deaths, migra-

tions, and immunisation status of all children born in Tari between 1989 and 1994. The study aims to determine the effect of DTP, BCG and measles vaccinations on mortality in the first two years of life. A manuscript has been submitted for publication.

Maternal immunisation with pneumococcal polysaccharide vaccine in the highlands of Papua New Guinea

D Lehmann, WS Pomat, YC Liu in collaboration with B Combs, T Dyke, MP Alpers (Papua New Guinea Institute of Medical Research).

Mortality due to invasive pneumococcal disease (pneumonia and meningitis) is very high in young children in non-industrialised countries. It is therefore necessary to identify appropriate interventions before birth or very soon after birth. One potential intervention is vaccination of pregnant women as has been done for the prevention of neonatal tetanus. The transfer of pneumococcal antibodies from mothers to their offspring has been investigated in Papua New Guinea. Pneumococcal polysaccharide (Pnc PS) vaccine was offered to women at 28-38 weeks gestation and blood samples for measurement of levels of antibody titres to 4 Pnc serotypes were collected before immunisation, at delivery and twice in first 6 months of life. Samples were also collected from unimmunised women and children at delivery and at similar times postpartum. To determine whether maternal immunisation might affect subsequent infant immunisation, the antibody response to Pnc Ps vaccine given to a subset of children at age 8-9 months was also assessed. There was a significant increase in antibody titres to Pnc serotypes 5, 14 and 23F in immunised women but not for serotype 7F. Antibody titres for serotypes 5 and 23F were higher in children of immunised women than in the unimmunised group up to age 2 months and for serotype 14 higher to age 4 months. Maternal immunisation did not affect children's capacity to mount antibody responses to immunisation with Pnc PS vaccine in infancy. Breast milk IgA to 4 serotypes was 1.1-1.8 times higher in immunised than unimmunised women for 6 months postpartum. This study together with studies in several other countries provides further support for the concept of maternal

Methodological Approach

Biostatistics and Genetic Epidemiology

Biostatistical analysis and support

N de Klerk, M Firth, J Hansen, P Jacoby, YC Liu.

This group continued collaborative work with other projects throughout the Division and the Institute as well as with the University Schools of Paediatrics & Child Health, Population Health, and Primary, Aboriginal and Rural Health Care, particularly in areas involving the analysis of complex longitudinal data and survival analysis. The group has also diversified into bioinformatics, in particular the analysis of genetic data from microarray experiments.

Nutritional determinants of childhood asthma and atopy

W Oddy, J Sherriff, JK Peat, N de Klerk.

Wendy Oddy has continued her post-doctoral fellowship at the Department of Nutrition, Dietetics and Food Science at Curtin University of Technology. This involves continued collaboration with the Institute in the analysis of data from various studies including the analysis of dietary data collected from the Raine Study 8 year follow-up. The work at Curtin has included a number of teaching responsibilities including delivery of lectures in the Nutritional Epidemiology 382 Unit; and supervision of Ph.D student Kathleen Graham on the Commonwealth funded Perth Infant Feeding Study, as well as acting on the Nutrition Reference Group at Ngala. Dr Oddy was

awarded an NHMRC Travel Award in 2002 and spent 6 months in Tucson, Arizona with Dr Anne Wright studying the association of breast milk cytokines and infant wheeze.

Suicide Prevention

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

The Institute supports a program of translational research in suicide prevention. This research aims to ensure that new knowledge on the etiology and epidemiology of suicide and suicidal behaviour can be applied in current policy and practice. An important component of this is the accommodation of the Ministerial Council for Suicide Prevention (MCSP) at the Institute. The MCSP reports through the Minister for Health to all other Ministers on the Cabinet Sub-committee for Social Policy and is responsible for advising government and supporting effort, across government and non-government agencies, to reduce the morbidity and mortality associated with suicide and self harm. The MCSP is also active in advancing scientific and community understanding of suicide and its prevention. The broad representation of government departments concerned with social policy, non-government agencies, and key community stakeholders on the Council has ensured that new knowledge in suicide prevention can be implemented in the policy and practice of these organisations.

The MCSP is responsible for maintaining the WA Coroner's Database on Suicide. This ongoing collection of epidemiological surveillance data on suicides by persons of all ages in Western Australia has provided some of the first Australian data delineating key risk and protective factors for suicide among young people. It is also used for the monitoring of emerging trends, such as a recently observed increase in illicit drug use associated with suicide among young people.

The MCSP also supports a program of research into the hospital and community management of deliberate self-harm. This includes the design and maintenance of a deliberate self-harm database within each of the three adult teaching hospitals in Perth. This database has been used to monitor trends in deliberate self-harm admissions and to monitor the progress of the implementation of the practice guidelines developed by the Royal Australian and New Zealand College of Psychiatry and the Australian College of Emergency Physicians. A recent report from this database at Fremantle Hospital described reasons for the changing pattern of hospital presentations and has been used to inform service planning decisions for improved community based emergency care (Northey et al 2003).

National Suicide Prevention Strategy (NSPS) funding over 18 months has enabled the MCSP to conduct a qualitative research suicide prevention project targeting males aged 17-35 years. The research project commenced in December 2002 and will utilise a consumer consultation process to inform and support the development and improvement of suicide intervention and prevention initiatives in Western Australia. Consultations will include consumers from the general population, at risk groups, carers and service providers. It is anticipated that these consultations will provide data on men's knowledge, attitudes and behaviours in relation to help seeking and service and support access.

The public education strategy secured corporate funding over three years from January 2002, through a joint proposal with Woodside Energy Ltd. and the Telethon Institute for Child Health Research, to assist in the development of a national information and resource system for suicide prevention. ASPiRE (Australian Suicide Prevention information and Resource

Exchange) was launched in November 2002 and provides access to over 2000 research articles and resources. The strategy includes dissemination of new information and targets both professionals and the community. ASPiRE is located on the MCSP website at www.mcsp.org.au.

The "Information and Support Pack for those bereaved by suicide or other sudden death" was produced by the MCSP with funding through the NSPS. The development of this key resource was informed by research on the information and support needs of families bereaved through suicide. The pack is distributed through the Coronial counselling service to all families bereaved by suicide or other sudden death. It is also available online at www.mcsp.org.au.

Prevalence data on suicidal behaviour and their risk associations are currently being explored through the 2000/2001 WA Aboriginal Child Health Survey. The early antecedents of depression and suicidal behaviour are also being investigated in the RASCALS study survey of a 10% random sample of recently delivered mothers from the 1995 and 1996 Western Australian birth cohorts. This study aims to elucidate the complex causal chain events from infancy and early childhood that lead to mental health problems and self-harm. The RAINE study data are also being used in a longitudinal study of the relationship of dietary factors (omega 3 fatty acid deficiency) in the etiology and onset of depression in adolescents. This study was initiated as a result of recent reports indicating an association between omega 3 FA and depression in adults. It hypothesised that the increasing proportion of children eating diets deficient in whole grains and fish may be a vulnerability factor for some children's adverse response to the stresses they encounter in the adolescent years.

The continuing high rates of suicide and self-harming behaviour in the Aboriginal population and some areas of WA are a major concern. The MCSP and the Institute has, for the past year, supported the secretariat of the WA Aboriginal Suicide Prevention Steering Committee. This group has been active overseeing the implementation of the State's strategy to reduce Aboriginal suicide and self-harm. During the past year it has been active lobbying for the establishment of an Aboriginal suicide prevention coordinator position in the Kimberley. This recommendation came from community consultation and representations identifying the need for practical administrative support to coordinate and progress the work of local agencies to address the high rates of suicide in that region. A proposal is currently under consideration by the State Government for this group to be re-convened as a working group of the MCSP.

Maternal and Child Health Research Data Base (MCHRDB).

This composite Data Base is the backbone of the work of the Division of Population Sciences, as virtually all studies emanating from the Division are informed by it in some way. Central to the Data Base is a register of all births in the State of Western Australia since 1980, based on the statutory reports submitted by the attending midwife. These reports provide information on a number of factors relating to each pregnancy and birth. This register is validated and completed by linkage to the Registrar General's register of births and deaths. In house manipulation of this data has identified maternal sibships for births 1980-95, by linking all births occurring to each woman since 1980.

This central register of every birth can be linked to the Birth Defects Registry, Cerebral Palsy Register, Mental Health Information System, and the Reproductive Technology Register which are believed to completely ascertain major birth defects, moderate and severe cerebral palsy, significant mental health

problems and conceptions following treatment at an infertility clinic respectively. It is also linked to a register of people requesting services for intellectual disability and to the surveys and studies conducted in association with the Centre for Child Health.

Thus the MCHRB:
• provides a sampling frame for epidemiological studies,
• allows comparisons between study samples and the general population to enable appropriate generalisation of results and
• identifies several important outcomes and exposures enabling many important studies to be conducted quickly and efficiently without the need for further data collection.

Furthermore, the ongoing nature of this data collection uniquely allows family effects to be investigated (using sibships) and in the very near future, when the earlier birth cohorts are themselves having children, will allow the investigation of inter-generational effects on maternal and child health.

Population Studies

The Western Australian Twin Register

J Hansen, P Alessandri, K Coleman, N de Klerk, M Croft, A James, P 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. 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.

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, 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. 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 average age of mothers at the time of the multiples' birth was 29.1 years (range 14-45 years). Mothers who were under 20 years of age at the time of the multiples' birth were more likely to have experienced the loss of one of their multiples compared with older mothers (15.1% vs. 6.2% ($p<0.0001$) for all childhood deaths; 12.7% vs. 5.5% ($p<0.001$) for perinatal deaths).

The WA Twin Child Health (WATCH) study

The aim of the WATCH study was to collect data from families on 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. We were able to trace over 90% of families of multiples born between 1980 and 1995, and invite them 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

(90.6% vs. 78.5%, p<0.001); and mothers who were under 20 at the time of the multiples' birth were less likely to reply to the introductory letter, and less likely to agree to participate than older mothers (45.2% vs. 79.1%, p<0.001; 41.7% vs. 70.4%, p<0.001); they were also less likely to return completed questionnaires (29.8% vs. 61.0%, p<0.001). There was no difference in the overall response rates between metropolitan and rural families (58.9% vs. 62.0%, 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 (79.9% vs. 75.5%, p=0.007), but no more likely to participate in the study (69.6% vs. 69.0%, p=0.76). Families who lived outside of Perth were more likely to return completed questionnaires (89.9% vs. 84.6%, p=0.002) than those who lived in Perth.

We were able to examine a number of asthma and atopy endpoints from the questionnaire data. All showed a greater concordance between MZ twins compared with DZ twins, suggesting evidence of a genetic component.

End point	Monozygous (MZ) twins	Dizygous (DZ) twins
Wheezing ever	.80	.55
Current wheeze	.61	.35
Wheezing before age 2	.83	.52
Wheezing before age 5	.81	.54
Doctor-diagnosed asthma by age 15	.76	.49
Current asthma	.61	.30
Hay fever ever	.80	.51
Current hay fever	.71	.46
Eczema ever	.70	.39
Current eczema	.58	.25
Atopy (asthma, hay fever or eczema)	.85	.64
Allergy (hay fever or eczema)	.80	.56

After adjusting for age, boys had a significantly higher prevalence of doctor diagnosed asthma before the age of 15 (p=0.0114), current asthma (p=0.0206), wheezing ever (p<0.0001), current wheeze (p=0.0001), when compared with girls. However, there was no difference in prevalence in hay fever ever, current hay fever, eczema ever, current eczema, atopy and allergy.

Generalised estimating equations were fitted to the data, modelling various respiratory end points with known or potential risk factors, individually and collectively, after adjustment of age and sex. Using doctor-diagnosed asthma before the age of 15 as an example, factors shown to increase risk include: having older siblings, low birth weight (<2,500g), premature birth (<32 weeks gestation), having an asthmatic mother or father, and being exposed to environmental tobacco smoke at home.

"WATCH for Asthma" study

The next phase of the WATCH study is to explore the complexity of the asthma phenotype in WA twin families, 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. Families of multiples born between 1990 and 1995 are invited to participate. They are asked to complete questionnaires about the respiratory health of all family members, and then attend a Clinic for a series of standard breathing, allergy and blood tests. We also offer free zygosity testing to families who are unsure of the zygosity of their twins. So far, 180 families have completed testing. We conducted a clinic in Busselton where 20 families were tested. We plan to conduct clinics in other rural areas in the future to involve families who live outside the Perth metropolitan area.

RASCALS Study

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

SR Zubrick, SR Silburn, JJ Kurinczuk (University of Leicester, 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 outstanding 82% of the questionnaires were returned completed. 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

RASCALS Study - Response Rates for the 1995 and 1996 birth cohorts

Birth cohort and questionnaires mailed	Year sent	Response rates
1995 birth cohort		
13 mth questionnaire	1996	76%
2 year questionnaire	1997	85%
3 year questionnaire	1998	91%
4 year questionnaire	1999	88%
5 year questionnaire	2000	88%
6 year questionnaire	2001	91%
7 year questionnaire	2002	87%
8 year questionnaire	2003	74% (as at 03/07/2003)
1996 birth cohort		
13 mth questionnaire	1997	80%
2 year questionnaire	1998	82%
3 year questionnaire	1999	76%
4 year questionnaire	2000	81%
5 year questionnaire	2001	87%
6 year questionnaire	2002	85%
7 year questionnaire	2003	73% (as at 03/07/2003)

and primary caregiver's mental well-being, will be used to identify possible causal factors and protective factors of mental health.

The RASCALS data will be used as a baseline for the prevalence of specific language disorders within the general population of the Western Australian community. Associate Professor Wendy Hall from the University of British Columbia Canada, has been analysing some of the data on sleep patterns, and this data suggests that children who are breastfed beyond their first birthday were more than twice as likely to have sleep problems in the following year. Although this is certainly not an argument against breastfeeding, it may reflect children who develop patterns of falling asleep while breastfeeding and are therefore unable to return to sleep by themselves. In July 2002 Associate Professor Hall presented more of her findings on sleep at the Third International Conference on Child and Adolescent Mental Health in Brisbane and is in the process of submitting her findings to a journal for publication.
Internationally, the RASCALS study is attracting great interest. One of the researchers, Associate Professor Leon Straker from Curtin University of Technology

Western Australia, reported preliminary findings of children's computer usage at John Hopkins University USA and was invited to speak at the National Institute of Working Life in Stockholm in November 2002.

The RASCALS study is one of a few key longitudinal studies in Australia. At this stage of our research we are now sending out the 1995 cohort eight year questionnaire and the 1996 cohort seven year old questionnaire which will be complemented by information attained from the study child's educational setting upon the consent of the primary caregiver. We are continuing with good response rates which are set out in a table below.

Western Australian pregnancy cohort (RAINE) study

G Kendall, K Moonen, S Hoey, L Clohessy, C Smargiassi, R Austin, K Blake, W Oddy, FJ Stanley, in collaboration with P Sly (Clinical Sciences), P Holt (Cell Biology), S Zubrick and S Silburn (Population Sciences)

The WA Pregnancy Cohort Study involves the questionnaire assessment and examination of over 2,000 children, now 10 years old, who have been followed from before birth. Intrauterine growth and other prenatal, perinatal, and postnatal characteristics have been related to a variety of developmental and health outcomes (asthma and atopy, respiratory morbidity, blood pressure and cardiovascular status). Current data collection is multifaceted and focuses on the psychosocial environment of the child and mental health, intellectual, and academic outcomes. Developmental assessment includes measures of: receptive vocabulary, non-verbal ability, neurological deficit, speech and language, and motor competence. In addition, both principal caregiver and teacher complete a checklist of emotional and behavioural functioning for the child and information regarding literacy and numeracy is obtained via the education department. The aim is to quantify the contribution that poor fetal growth makes to mental health outcomes in childhood.

Data collection at 10 years was completed at the close of 2002. Approx 1,700 children/families attended and questionnaire data are available for approx 2,100 children/families. The data are being prepared for analysis which will begin in 2003. Planning is well underway for the next follow-up at 13 years. This phase of the study seeks to address 5 independent but interrelated objectives. The first objective relates to the valid measurement of physical activity levels, physical fitness, and motor competence. We are interested to know how level of physical activity, measured by pedometer,

relates to various aspects of fitness and motor competence, but our interest goes beyond this. We would like to understand more about the complex relationship between physical activity and the development of the precursors to cardiovascular disease. We would also like to understand more about psychosocial factors that influence physical activity levels, and the mental health correlates of inactivity in this age group. A clearer understanding of the physiological, immunological, psychological, and genetic, mechanisms that drive the development of asthma is our fourth objective. Our fifth objective, to assess the responsiveness of the hypothalamo-pituitary-adrenal (HPA) axis and relate this to asthma at age 13, will be made possible because of data collection associated with the above objective.

The study continues with an increasingly busy program of analyses to investigate the fetal and early life origins of childhood diseases. A highlight in 2002 was the finding of a genetic trigger that explains why some children develop severe asthma.

WA Mortality Study

J Freemantle, N de Klerk, A Read, E Blair, L Alessandri (deceased) in collaboration with M Divitin (Department of Population Health, University of Western Australia) and the Path Centre, Western Australia.

This study formed the major component of the doctoral research completed by Jane Freemantle in February 2003. The study comprises a longitudinal birth cohort study, the primary data source being the MCHRDB. Data included on the MCHRDB are complete for all births in WA from 1980 onwards, with new birth cohorts linked on an annual basis. Maternal and infant variables and the geographical location of the residence and the time of birth and death were included in the descriptive and multivariate analyses. Each infant and childhood death was coded using a three-digit code developed primarily for research purposes.

The descriptive analyses of mortality referred to the probability of dying in infancy and in childhood as the cumulative mortality risk (CMR), for various diseases and various population subgroups. Age-specific childhood rates were also calculated. The results of multivariate analyses included the fitting of Cox and Poisson regression models, and estimates of effect were represented as hazard ratios (Cox regression) and relative rates (Poisson regression).

Results

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 to 9% of non-Indigenous births. Indigenous mothers gave birth at an earlier age (30% of births were to teenage mothers compared to 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 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 the birth year groups 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). This profile differed from that for non-Indigenous 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 potentially preventable. These causes were infection followed by Sudden Infant Death Syndrome (SIDS), which differed from the main causes for non-Indigenous infants, 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. Furthermore, 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 was more than three times higher than for non-Indigenous children. This risk was significantly increased when most of the perinatal maternal and infant variables were considered. Accident and injury, and infection were the main causes of mortality amongst Indigenous children. For non-Indigenous children the main causes were also accident and injury, followed by infection and cancer. However, the risk of accidental death for Indigenous children was nearly 4_ times higher, and death due to infection nearly seven times higher, than for non-Indigenous children. The childhood mortality rate in Indigenous children was highest in those born in remote areas, and in rural areas for non-Indigenous children.

Conclusion
A unique total population based mortality profile of all infants and children born in Western Australia between 1980 and 1997 and who died before 1998 has been constructed. The high mortality rate in Indigenous children, and particularly the absence of significant improvement in these rates, and the increasing disparity in the risk of mortality between Indigenous and non-Indigenous infants and children are all unacceptable. These data will inform recommendations aimed at decreasing infant and childhood mortality, particularly for the Indigenous population, through further research and the implementation of strategically focussed prevention programs and policies.

Population Screening for Cystic Fibrosis: Knowledge and Emotional Consequences 18 Months Later

C Gordon, S Zubrick, C Bower, I Walpole (WA Genetic Services).

We assessed cystic fibrosis (CF) knowledge and emotional consequences of CF population testing 18 months after screening was offered. Questionnaires were sent to 593 individuals and 353 responded (59.5%). All respondents had sound knowledge of CF disease, although carriers were more likely to correctly state the pattern of CF inheritance and CF carrier rate in Australia. Eleven of 47 carriers falsely believed they were only very likely to be carriers, while nearly a third of test negative individuals falsely believed they were definitely not carriers. Imprecise recall of the meaning of results may be due to memory loss over time, simplification of result meaning and minimisation of risk. The Health Orientation Scale was used to assess emotional consequences of CF carrier testing 18 months after testing. Both carriers and test-negative individuals thought most carriers would experience more negative feelings than most non-carriers. Carriers experienced less positive feelings about their test result compared to non-carriers. Interestingly, carriers

own feelings about their result were more positive compared to how they thought most carriers would feel. These results suggest that carriers experience minimal adverse psychological effects, although a negative social stigma may be attached to carrying the CF gene mutation.

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C Bower. Member. Scientific Sub-Committee of the Human Research Ethics Committee, Curtin University of Technology
C Bower. Member. Western Australian Genetics Council, Department of Health WA
C Bower. Member. Prenatal Diagnosis Committee, Department of Health WA
C Bower. Member. Health Standards and Surveillance Council, WA Department of Health
C Bower. Working Group for the Health Standards and Surveillance Council, WA Department of Health

C Bower. Member. Australian Paediatric Surveillance Unit Board
C Bower. Member. Australian Paediatric Surveillance Unit Scientific Review Panel
C Bower. Member. Perinatal and Infant Mortality Committee
C Bower. Member. Midwives' Notification System Advisory Committee
C Bower. Member. Confidentiality of Health Information Committee
C Bower. Member. Human Genetics Society of Australasia
C Bower. Member. Public Health Association of Australia
C Bower. Member. Teratology Society (USA)
C Bower. Member. Perinatal Society of Australia and New Zealand
C Bower. Member. Australian Birth Defects Society
C Bower. Member. Australasian Epidemiological Association
C Bower. Member. Society for Pediatric Epidemiologic Research
C Bower. Member. Australian Association of Health Promotion Professionals
N de Klerk. Member. NHMRC New Program Grants Committee
N de Klerk. Member. Busselton Population Research Foundation Board of Directors
N de Klerk. Member. Perth Respiratory Epidemiology Group (P.R.E.G.) Executive Committee
N de Klerk. Member. Scientific Advisory Committee of the Busselton Research Foundation Board
N de Klerk. Member. Australian Radiation Health and Safety Advisory Council
N de Klerk. Member. Clinical Drug Trial Committee, Sir Charles Gairdner Hospital
N de Klerk. Member. Mesothelioma Committee of Western Australia
N de Klerk. Member. Western Australian Air Quality Coordinating Committee Health Issues Group
N de Klerk. Member. Management Committee, Data Linkage Project, Health Department of WA
N de Klerk. Member. Executive Committee, Australian Twin Register
N de Klerk. Member. Medical and Scientific Advisory Panel, Cancer Foundation of WA
N de Klerk. Member. Australian Working Group developing Radiation Protection Standard for Exposure to ELF
D Elsbury. Ear Health Committee, Kalgoorlie.
J Finucane Ear Health Committee, Kalgoorlie.
J Freemantle. National Secretary. Public Health Association of Australia
J Freemantle. Chair. Lady Lawley Cottage Management Committee, Australian Red Cross
J Freemantle. Member. Perinatal Society of Australia and New Zealand
J Freemantle. Fellow. Guildford Grammar School
J Freemantle. Member. Public Health Association of Australia
J Freemantle. Member. Australasian Epidemiological Association
J Freemantle. Member. Perth Epidemiology Group
J Freemantle. Member. Australia/New Zealand Health Services Research and Policy Association
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. Member. Australasian Epidemiological Association
D Lehmann. Member. Medical Society of Papua New Guinea
D Lehmann. Member. Public Health Association of Australia
D Lehmann. Member. Australasian Society of Human Biology
D Lehmann. Member. Australasian Society for Infectious Diseases
D Lehmann. Member. Australian Society for Microbiology
D McAullay. Member. Western Australian Aboriginal Health Information and Ethics

Committee.
D McAullay. Member. Healthway Health Advisory Committee
D McAullay. Member. AHEC working party to revise the Guidelines on Ethical Matters in Aboriginal and Torres Strait Islander Health
D McAullay. Member. NHMRC Indigenous Health Research Panel
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, Cancer Foundation of WA Skin Cancer Control Steering Committee
E Milne. Member, Clinical Oncology Society of Australia conference organising committee
W Oddy. Chairperson. Baby Friendly Hospital Initiative, WA Branch
W Oddy. Member. International Society for Research into Human Milk and Lactation
W Oddy. Member. Public Health Association of Australia Executive Child Health Special Interest Group - Invited position
W Oddy. Member. National Food and Nutrition Monitoring Project, Breastfeeding Reference Group (Australia) Invited position
W Oddy. Member. Breastfeeding Public Health Action Group, Health Department of Western Australia (HDWA)
W Oddy. Member. Nutrition Advocacy Group, with Cancer Foundation of WA, Perth, Western Australia
W Oddy. Member. Pediatric Research Society of Australia and New Zealand
SR Silburn. Member. National Advisory Council on Suicide Prevention
SR Silburn. Member. Ministry of Justice Suicide Prevention Taskforce Steering Committee.
SR Silburn. Chairperson Ministerial Council for Suicide Prevention
SR Silburn. Member. Research Subcommittee, Western Australian Health Promotion Foundation
SR Silburn. Member. Consultants Advisory Group for the Longitudinal Study of Australian Children, Australian Institute of Family Studies
SR Silburn. NHMRC Grant Reviewer
A Williams. Member. National Working Party on Mental Health Prevention and Promotion, Commonwealth Department of Health and Aged Care.
A Williams. Member. Management Committee Auseinet: The Australian Network for Promotion, Prevention and Early Intervention for Mental Health
SR Zubrick. Member. National Working Party on Mental Health Prevention and Promotion, Commonwealth Department of Health and Aged Care.
SR Zubrick. Chairperson. Management Committee Auseinet The Australian Network for Promotion, Prevention and Early Intervention for Mental Health
SR Zubrick. Chairperson. Consultants Advisory Group for the Longitudinal Study of Australian Children, Australian Institute of Family Studies
SR Zubrick. Member. Ministerial Council for Suicide Prevention
SR Zubrick. Member. Professional Advisory Committee Ngala Family Resource Centre
SR Zubrick. Member. National Research Partnership for Promotion and Development of Health and Wellbeing
SR Zubrick. Member. Health Promotion Advisory Committee Curtin School of Public Health

Invited Presentations

E Blair. Trends in cerebral palsy. Malaysian Perinatal Society. Genting, Malaysia
E Blair. Antecedents of cerebral palsy. Plenary session. Malaysian Perinatal Society. Genting, Malaysia
E Blair. CP Aetiology research at ICHR. Weekly Perinatal seminar at Newborn Services, KEMH, WA
E Blair. Who should be responsible for funding disability? Futures Forum Opening Symposium for Cerebral Palsy Week 2002. Curtin Technology Park, Bentley, WA
E Blair. What's this stuff about causal pathways? Example from Cerebral Palsy Aetiology

Research. Pop Sci. weekly meeting
E Blair. Who should be funding disability? Australian Cerebral Palsy Association Conference, Sydney
E Blair. Who should be responsible for funding disability? Early Intervention Interest Group, Perth
E Blair. Birth asphyxia – what is it? Opening plenary session. Malaysian Perinatal Society. Genting, Malaysia
C Bower. What is the use of the WA Birth Defects Registry? King Edward Memorial Hospital
C Bower. Relationship between support groups and research. WA Genetic Support Council (Inc) Inaugural Annual meeting
C Bower. Preconception counselling and pre-natal screening. WA Centre for Remote and Rural Medicine – Rural Doctors Program (TV broadcast) Perth
C Bower. Approaches to the prevention of birth defects: striking a balance. WA Genetic Research Forum, Perth.
C Bower. What can we do to prevent spina bifida? Department of Health Symposium, Perth.
C Bower. What is the use of the WA Birth Defects Registry? Department of Public Health, UWA Seminar Series. Perth.
C Bower. The Western Australian Birth Defects Registry. International Clearinghouse for Birth Defects Monitoring Systems Annual Meeting. Lake Lanier Georgia, USA.
C Bower. ICSI, IVF and major birth defects: studies from Western Australia (keynote address). Fertility Society of Australia 21st Annual Scientific Meeting. Gold Coast.
N de Klerk. Air pollution and admissions to hospital for asthma in children in the Perth metropolitan area. Perspectives in Child Health, Institute for Child Health Research, Perth, 2002.
N de Klerk. Epidemiology of mesothelioma in Western Australia. International Mesothelioma Interest Group Meeting, Perth, 2002 (and session chair).
N de Klerk. Familial aggregation of exhaled nitric oxide. Australian Twin Register, Annual Conference, Hobart, 2002.
N de Klerk. Asthma in Childhood Twins. Asthma and Allergy Research Institute, Perth, 2002
E d'Espaignet. The Scientific Work Program of the Population Science Division of the Institute. Strategic Inter-Governmental Nutrition Alliance
E d'Espaignet. Indicators of Social and Family Functioning. Department of Health Science, University of Mauritius
C Jeffries-Stokes. An Aboriginal Perspective of Middle Ear Disease in the Arid Zone of Western Australia. Pina health. Goldfields Ear Health Conference. Kalgoorlie, WA, Oct 31, 2002.
D Lehmann. Pina Health. Goldfields Ear Health Conference. Kalgoorlie, Western Australia
D Lehmann. International Symposium on Protection of newborns through maternal immunization. Fondation Mérieux, Veyrier du Lac, France. December
W Oddy. 31st American Public Health Association Annual Meeting, Philadelphia, Breastfeeding, body mass index and asthma in childhood.
W Oddy. XVI IEA World Congress of Epidemiology, Breastfeeding and cognitive development in childhood, Montreal, Canada.
W Oddy. International Society for Research in Human Milk and Lactation, 13th International Conference, Breastfeeding and cognitive development in childhood: a prospective birth cohort study and Breastfeeding, body mass index and asthma in childhood, Mexico City
W Oddy. Australian Society for Medical Research Inaugural Symposium, Breastfeeding and cognitive development in childhood: a prospective birth cohort study, Perth, WA
W Oddy. Arizona Respiratory Center, University of Arizona, Breastfeeding, body mass index and asthma in childhood
W Oddy. Department of Nutrition Seminar, Breastfeeding, body mass index and asthma in childhood, Curtin University of Technology, Perth
W Oddy. Bunbury Health Service Community Nurses, Mechanisms through which breastfeeding contributes to optimal long-term health, Bunbury WA
SR Silburn. Education as an Equalizer? Annual

Meeting of the Academy of Social Sciences in Australia "Creating a Better Future for Australia's Children", Australian National University, Canberra
SR Silburn. Creating Resilience in Community: A focus on the role of schools in developing resilience in students to enhance their mental health, well-being and learning. Invited keynote address presented at the National Conference of the Council of Independent Schools Association Conference, Fremantle WA
SR Silburn. Translating Research into Policy and Practice. Academy of Social Sciences in Australia workshop "Investing in our Children", University of Melbourne
M Tennant: Impact of Introduction of Swimming Pools on health of Aboriginal children and adolescents living in remote areas of Western Australia 57th State Environmental Health Conference. Perth, WA, Sept 2002.
SR Zubrick. Forecasting the mental health futures of Australian children: Advances in epidemiology and prevention science. Keynote address, 3rd International Conference on Child and Adolescent Mental Health, Brisbane.
SR Zubrick and Williams AW. Social determinants of mental health. 3rd International Conference on Child and Adolescent Mental Health, Brisbane.
SR Zubrick. Challenges of measurement in child and family focused research. Invited paper delivered to a workshop of the Australian Academy of Social Sciences, University of Melbourne
SR Zubrick. Putting it all together: a national forum on Promotion, Prevention and Early Intervention for Mental Health. Auseinet Forum, Adelaide.

Awards

N Badawi. NHMRC Clinical Career Development Award.
C Bower. NH&MRC Senior Research Fellowship 2000-2004

Theses Passed

Maxine Croft PhD
G. Dixon. The Potential Value of Parent Completed Screening for Developmental Delay. Master of Psychology (Clinical), UWA
G Zawalski, BSc(Hons), Dept of Microbiology UWA
C Gordon, BMedSci, University of Melbourne
K Sivwright, M Clin Audiol, UWA

Special Acknowledgements

The Western Australian Department of Health, particularly Mrs V Gee (Midwives' Notification System) and the Health Information System (Hospital Morbidity data), the Registrar General's Office, The Australian Bureau of Statistics, the Western Australian Birth Defects Registry, King Edward Memorial Hospital and the Disability Services Commission provide data crucial to the Maternal and Child Health Research Data Base – our work could not proceed without their continued contribution and valued collaboration.
We are also grateful to the WA Department of Health and the University of Western Australia Data Linkage Unit for assisting with the provision of data and continued support.

Division of Virology

Overview

Current research projects within the Division of Virology focus 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.

Murray Valley Encephalitis

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

Peter McMinn, Melissa Kroeger, 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 effect 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 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.

Enterovirus encephalitis

Molecular epidemiology of enterovirus 71 in the Asia – Pacific region

Peter McMinn, Lara Herrero, Sharon Sanders, Mary Jane Cardosa (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)

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.

In response to these outbreaks, we are actively contributing to a collaborative study of the molecular epidemiology and evolution of EV71 in the Asia-Pacific region. The collaborators are: Professor Jane Cardosa, Institute of Health and Community Medicine, University Malaysia Sarawak, A/Professor KB Chua, Department of Microbiology, University of Malaya and Dr Kwai Peng Chan, Department of Virology, Singapore General Hospital. The study has commenced with the analysis of 180 EV71 isolates obtained from Perth (25), Sarawak (40), Peninsular Malaysia (54), Singapore (25), Indonesia (22; Java and Bali), Taiwan (11) and Melbourne (3). Further EV71 strains isolated in the Asia-Pacific region will also be analysed over the coming years.

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.

Studies on the molecular genetics of enterovirus 71 encephalitis

Peter McMinn, Chee Choy Kok, Lara Herrero, Beng Hooi Chua, Robert Hurrelbrink, 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 my research group has considerable expertise. The first step in EV71 vaccine development has been the construction of an infectious cDNA clone. I currently have 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 patho-

genesis of EV71 encephalitis, as we have done for MVEV. 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.

Staff and Students

Head of Division

Peter McMinn BMedSc (Hon) MB, BS PhD FRCPA FRCPath
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
NHMRC Senior Research Fellow

Students

Beng Hooi Chua BS	PhD candidate
Lara Herrero BSc (Hon)	PhD candidate
Melissa Kroeger BSc (Hon)	MSc candidate
Kimberley Harris	BSc (Hon) candidate
Ashwenia Krishnan	BSc (Hon) candidate
Sharon Sanders	BSc (Hon) candidate

Visitors

Doosung Cheon, division of Virology, National Institute for Health, Seoul, Korea
Jane Cardosa, Director, Institute for Health and community Medicine, UNIMAS, Sarawak, Malaysia

Theses passed

Melissa Kroeger	MSc
Kimberley Harris	BSc with Second Class honours, Division A

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

Invited Presentations

Peter McMinn. Community surveillance for enterovirus 71 and other neurotropic enteroviruses by rapid molecular typing. Communicable Disease Control Conference, Canberra, Australia.
Peter McMinn. Community surveillance for enterovirus 71 and other neurotropic enteroviruses by rapid molecular typing. Infection Control Association of WA Annual Conference, Perth, WA, Australia.
Peter McMinn. The molecular epidemiology of enterovirus 71 in the Asia – Pacific region, 1997-2001. ASM Annual Scientific Conference, Perth, WA.
Peter McMinn. The molecular basis of viral encephalitis: a study of two models. Australian Virology Conference, Qld, Australia.
Peter McMinn. Community surveillance for enterovirus 71 and other neurotropic enteroviruses by rapid molecular typing. Department of Infectious diseases, Fremantle Hospital, WA.

Publications 2002

Bailey HD, Bower C, Gifkins K, Coates HL. Prevalence of permanent childhood hearing impairment - Pilot programme in Australia shows promising results (letter). British Medical Journal 2002;324:172.

Bailey HD, Bower C, Krishnaswamy J, Coates HL. Research: Newborn hearing screening in Western Australia. Medical Journal of Australia 2002;177:180-5.

Basser RL, O'Flaherty E, Green M, Edmonds M, Nichol J, Menchaca DM, Cohen B, Begley CG. Development of pancytopenia with neutralizing antibodies to thrombopoietin after multicycle chemotherapy supported by megakaryocyte growth and development factor. Blood 2002;99:2599-602.

Blair E, Stanley F. Causal pathways to cerebral palsy. Current Paediatrics 2002;12:179-85.

Blair E, Stanley F. New thoughts on the aetiology of cerebral palsy. In: Sturdee D, Oláh K, Purdie D, Keane D, editors. The Yearbook of Obstetrics and Gynaecology. London: RCOG Press; 2002. p. 357-67.

Blake KV, Gurrin LC, Beilin LJ, Stanley FJ, Kendall GE, Landau LI, Newnham JP. Prenatal ultrasound biometry related to subsequent blood pressure in childhood. Journal of Epidemiology and Community Health 2002;56:713-8.

Bower C, Rudy E, Ryan A, Cosgrove P. Report of the Birth Defects Registry of Western Australia 1980-2001. Perth: King Edward Hospital, Centre for Women's Health; 2002 November 2002. Number 9.

Bower C, Ryan A, Rudy E, Miller M. Trends in neural tube defects in Western Australia. Australian and New Zealand Journal of Public Health 2002;26:150-1.

Brake RL, Kees UR, Watt PM. A complex containing PBX2 contributes to activation of the proto-oncogene HOX11. Biochemical and Biophysical Research Communications 2002;294:23-34.

Brameld KJ, Holman CDJ, Threlfall TJ, Lawrence DM, De Klerk NH. Increasing 'active prevalence' of cancer in Western Australia and its implications for health services. Australian and New Zealand Journal of Public Health 2002;26:164-9.

Burton PR. Comment on "ascertainment adjustment in complex diseases. Genetic Epidemiology 2002;23:214-8.

Burton PR, Palmer LJ, Keen KJ, Olson JM, Elston RC. Response to Epstein et al. (letter). American Journal of Human Genetics 2002;71:441-2.

Croft ML, Read AW, de Klerk N, Hansen J, Kurinczuk JJ. Population based ascertainment of twins and their siblings, born in Western Australia 1980 to 1992, through the construction and validation of a maternally linked database of siblings. Twin Research 2002;5:317-23.

d'Espaignet E, Measey M-A, Del Grande E. Northern Territory Health and wellbeing Survey Non-Indigenous Population. Darwin: Department of Health and Community Services; 2002 December 2002. ISBN 0 7245 4625 1.

De Klerk NH, Ambrosini GL, Pang SC, Musk AW. Silicosis compensation in Western Australian gold miners since the introduction of an occupational exposure standard for crystalline silica. Annals of Occupational Hygiene 2002;46:687-92.

de Klerk NH, Musk AW. Epidemiology of Mesothelioma. In: Robinson BWS, Chahinian P, editors. Mesothelioma. London: Martin Dunitz; 2002. p. 339-49.

Dixon G, Badawi N, Kurinczuk JJ, Keogh JM, Silburn SR, Zubrick SR, Stanley FJ. Early developmental outcomes after newborn encephalopathy. Pediatrics 2002;109:26-33.

Epton MJ, Hales BJ, Thompson PJ, Thomas WR. T cell cytokine responses to outer membrane proteins of Haemophilus influenzae and the house dust mite allergens Der p 1 in allergic and non-allergic subjects. Clinical and Experimental Allergy 2002;32:1589-95.

Epton MJ, Smith W, Hales BJ, Hazell L, Thompson PJ, Thomas WR. Non-allergenic antigen in allergic sensitization: responses to the mite ferritin heavy chain antigen by allergic and non-allergic subjects. Clinical and Experimental Allergy 2002;32:1341-7.

Ernst M, Inglese M, Scholz GM, Harder KW, Clay FJ, Bozinovski S, Waring P, Darwiche R, Kay T, Sly PD, Collins R, Turner DJ, Hibbs ML, Anderson GP, Dunn AR. Constitutive activation of the Src-family kinase Hck results in spontaneous pulmonary inflammation and an enhanced innate immune response. Journal of Experimental Medicine 2002;196:589-604.

Grant SL, Hammacher A, Douglas AM, Goss GA, Mansfield RK, Heath JK, Begley CG. An unexpected biochemical and functional interaction between gp130 and the EGF receptor family in breast cancer cells. Oncogene 2002;21:460-74.

Greene WK, Ford J, Dixon D, Tilbrook PA, Watt PM, Klinken SP, Kees UR. Enforced expression of HOX11 is associated with an immature phenotype in J2E erythroid cells. British Journal of Haematology 2002;118:909-17.

Gurrin LC, Sly PD, Burton PR. Using imprecise probabilities to address the questions of inference and decision in randomized clinical trials. Journal of Evaluation in Clinical Practice 2002;8:255-68.

Hales BJ, Hazell LA, Smith W, Thomas WR. Genetic variation of Der p 2 allergens: effects on T cell responses and immunoglobulin E binding. Clinical and Experimental Allergy 2002;32:1461-7.

Hall GL, Hantos Z, Wildhaber JH, Sly PD. Contribution of nasal pathways to low frequency respiratory impedance in infants. Thorax 2002;57:396-9.

Hallmayer J, Glasson EJ, Bower C, Petterson B, Croen L, Grether J, Risch N. On the twin risk in autism. American Journal of Human Genetics 2002;71:941-6.

Hansen M, Kurinczuk JJ, Bower C, Webb S. The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. New England Journal of Medicine 2002;346:725-30.

Heidari M, Rice KL, Kees UR, Greene WK. Expression and purification of the human homeodomain oncoprotein HOX11. Protein Expression and Purification 2002;25:313-8.

Hiller R, Laffer S, Harwanegg C, Huber M, Schmidt WM, Twardosz A, Barletta B, Becker WM, Blaser K, Breiteneder H, Chapman A, Crameri R, Duchene M, Ferreira F, Fiebig H, Hoffmann-Sommergruber K, King TP, Kleber-Janke T, Kurup VP, Lehrer SB, Lidholm J, Müller U, Pini C, Reese G, Scheiner O, Scheynius A, Shen H-D, Spitzauer S, Suck R, Swoboda I, Thomas W, Tinghino R, Van Hage-Hamsten M, Virtanen T, Kraft D, Müller MW, Valenta R. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. FASEB Journal 2002;16:414-6.

Hinwood AL, Sim MR, de Klerk N, Drummer O, Gerostamoulos J, Bastone EB. Are 24-hour urine samples and creatinine adjustment required for analysis of inorganic arsenic in urine in population studies? Environmental Research 2002;88:219-24.

Holt PG. Postnatal maturation of immune and inflammatory functions. In: Silverman M, editor. Childhood asthma and other wheezing disorders. second ed. London: Arnold; 2002. p. 69-84.

Holt PG. The role of airway dendritic cell populations in regulation of T-cell responses to inhaled antigens: Atopic asthma as a paradigm. Journal of Aerosol Medicine-Deposition Clearance and Effects in the Lung 2002;15:161-8.

Holt PG, Sly PD. Interactions between respiratory tract infections and atopy in the aetiology of asthma. European Respiratory Journal 2002;19:538-45.

Holt PG, Sly PD. Interactions between RSV infection, asthma, and atopy: Unraveling the complexities. Journal of Experimental Medicine 2002;196:1271-5.

Ikegami M, Weaver TE, Conkright JJ, Sly PD, Ross GF, Whitsett JA, Glasser SW. Deficiency of SP-B reveals protective role of SP-C during oxygen lung injury. Journal of Applied Physiology 2002;92:519-26.

Jarnicki AG, Thomas WR. Stimulatory and inhibitory epitopes in the T cell responses of mice to Der p 1. Clinical and Experimental Allergy 2002;32:942-50.

Jarnicki AG, Tsuji T, Thomas WR. Hypersensitivity reactions after respiratory sensitization: Effect of intranasal peptides containing T-cell epitopes. Journal of Allergy and Clinical Immunology 2002;110:610-6.

Jecker P, Mann WJ, McWilliam AS, Holt PG. Dendritic cell influx differs between the subglottic and glottic mucosae during acute laryngotracheitis induced by a broad spectrum of stimuli. Annals of Otology, Rhinology and Laryngology 2002;111:567-72.

Jongeling BR, Badawi N, Kurinczuk JJ, Thonell S, Watson L, Dixon G, Stanley FJ. Cranial ultrasound as a predictor of outcome in term newborn encephalopathy. Pediatric Neurology 2002;26:37-42.

Kovar J, Sly PD, Willet KE. Postnatal alveolar development of the rabbit. Journal of Applied Physiology 2002;93:629-35.

Kroeger MA, McMinn PC. Murray Valley encephalitis virus recombinant subviral particles protect mice from lethal challenge with virulent wild-type virus. Archives of Virology 2002;147:1155-72.

Kurinczuk JJ, Bower C, Lewis B, Byrne G. Congenital hypothyroidism in Western Australia 1981-1998. Journal of Paediatrics and Child Health 2002;38:187-91.

Kurinczuk JJ, Hansen M. Major birth defects after assisted reproduction - Reply (letter). New England Journal of Medicine 2002;347:1450-1.

Kuter DJ, Begley CG. Recombinant human thrombopoietin: basic biology and evaluation of clinical studies. Blood 2002;100:3457-69.

Lee YC, de Klerk N, Henderson D, Musk AW. Malignant Mesothelioma. In: Hendrick DJ, Beckett W, Burge PS, Churg A, editors. Occupational Disorders of the Lung. London: Harcourt; 2002. p. 359-79.

Lehmann D, Pomat WS, Combs B, Dyke T, Alpers MP. Maternal immunization with pneumococcal polysaccharide vaccine in the highlands of Papua New Guinea. Vaccine 2002;20:1837-45.

Lehmann* D. Demography and causes of death among the Huli in the Tari basin. PNG Medical Journal 2002;45:51-62.

Leonard H, Wen XY. The epidemiology of mental retardation: Challenges and opportunities in the new millennium. Mental Retardation and Developmental Disabilities Research Reviews 2002;8:117-34.

Leonard S, Msall M, Bower C, Tremont M, Leonard H. Functional status of school-aged children with Down syndrome. Journal of Paediatrics and Child Health 2002;38:160-5.

Lécuyer E, Herblot S, Saint-Denis M, Martin R, Begley CG, Porcher C, Orkin SH, Hoang T. The SCL complex regulates c-kit expression in hematopoietic cells through functional interaction with Sp1. Blood 2002;100:2430-40.

Liu YC, Blair EM. Predicted birthweight for singletons and twins. Twin Research 2002;5:529-37.

Lum LCS, Chua KB, McMinn PC, Goh AYT, Muridan R, Sarji SA, Hooi PS, Chua BH, Lam SK. Echovirus 7 associated encephalomyelitis. Journal of Clinical Virology 2002;23:153-60.

Macaubas C, Lee PT, Smallacombe TB, Holt BJ, Wee C, Sly PD, Holt PG. Reciprocal patterns of allergen-induced GATA-3 expression in peripheral blood mononuclear cells from atopics vs. non-atopics. Clinical and Experimental Allergy 2002;32:97-106.

Manners PJ, Bower C. Worldwide prevalence of juvenile arthritis - Why does it vary so much? Journal of Rheumatology 2002;29:1520-30.

McMinn PC. An overview of the evolution of enterovirus 71 and its clinical and public health significance. FEMS Microbiology Reviews 2002;26:91-107.

Morahan G, Huang DX, Wu M, Holt BJ, White GP, Kendall GE, Sly PD, Holt PG. Association of IL12B promoter polymorphism with severity of atopic and non-atopic asthma in children. Lancet 2002;360:455-9.

Morley R, Owens J, Blair E, Dwyer T. Is birthweight a good marker for gestational exposures that increase the risk of adult disease? Paediatric and Perinatal Epidemiology 2002;16(3):194-9.

Musk AW, de Klerk NH. The qualitative risks of mesothelioma and lung cancer in relation to asbestos exposure: the Wittenoom Data. (Reply). Annals of Occupational Hygiene 2002;46:128-9.

Musk AW, de Klerk NH, Olsen NJ, Hansen J, Ambrosini GL, Fritschi L, Merlere E, Hobbs MST. Mortality in miners and millers of crocidolite in Western Australia: follow-up to 1999. Annals of Occupational Hygiene 2002;46(S1):90-2.

Ng T-W, Holt PG, Prescott SL. Cellular immune responses to ovalbumin and house dust mite in egg-allergic children. Allergy 2002;57:207-14.

O'Flaherty E, Cook W, Boyd J, Grant SL, Kurek J, Begley CG. Cytotoxicity of paclitaxel or cisplatin on carcinoma cell lines is not inhibited by leukemia inhibitory factor (LIF). Growth Factors 2002;20:141-5.

Oddy WH, de Klerk NH, Sly PD, Holt PG. The effects of respiratory infections, atopy, and breastfeeding on childhood asthma. European Respiratory Journal 2002;19:899-905.

Oddy WH, Peat JK, de Klerk NH. Maternal asthma, infant feeding, and the risk of asthma in childhood. Journal of Allergy and Clinical Immunology 2002;110:65-7.

Peták F, Habre W, Hantos Z, Sly PD, Morel DR. Effects of pulmonary vascular pressures and flow on airway and parenchymal mechanics in isolated rat lungs. Journal of Applied Physiology 2002;92:169-78.

Pillow JJ, Sly PD, Hantos Z, Bates JHT. Dependence of intrapulmonary pressure amplitudes on respiratory mechanics during high-frequency oscillatory ventilation in preterm lambs. Pediatric Research 2002;52:538-44.

Read AW. What are the national rates for sudden infant death syndrome for Aboriginal and Torres Strait Islander infants? Journal of Paediatrics and Child Health 2002;38:122-3.

Reutens AT, Begley CG. Endophilin-1: a multifunctional protein. International Journal of Biochemistry and Cell Biology 2002;34:1173-7.

Sanson A, Nicholon J, Ungerer J, Zubrick S, Wilson K, Ainley J, Berthelsen D, Bittman M, Broom D, Harrison L, Rodgers B, Sawyer M, Silburn S, Strazdins L, Vimpani G, Wake M. Introducing the longitudinal study of Australian children. Canberra: Australian Institute of Family Studies; 2002.

Scher AI, Petterson B, Blair E, Ekkenberg JH, Grether JK, Haan E, Reddihough DS, Yeargin-Allsopp M, Nelson KB. The risk of mortality or cerebral palsy in twins: a collaborative population-based study. Pediatric Research 2002;52:671-81.

Silburn S. Translating research into policy and practice. In: Prior M, editor. Investing in our Children. Developing a research agenda. Canberra: Academy of Social Sciences; 2002. p. 130-42.

Slack-Smith LM, Read AW, Stanley FJ. Experience of respiratory and allergic illness in children attending childcare. Child: Care, Health and Development 2002;28:171-7.

Slack-Smith LM, Read AW, Stanley FJ. A prospective study of absence for illness and injury in childcare children. Child: Care, Health and Development 2002;28:487-94.

Sly PD. Current perspectives in management of chronic asthma in children. Indian Pediatrics 2002;39:719-23.

Sly PD. Croup and Stridor. In: Robertson DM, Robinson M, editors. Practical Paediatrics. 5 ed: Churchill Livingstone; 2002. p. 449-53.

Sly PD, Flack FS. Lung function. In: Silverman M, editor. Childhood asthma and other wheezing disorders. second ed. London: Arnold; 2002. p. 125-43.

Sly PD, Flack FS. What is the role of home monitoring of lung function in managing asthma in children? Italian Journal of Paediatrics 2002;28:281-4.

Sly PD, Holt PG. Breast is best for preventing asthma and allergies - or is it? Lancet 2002;360:887-8.

Smith E, Hargrave M, Yamada T, Begley CG, Little MH. Brief Communications: Coexpression of SCL and GATA3 in the V2 interneurons of the developing mouse spinal cord. Developmental Dynamics 2002;224:231-7.

Stanley F. From Susser's causal paradigms to social justice in Australia? International Journal of Epidemiology 2002;31:40-5.

Stanley F. A new research paradigm for addressing social justice in Australia. In: Prior M, editor. Investing in our Children. Developing a research agenda. Canberra: Academy of Social Sciences; 2002. p. 143-53.

Stanley FJ, Sanson A, McMichael T. New ways of causal pathways thinking for public health. In: Sanson A, editor. Children's Health and Development: New Research Directions for Australia. Melbourne: Australian Institute of Family Studies; 2002. p. 7-13.

t' Mannetje A, Steenland K, Attfield M, Boffetta P, Checkoway H, DeKlerk N, Koskela RS. Exposure-response analysis and risk assessment for silica and silicosis mortality in a pooled analysis of six cohorts. Occupational and Environmental Medicine 2002;59:723-8.

t' Mannetje A, Steenland K, Checkoway H, Koskela RS, Koponen M, Attfield M, Chen JQ, Hnizdo E, DeKlerk N, Dosemeci M. Development of quantitative exposure data for a pooled exposure-response analysis of 10 silica cohorts. American Journal of Industrial Medicine 2002;42:73-86.

Taylor A, Daly A, d'Espaignet E, Wilson D, Eshpeter J, Measey M-A. Collaborative health and wellbeing CATI survey of adults living in Western Australia, Northern Territory and South Australia: Report I: Summary of results by state/territory. Adelaide: South Australian Department of Human Services; 2002 September 2002. ISBN 0 7308 9207 7.

Thomas WR, Smith W. How good are carbohydrates as allergens? Clinical and Experimental Allergy 2002;32:658-61.

Thomas WR, Smith WA, Hales BJ, Mills KL, O'Brien RM. Characterization and immunobiology of house dust mite allergens. International Archives of Allergy and Immunology 2002;129:1-18.

Tulic MK, Holt PG, Sly PD. Modification of acute and late-phase allergic responses to ovalbumin with lipopolysaccharide. International Archives of Allergy and Immunology 2002;129:119-28.

Upham JW, Lee PT, Holt BJ, Heaton T, Prescott SL, Sharp MJ, Sly PD, Holt PG. Development of interleukin-12-producing capacity throughout childhood. Infection and Immunity 2002;70:6583-8.

van Asperen PP, Mellis CM, Sly PD. The role of corticosteroids in the management of childhood asthma. Medical Journal of Australia 2002;176:168-74.

White GP, Watt PM, Holt BJ, Holt PG. Differential patterns of methylation of the IFN-gamma promoter at CpG and Non-CpG sites underlie differences in IFN-gamma gene expression between human neonatal and adult CD45RO(-) T cells. Journal of Immunology 2002;168:2820-7.

Willet KE, Kramer BW, Kallapur SG, Ikegami M, Newnham JP, Moss TJ, Sly PD, Jobe AH. Pre- and postnatal lung development, maturation, and plasticity: Intra-amniotic injection of IL-1 induces inflammation and maturation in fetal sheep lung. American Journal of Physiology - Lung Cellular and Molecular Physiology 2002;282:L411-L20.

Willet KE, Sly PD. Developmental physiology. In: Silverman M, editor. Childhood asthma and other wheezing disorders. second ed. London: Arnold; 2002. p. 57-68.

Woodruff PG, Prescott SL, Holt PG, Fahy JV. Antenatal factors in the development of atopy and asthma. In: Johnston SL, Holgate ST, editors. Asthma: Critical Debates. Oxford: Blackwell Science Ltd; 2002. p. 116-37.

Zubrick S. Challenges of measurement in child and family focused research. In: Prior M, editor. Investing in our Children. Developing a research agenda. Canberra: Academy of Social Sciences; 2002. p. 119-29.