



Scientific Supplement²⁰⁰¹

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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. Our studies on the signals a cell receives from its environment are examining a growth factor (or hormone) that we discovered causes breast cancer cells to stop growing. This growth factor is called Oncostatin M (or OSM). Again we need to understand both the normal function of this growth factor and how its action on cancer cells can be exploited to therapeutic advantage. Our studies are designed to address both these important questions.

The role of SCL in hemopoietic development and leukemogenesis

'Proteomic' analysis of the SCL transcription-factor complex

U Schmidt

Transcription is a tightly regulated process that involves the binding of multi-component protein complexes to specific DNA sequences within promoter and enhancer regions of the genome¹. The basic helix-loop-helix (bHLH) transcription factor SCL is no exception and has been shown to be part of a larger protein complex. SCL interacts directly with ubiquitously expressed products of the E2A gene forming a hetero-dimer that binds to E-box DNA motifs. The bHLH domain of SCL binds to the LIM domain of Lmo2, a LIM-only Zn-finger protein. It has also been demonstrated that the transcription factor GATA-1 (binding to GATA-sites on the DNA) and the LIM-binding protein Ldb1 are part of the complex^{2,3} (Figure 1)

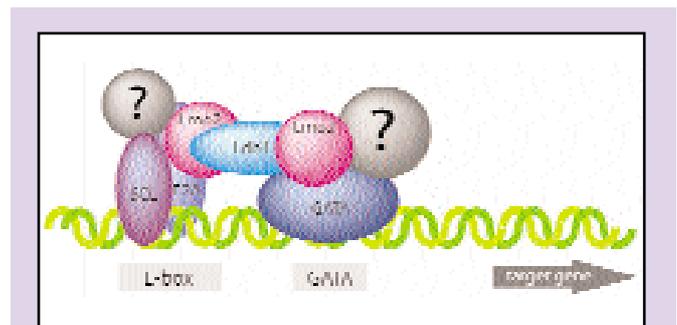


Figure 1. SCL is part of a multi-component transcription factor complex that binds to E-box and GATA sequences^{2,3}. There are most likely more, yet unidentified, proteins present in the complex.

It is expected that more proteins are involved to facilitate the complex roles that SCL and its interacting partners play, for example proteins for chromatin remodelling, like histone acetylases and histone deacetylases. In order to identify novel components in an unbiased way the protein complex is isolated from nuclear extracts of hematopoietic cell lines by its specific binding to E-box/GATA DNA motifs. The

proteins are then separated by 2D gel electrophoresis and subsequently identified by tandem mass spectrometry. Alternatively, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is used to separate and analyse the proteins. Nuclear extracts of different leukemic cell lines are analysed in this way to decipher the complex networks of SCL transcriptional regulation in the normal and malignant state.

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Regulated deletion of gene function in hematopoietic stem cells

JR Goethert, SE Gustin, KA 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

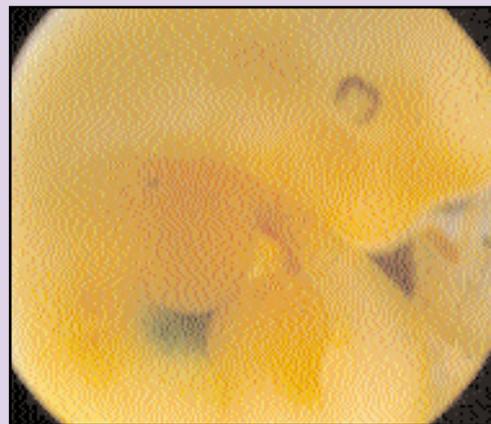


Figure 2. Murine embryo of a cross between a CreERT-3'enhancer transgenic mouse and a LacZ-Cre-reporter mouse. Tamoxifen was administered to the pregnant female with the following regimen: E9.5 0.5 mg, E10.5 1 mg, E11.5 2 mg. On day E12.5 whole-mount LacZ-staining was performed. The fetal liver is positive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

way we were able to demonstrate function of the Cre-recombinase in fetal liver and bone marrow (Figure 2). 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.

Function of SCL in endothelial development

JR Goethert, SE Gustin, KA 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 3). 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.

The role of SCL in Neural Development

JAM van Eekelen, CK Bradley and CG Begley in collaboration with A Harvey, Department of Anatomy and Human Biology, UWA.

SCL is expressed in the central nervous system (CNS) of the mouse during normal development and in adulthood. Expression is confined to specific mid-brain and hindbrain regions and spinal cord. It is also highly regulated by a specific 3.8 kb long regulatory DNA sequence stretching from -0.9 kb upstream of the SCL coding region to exon 3 (5'SCL

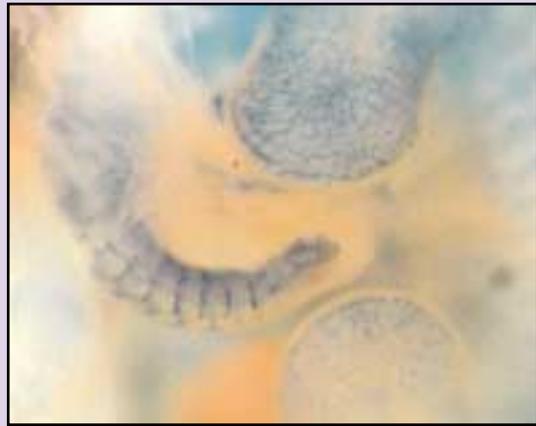


Figure 3. Murine embryo (of a cross between a SCL endothelial enhancer CreERT transgenic mouse and a LacZ-Cre-reporter mouse. Tamoxifen was administered to the pregnant female with the following regimen: E8.5 0.5 mg, E9.5 1 mg, E10.5 2 mg. On day E11.5 whole-mount LacZ-staining was performed. The embryonic blood vessels are positive for LacZ-staining (turquoise/blue). Lateral view demonstrating LacZ-staining in small capillaries of the hand-, footplate and the tail.

neural enhancer). We hypothesize that such highly regulated expression of SCL in the murine brain implies an important, as yet undefined role for SCL in neural cells. To address this function of SCL, we perform neuroanatomical mapping studies to document the precise pattern of SCL expression in the CNS and to identify the neurochemical phenotype of these SCL-expressing cells. In these studies, the SCL-LacZ knock-In mouse, which has the reporter gene LacZ integrated in the SCL locus, is a very useful model to monitor SCL expression. The expression of LacZ in these mice faithfully reproduces the expression of SCL. In our recent LacZ staining experiments on mouse brain tissue fixed by perfusion and cryosectioned, we have demonstrated a widespread expression of SCL in the diencephalon, mesencephalon and metencephalon, including previously unrecognised brain regions (Figure 4). We have also shown 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. We will further investigate central SCL expression during development and in adulthood by comparative analysis of

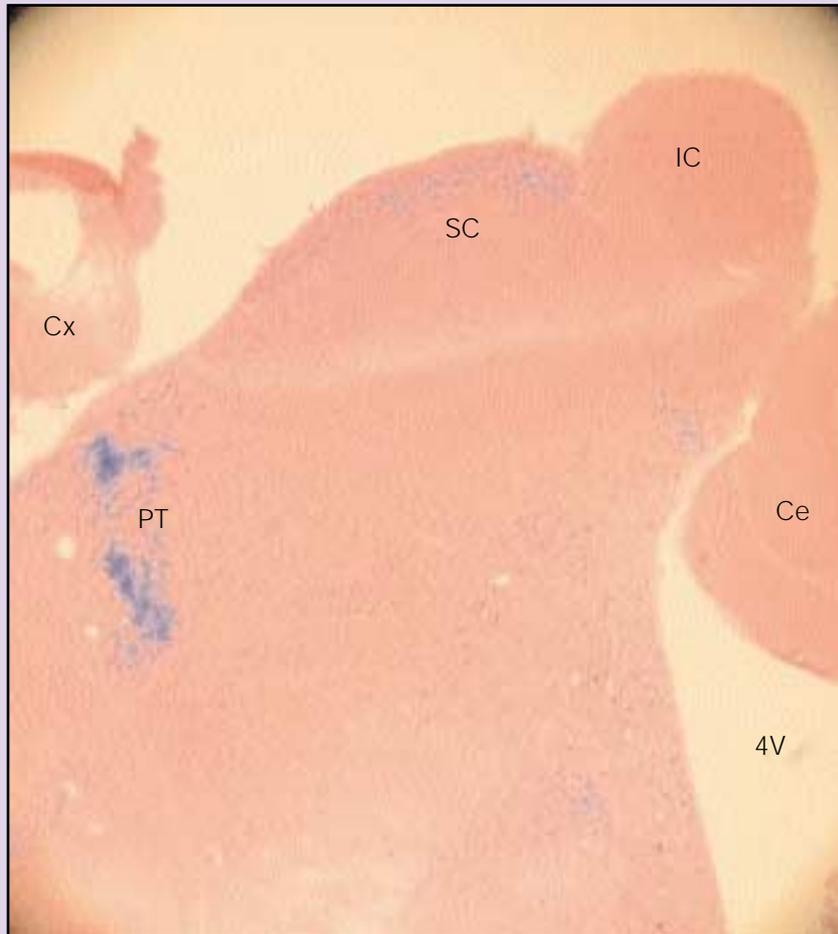


Figure 4. LacZ expression in a 20µm sagittal adult mouse brain section showing the tectum and part of tegmentum in the midbrain. Blue stained cells representing SCL expressing neurons are detected in the pretectal area (PT), the dorsal visual layers of the superior colliculus (SC), the cortex of the inferior colliculus (IC) and scattered cells along the Ventral side of the 4th ventricle (4V). Cerebellum (Ce) and cerebral cortex (Cx) are devoid of LacZ expression.

its pattern of expression with the expression pattern of other transcription factors, like MASH1 and GATA-3. These two transcription factors are known to interact with SCL, they have been shown to be expressed in the brain and have been described as having a role in neuronal development.

In parallel, we are generating 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 Knock Out 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. We aim to express cre recombinase under control of the 5'SCL neural enhancer. The 5'SCL neural enhancer - Cre recombinase construct is fused to a mutated estrogen receptor ligand binding domain showing only

affinity to tamoxifen [ER(T)]. This combination assures cre-recombinase expression only in SCL expressing neurons, which will be activated and translocated to the nucleus to act as a recombinase upon tamoxifen administration to the animal. To confirm specific expression of cre recombinase in SCL expressing neurons of the CNS, we are in the process of analysing offspring from an intercross between the 5'SCL neural enhancer-creER(T) and the Gtosa26-LacZ reporter mouse, in which cre recombinase is monitored by LacZ reporter gene expression. To specifically ablate SCL function, 5'SCL neural enhancer-creER(T) are to be intercrossed with transgenic SCLfloxed mice having the lacZ reporter gene integrated in one SCL allele and two lox P sites integrated in the second SCL allele. As a result of this loss of function approach a specific functional or behavioural mouse phenotype is anticipated.

The role of OSM in normal breast development and possible tumour suppression

Investigation of the effects of oncostatin M (OSM) on mammary gland development and neoplasia

AM Reutens and K Smith

Oncostatin M (OSM) is a pleiotropic cytokine that signals through heterodimeric cell surface receptors via the JAK/STAT and Ras/MAPK signal transduction pathways. Previous *in vitro* studies with cultured cells showed that OSM regulated various processes implicated in breast cancer development and progression, such as cell proliferation, differentiation, angiogenesis and degradation of extracellular matrix. The studies proposed in this project will use *in vivo* mouse models for exploring the role of OSM in normal mammary gland development and in prevention and treatment of breast cancer. One approach will be to compare mammary gland development in female osmb receptor knockout mice and wild type control mice. A second approach will be to examine the effect of OSM induction on growth and metastasis of human breast cancer cells implanted into the mammary fat pads of female nude mice. A third approach will employ transgenic mice that develop mammary tumours as a result of the expression of *myc* or *neu* oncogenes in the mammary epithelium, oncogenes that are frequently upregulated in human breast cancer. To address the hypothesis that OSM will reduce tumour development and growth, these oncogenic mice will be crossed with osmb receptor knockout mice. The resulting mouse lines will allow examination of the effect of lack of OSM action. These proposed studies will extend knowledge of OSM effects on breast cancer and permit dissection of its effects in a whole animal setting.

The Actions of Oncostatin M in Breast Cancer

SL Grant

Oncostatin M (OSM) is a member of the interleukin-6 (IL-6) family of pleiotropic cytokines which utilise the signal-transducing receptor subunit, gp130. OSM was originally identified as a cytokine which could inhibit the proliferation of a number of cancer cell lines such as melanoma cells (Grant and Begley, 1999; Horn et al., 1990). In our studies, the

actions of OSM on breast cancer cells have been characterised.

We have demonstrated that treatment of estrogen receptor (ER) positive MCF-7 and ER negative MDA-MB-231 cells with OSM resulted in inhibition of proliferation and decreased clonogenicity. This was associated with decreased cell cycle progression; exposure of breast cancer cells to OSM resulted in a decreased the proportion of cells in the S phase of the cell cycle, with a corresponding, increase in cells in the G0/G1 phase of the cell cycle.

Treatment of cells with OSM resulted in striking alterations in cell morphology (Figure 5A). Following exposure to OSM cells were enlarged with heterogeneous morphology and loss of cell-cell adhesion. Associated with these morphological changes, there was accumulation of neutral lipid droplets in the cytoplasm of these cells, a characteristic of breast cell differentiation (Figure 5B). In addition, OSM treatment resulted in changes in expression of several proto-oncogenes, growth factors and receptors including decreased expression of ER and an increase in *c-myc*, *c-fos*, TGF α and EGF receptor mRNA levels in breast cancer cell lines. Taken together, these changes in ER positive and negative breast cancer cells implied a change in differentiation status that was coupled with decreased proliferative capacity (Douglas et al., 1998).

A xenograft model in nude mice has also been used to examine the actions of OSM on MCF-7 tumour growth *in vivo*. When OSM was administered to mice for 14 days in a preventative model, the onset of tumour formation and progression of tumours was delayed significantly compared to tumours in the placebo group of mice (Figure 6). Thus, consistent with the actions *in vitro*, OSM also inhibited growth of MCF-7 tumours *in vivo*. We are currently generating MCF-7 cells which express OSM under the control of a tetracycline response promoter (tet-off). These cells will allow us to further investigate the actions of OSM on tumour growth *in vivo*, particularly following long-term OSM exposure.

In addition to examining breast cancer cell lines, the actions of OSM and LIF on normal human breast epithelial cells was examined. Receptors for OSM and LIF were expressed on normal breast samples and cell lines derived from normal breast tissue. Consistent with the actions of OSM on breast cancer cells, OSM inhibited the proliferation of normal mammary epithelial cells. This inhibition was also seen

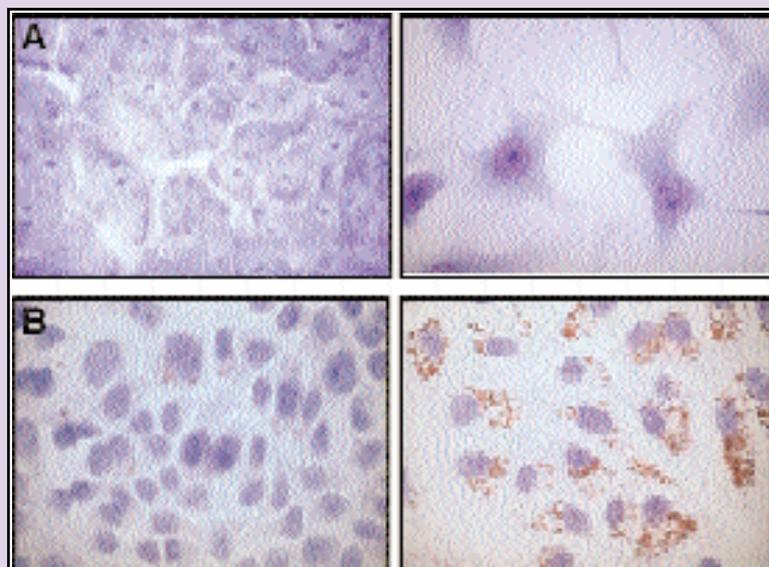


Figure 5. Treatment with OSM alters cell morphology and induces differentiation. MCF-7 cells were cultured for 7 days in the absence or presence of OSM (50 ng/ml). Slides of control (left) and OSM treated (right) MCF-7 cells are shown. (A) Slides were fixed, stained with May Grunwald/Giemsa and photographed at 400X magnification for visualisation of cell morphology. (B) Slides were stained with Oil-Red-O and photographed for visualisation of neutral lipids.

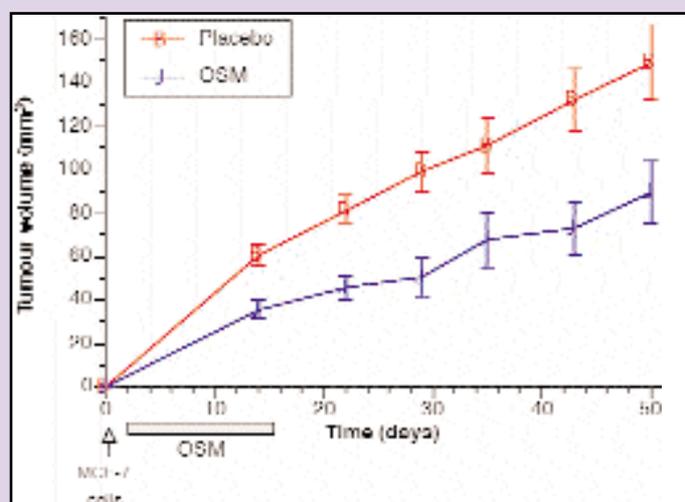


Figure 6. OSM treatment inhibits MCF-7 tumour growth in mice. Female Balb/c nude mice were inoculated with 2×10^7 MCF-7 cells. Mice were injected with OSM (10 μ g), 3 times daily for 14 days. Tumours were measured weekly and mean tumour volumes for each group of mice is plotted. Results are from 32 mice in the placebo group and 28 mice in the OSM-treated group.

in response to LIF. These results implied that OSM and LIF may have a physiological role in the regulation of mammary development (Grant et al., 2001). We have recently obtained OSM receptor knockout mice (OSMR^{-/-}) and are currently investigating the role of OSM in murine mammary development.

In addition to characterising the actions of OSM alone, the effects of OSM in combination with the breast cell mitogen, EGF was examined. Paradoxically, EGF enhanced the OSM-induced inhibition of proliferation and induction of cellular differentiation in breast cancer cells. This functional synergism was also seen with heregulin but not SCF, PDGF or IGF-1, indicating that it was specific to EGF-related growth factors. Immunoprecipitation experiments revealed that OSM receptor b-chain

and gp130 were associated with several EGF receptor family members (Figure 7A). In addition, EGF induced tyrosine phosphorylation of gp130 (Figure 7B). The signalling pathways activated in response to OSM and EGF were also examined (Grant et al., 2002). These findings implicate a novel cross-talk mechanism between OSM and EGF and their receptors in breast cancer cells.

To gain a clearer understanding of the changes in gene expression involved in the responses induced by OSM and OSM plus EGF in MCF-7 cells, cDNA microarray experiments were performed. This study has identified approximately 90 genes that were differentially regulated in response to OSM, EGF and/or OSM plus EGF at 4 time points (30 minutes, 1 hour, 4 hours and 24 hours). A number of these

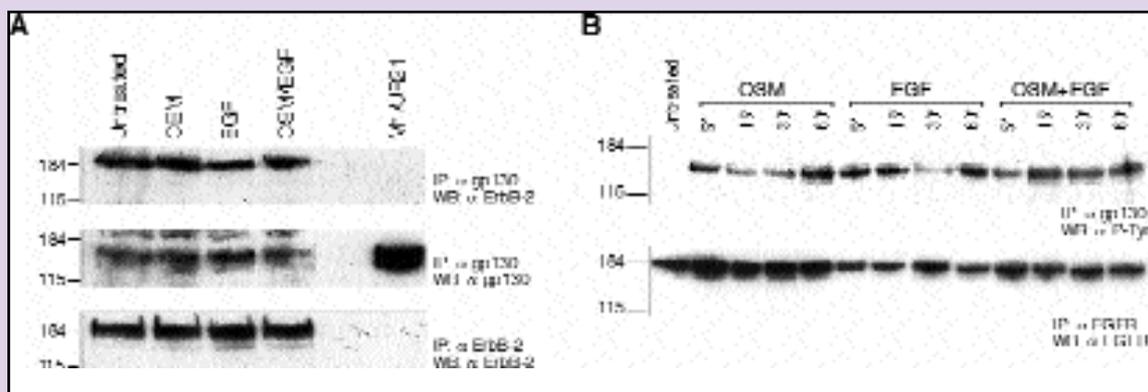


Figure 7. Association of ErbB-2 with gp130 in breast cancer cells and tyrosine phosphorylation of gp130 by OSM and EGF. (A) Co-immunoprecipitation of ErbB-2 with gp130 in untreated MCF-7 cells and cells treated with OSM, EGF and OSM plus EGF. Lysate from M1/UR21 cells transfected with human gp130 were used as a negative control. These cells contain no endogenous ErbB-2. Membranes were stripped and reprobed to confirm equal loading of gp130 (centre). The same lysates were immunoprecipitated and western blotted with anti-ErbB-2 to confirm equal levels of ErbB-2 in each lysate sample (bottom). Similar results were seen in 4 independent experiments and in MDA-MB-231 cells. (B) Time course of gp130 tyrosine phosphorylation in MDA-MB-231 cells. Note that EGFR was used as a control for protein loading in this experiment. Similar results were seen in 3 experiments and in MCF-7 cells.

90 genes are important in cell differentiation, cell cycle, transcription, cell adhesion and metastasis and thus represent interesting candidates. Of these genes, 15 were regulated by the combination of OSM plus EGF only. Further studies are currently in progress to address the significance of these genes in the response to OSM and EGF in breast cancer cells.

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Discovery of SCL target and regulatory genes

CK Bradley, DJ Izon, RL Brake and U Schmidt, in collaboration with Pfizer Inc., Groton, NY, USA

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. 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.

Using gene array technology, two different strategies to uncover new genes that regulate early blood development and hematopoietic cell lineage specification are being conducted in the laboratory:

- Expression profiling of differentiating DU528 cells
- Expression profiling of differentiating SCL-null and WT ES cells

Discovery of genes regulating hematopoietic cell lineages and leukemogenesis using the DU.528 cell line

The human stem cell line DU.528 was established from a 16-year-old boy with T-cell acute leukemia. When the boy was treated with the adenosine deaminase inhibitor, 2'deoxycoformycin, his lymphoid leukemic cells transformed into myeloid cells over the course of 3-4 days. This was an amazing and unexpected response. The cell line DU.528 also responds to 2'deoxycoformycin by undergoing this dramatic change in phenotype.

The DU.528 cell line therefore represents a highly tractable system to allow identification of genes critically important in the stem cell decision to differentiate into myeloid rather than lymphoid cells. The differentiation of the DU.528 cells is stimulated by various combinations of reagents and growth factors. Cell morphology, surface antigen expression, and the expression of several hematopoietic marker genes including SCL is used to characterise the nature and time-course of cell differentiation. Processes involving SCL are then analysed by microarrays in order to find potential SCL target and regulatory genes. Although SCL is clearly established as a key master hematopoietic regulator gene, it is likely that there are additional genes important in this unusual phenotype and highlighted by their involvement in additional translocation events within the DU.528 cells. This strategy also offers a direct approach to identifying those genes, which are likely to be important in both the stem cell phenotype of DU.528 and in the oncogenic steps that have occurred within these cells.

Discovery of genes regulating early blood development by comparison of gene expression in SCL null and wild type embryoid bodies

To identify hematopoietic genes regulated by SCL, we are comparing gene expression profiles between differentiating SCL-null and parental wild-type embryonic stem (ES) cells. Totipotent ES cells, derived from the mouse blastocyst, differentiate into many different cell types under specific culture conditions. When cultured in vitro ES cells form spherical structures, known as embryoid bodies, which progress through a primitive (embryonic) and then definitive (adult) hematopoiesis over a 10-12 day period. This differentiation can be followed by RT-

PCR of known hematopoietic genes.

As ES cell 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 ES cells, derived by sequential targeting of each allele of the SCL locus, do not give rise to blood cells when cultured in vitro or express genes normally transcribed only in early hematopoietic cells. By comparison of SCL null and WT ES cell expression profiles, genes that SCL regulates, as well as the mechanism by which SCL itself is regulated may be identified, which will contribute significantly to our understanding of normal and aberrant hematopoiesis.

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Thesis passed

Suzie L Grant. The Actions of Oncostatin M in
Breast Cancer. PhD Department of Medicine, The
University of Melbourne, 2001.

Awards

AT Reutens. Peter Doherty Fellowship, 2002-2005,
NH&MRC.
SL Grant. WAIMR Publication Award 2001, Western
Australian Institute for Medical Research.

Invited Presentations

CG Begley. Invited speaker at the Childhood
Cancer Conference, Sydney, NSW.
CG Begley. Session Chairman and invited speaker,
The Haematology Society of Australia, Brisbane,
QLD
JR Goethert. The Stem Cell Leukemia gene SCL is
a key regulator in leukemia and during normal
hematopoietic development. Invited speaker at the
Institute of Toxicology, University of Mainz, Germany.
JR Goethert. The Role of SCL in Leukemogenesis.
Invited speaker at the Department of Hematology,

University of Essen, Germany.

SL Grant. The Actions of Oncostatin M in Breast
Cancer. Invited speaker at the Walter and Eliza Hall
Institute for Medical Research, Melbourne, Victoria.

SL Grant. The Actions of Oncostatin M in Breast
Cancer. Invited speaker at the Department of
Medicine, University of Melbourne, Victoria.

DJ Izon. Notch signalling and the T-B cell fate deci-
sion. Invited speaker at the Division of Veterinary
and Biomedical Sciences, Murdoch University, Perth
DJ Izon. Notch signalling in lymphopoiesis. Invited
speaker at the Walter and Eliza Hall Institute for
Medical Research

External Committees

CG Begley. Scientific Advisory Committee of the
Cooperative Research Centre for
Gene Discovery, Melbourne, Victoria

CG Begley. External Scientific Committee of
Queensland Institute for Medical
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Division of Cell Biology

Overview

The principal research theme within the division is elucidation of mechanisms that underlie the initiation and progression of inflammatory diseases of the respiratory tract, with particular emphasis on asthma. This work involves a series of integrated research projects utilising material from both experimental animal and human sources. The former studies focus upon mechanism(s) responsible for regulation of T-cell mediated immune responses in respiratory tract tissues and associated regional lymph nodes. Of particular interest are populations of Dendritic Cells (DC) in respiratory tract tissues, especially within the conducting airways, which have been identified in earlier work within the division as the principal resident antigen presenting cells in these tissues. It is clear from our studies that the DC regulate the intensity and duration of local immune responses to inhaled antigens, which include not only environmental allergens but also viral pathogens, and they also determine the nature of ensuing programming of immunological memory.

Our second major research stream focuses upon postnatal development of T-cell immunity to environmental antigens in humans, in particular airborne allergens relevant to asthma pathogenesis. We have previously described an important genetically determined deficiency in postnatal maturation of Th1 function in children at high risk for atopy, which we believe increases susceptibility to allergic sensitisation and also to respiratory viral infection. We have also recently collaborated with the Martinez group in the US in the identification of a polymorphism in the CD14 gene which is associated with intensity of allergy, and which may also be involved in the Th1 maturation defect referred to above. We additionally have a rapidly growing interest in paediatric vaccine immunology, and in particular in the role of developmental-associated variations in immune function as determinants of vaccine responsiveness.

Immunobiology of Dendritic Cell populations

The role of respiratory tract Dendritic Cells (RTDC) in T-cell activation

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

Since the initial discovery several years ago by our group of the highly developed RTDC network within airway mucosal tissues, it has been widely hypothesised that these cells may be the triggers for local T-cell activation in the late phase response (LPR) in asthma. This hypothesis appears increasingly more plausible as more information comes to light (see particularly "Human RTDC" below). However, in virtually all of the experimental models available, resident airway RTDC express the "sentinel" phenotype i.e. they are specialised for uptake and processing of inhaled antigens, but lack the capacity to function

as efficient antigen presenting cells (APC) for T-cell activation, and normally do not mature into APC until after migration to lymph nodes. Our current studies show promise of resolving this enigma. We have recently demonstrated in a rat asthma model, that following aerosol challenge of sensitised animals with allergen, resident RTDC acquire allergen by endocytosis, and confocal microscopy studies indicate that they then cluster with local T-cells.

Providing allergen-specific T-memory cells are present, the RTDC are themselves rapidly activated and switch briefly to APC mode, before migrating to regional lymph nodes where the bulk of their antigen presentation activities are performed. However during the brief temporal window period between allergen uptake and exiting airway tissue via draining lymph, which is <24 hrs duration but may be shorter, they express sufficient APC activity to activate significant numbers of local T-helper cells, thus triggering the LPR. In ongoing studies we are focusing on the nature of the mechanism(s), such as co-stimulator expression, involved in transient upregulation

of APC functions in RTDC following aerosol challenge, as well as the role of various RTDC subsets in the process, and the link between local T-cell activation and development of hyperresponsiveness to inhaled methacholine.

Responses of rat RTDC subsets to microbial challenge

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

In parallel studies employing alternative airborne inflammatory agents, a series of experiments have been performed to determine the recruitment kinetics and function activities of RTDC subsets in response to inhalation of bacterial stimuli. We have employed an experimental model comprising aerosol challenge of naive rats with heat-killed *Moraxella Catarrhalis*. At a series of time points post-challenge (2, 12, 14, 48, 96 hours), airway wall DC subsets and those of the draining lymph nodes have been analysed for phenotypic and functional changes by confocal microscopy and flow cytometry.

These studies suggest that the early response (within 1-2 hours post challenge) is characterised by rapid mobilisation of a resident subset of RTDC that is high for both MHC class II expression and endocytic activity, and these migrate from the airway wall to the draining lymph nodes. This early response is also associated with upregulation of co-stimulatory molecules (CD86 in particular), and we are currently addressing questions relating to the APC activity of the DC during this period. This initial response is followed at later time points by an influx of precursor cells that re-populate the airway wall. Interestingly, unlike our experience in the allergen-driven asthma model above, RTDC recruitment kinetics are still enhanced at later time points after challenge (up to 96h), suggesting a sustained alteration of the airway wall microenvironment.

Postnatal development of RTDC networks

DJ Strickland, PA Stumbles, I Tobagus, JA Thomas, PG Holt in collaboration with JC Huh (Niigata University School of Medicine, Niigata).

It is now known that key events in the establishment of Th-memory against environmental allergens occur

during the early postnatal period. With respect to inhalant allergens, the route for exposure of the immune system is via the airway mucosa, and hence via RTDC, and it is accordingly important that we gain a detailed understanding of how the functions of these cells are regulated during this period. Our ongoing studies in the rat model are tracking the slow postnatal maturation of RTDC networks between birth and weaning. We have confirmed our earlier findings on generally low level expression of function-associated molecules such as MHC class II during this period, and have also shown that capacity to respond locally to cognate antigen signals is attenuated. However, recent observations additionally have identified a brief window period in the first 48 hrs following birth during which MHC class II-high and CD86-high RTDC are transiently present in small but significant numbers in the airway wall, but disappear by day 3 of life; MHC class II-high RTDC progressively reaccumulate thereafter, attaining adult-equivalent levels around 1-2 weeks post weaning. Our most recent studies indicate that this initial perinatal wave of RTDC are functionally competent, as local (intranasal) antigen exposure at day 1 or day 2 after birth primes the animals for subsequent IgG responses, whereas exposure on day 3 or 4 elicits no detectable priming. The mechanism(s) and biological significance of this observation are presently unknown, but it may be related to epidemiological observations linking risk for pollen sensitisation to birth in spring i.e. a time during which early postnatal allergen exposure is most likely.

Human RTDC

JW Upham, BJ Holt, PG Holt in collaboration with F Jahnsen (Institute of Pathology, Rikshospitalet, Oslo).

We have now completed and published (Thorax 56:823-826, 2001) a landmark study which is the first direct evidence of RTDC participation in the late phase response in human atopic asthma. In collaboration with colleagues in Dublin and Oslo, we have shown rapid recruitment of RTDC into the airway mucosa of atopic asthmatics following bronchial challenge. We have additionally shown that the recruited DC are exclusively from the myeloid lineage; this differs markedly from responses in the nasal mucosa in which plasmacytoid DC are prominent. Follow-up studies are being initiated with colleagues in Scandinavia, which will focus on RTDC in airway biopsies from children.

Studies on human blood derived DC in childhood and adolescence

JW Upham, BJ Holt, PA Stumbles, PG Holt in collaboration with SL Prescott (Department of Paediatrics, University of Western Australia).

While it appears that infants have a reduced capacity to initiate Th1 immune responses, the mechanisms responsible remain poorly defined. Published studies in animal models and in the human system have implicated antigen presenting cell (and in particular, DC) function as one of the rate-limiting factors in postnatal development of immune competence, particularly in relation to expression of Th1 function. Additionally, DC function has been hypothesised to be a factor determining the level of expression of allergic disease in vivo. Accordingly, we have studied the ontogeny of the Th1-trophic cytokine IL-12, by examining circulating mononuclear cells from cord blood, 5 year olds, 12 year olds and adults, following optimal stimulation with LPS and IFN γ . Our findings indicate that human cord blood cells are very poor producers of bioactive IL-12, and that capacity for IL-12 synthesis matures slowly during childhood. Surprisingly, the capacity to synthesise adult levels of IL-12 does not develop until after the age of 12 years. In contrast, neonatal cells synthesise relatively higher amounts of IL-10, such that IL-10:IL-12 ratios are higher in neonates than in older children and adults. The preferential production of IL-10 by neonates may explain their predilection towards the Th2-polarised immunity.

Depletion experiments have indicated that the IL-12 synthesis in peripheral blood arises predominantly from HLA-DR $^{+}$ cells that lack surface markers for monocytes, B-cells and NK cells, and are thus putative DC. Current studies are therefore examining the ontogeny of myeloid and lymphoid DC subsets, in order to determine whether the number and phenotype of these cells varies with age. It appears that numbers of both DC subsets are reduced in neonates, compared to adults, and we are in the process of extending these studies into prospective birth cohorts of older children that will provide unique longitudinal data on DC ontogeny. Emerging data is also suggesting significant relationships between circulating DC in the post-natal period, and the subsequent development of allergic sensitisation.

Phenotypic and functional analysis of RTDC subsets in the mouse

PA Stumbles, I Tobagus, PG Holt.

In addition to our continuing rat studies, new investigations have commenced on airway wall and parenchymal lung RTDC in the mouse. Studies in mice have been initiated in order to take advantage of genetically modified strains that are available for this species, including cytokine knock-out and T cell receptor transgenic animals. A similar panel of functional markers will be included as for those used in rats, as well as additional markers that have been described in the literature to identify murine DC subsets.

DC function in germ-free mice

L Bowman, PG Holt in collaboration with B Björkstén (Karolinska Institute, Stockholm).

Ongoing studies in the division on the relationship between early postnatal exposure to microbial stimuli and maturation of adaptive immune function(s) were broadened during 2001 to include collaborative investigations on APC function in germ free (GF) mice, carried out by senior postgraduate student Lara Bowman in the laboratory of Professor Bengt Björkstén at the Karolinska Institute. The results of these studies are still being evaluated, but some notable findings are already apparent. In particular, it is evident that T-cell immunity in GF-mice is Th2 polarised relative to their microbiologically conventionalised (CV) counterparts, and in vitro crossover experiments with T-cells and DC from DC and CV animals suggest that the dominant signal determining the polarity of Th-cell responses in the GF animals is provided by their DC.

Aetiology and pathogenesis of allergy and asthma

Regulation of Interferon gamma (IFN γ) gene expression

GP White, A Bosco, BJ Holt, PG Holt in collaboration with M Kusel and PD Sly (Division of Clinical Sciences, Telethon Institute for Child Health Research), MJ Sharp and J Rowe (Division of Cell Biology, Telethon Institute for Child Health Research), P Watt (Children's Leukaemia and Cancer Research, Telethon Institute for Child Health Research), SL Prescott (Department of Paediatrics, The University of Western Australia).

We have recently published novel findings that indicate that attenuated capacity to produce IFN γ during the perinatal period in humans is associated with hypermethylation of CpG sites within and adjacent to the promoter of the IFN γ gene. Promoter hypermethylation of this nature has been shown to be associated with negative regulation of gene expression in a number of systems. We have additionally shown that this promoter hypermethylation is restricted to neonatal CD4 $^{+}$ T-helper cells, in contrast to CD8 $^{+}$ T-cells and NK cells in which methylation levels are equivalent to those seen in adulthood. Our most recent studies have focused on the nature and dynamics of changes in CpG methylation patterns in the IFN γ promoter during in vitro cytokine driven Th1 and Th2 polarisation of cord blood versus adult CD4 $^{+}$ T-cells. Analogous to findings in the mouse we have observed rapid demethylation in association with Th1 polarisation, in contrast to maintenance of hypermethylation during Th2 polarisation. Interestingly, the levels of hypomethylation achieved in Th1 lines derived from neonatal CD4 $^{+}$ CD45RA $^{+}$ T-cells are more profound than in their adult counterparts. Additionally, promoter methylation does not play an equivalent role in regulation of the IL-4 gene, as CpG methylation patterns in the IL-4 promoter appear unrelated to gene expression during Th1/Th2 differentiation.

In ongoing studies, we are investigating the possibility that variations in IFN γ promoter methylation patterns may underlie the differences in IFN γ production capacity in children at low versus high risk to atopic disease. These include assessment of age-related changes in prospective cohort studies, and cross-sectional studies in children and adults.

Studies on responses to microbial stimuli by cord blood cells

MJ Sharp, J Rowe, D Suriyaarachchi, T Heaton, PG Holt in collaboration with M Kusel and PD Sly (Division of Clinical Sciences, Telethon Institute for Child Health Research) and D Mallon (Princess Margaret Hospital for Children and Fremantle Hospital).

One of the major ongoing research interests in our group is the role of the microbial environment in driving the postnatal maturation of immune function, and the impact of this process on susceptibility to development of atopy and asthma. As part of these studies we have recently shown that PBMC from

adults with active allergic disease, in particular atopic dermatitis (AD), are hyperresponsive to bacterial superantigen, especially SEB from staphylococcus, and furthermore these effects are directed selectively towards the Th2 cytokine IL-5. In follow-up studies we are addressing the issue of whether susceptibility to SEB is acquired or inherent, and whether SEB reactivity associates with susceptibility to atopic disease in infants. Our recent findings indicate a consistent association between responsiveness to SEB in cord blood as determined by IL-5 production, and development of AD up to age 3 years. Additionally, risk for AD development was also associated with hyperresponsiveness to mycobacterial-derived PPD, but in this case associated with IL-10 and IFN γ production. Follow-up studies are in progress to track these responses through to age 5 years, to ascertain their relationship to development of SPT reactivity to dietary and inhalant allergens, and to determine the nature of the triggering stimuli and target cells in relation to neonatal PPD reactivity.

The role of infections in the aetiology of atopy and asthma

J Rowe, T Heaton, D Suriyaarachchi, BJ Holt, JA Thomas in collaboration with PD Sly and M Kusel (Division of Clinical Sciences, Telethon Institute for Child Health Research), N de Klerk and W Oddy (Population Sciences: Biostatistics & Genetic Epidemiology, Telethon Institute for Child Health Research), P Openshaw (St Mary's Hospital, London), S Johnston (Imperial College, London) and P Beverley (Edward Jenner Institute for Vaccine Research, Compton).

Our research interests in this area span a broad spectrum including the nature of the potential overlap between susceptibility to allergic sensitisation and respiratory viral infection in infancy, host defence mechanisms in RSV infection in infants and how these relate to subsequent asthma development, and association between type and frequency of infection in infancy and subsequent development of asthma and atopy.

We will soon publish the results of analyses on the RAINE study cohort (n~2000) at the 6 yr follow-up, which addresses the interactions between atopy and infections in infancy. The findings to be reported provide clear evidence of synergistic interactions between severe wheezing lower respiratory tract ill-

ness (WLR) during the first year of life (most likely to be viral induced), atopic sensitisation to inhalants, and asthma at 6 yrs. The most notable interactions occur in subjects with multiple WLRs, and our hypothesis is that the same genetically determined developmental deficiency in Th1 function during infancy underlies susceptibility to both infection and atopy.

In addition to this cohort, we are following the progress of a cohort (n~240) of children at high risk of asthma, up to their 5th birthday, collecting blood regularly and cryobanking PBMC, and cryobanking postnasal aspirates (PNA) or swabs taken at every episode of reported respiratory infection. Samples spanning the first year of PNA collections (n=1300) are currently undergoing PCR analyses in Sebastian Johnston's lab in London for typing of pathogens, and ongoing studies in our lab are focusing on innate and adaptive immune responses in the cryobanked blood samples collected between birth and age 1 year. The latter studies include responses to polyclonal stimuli, microbial antigens, and allergens. We have also initiated studies on acute host responses to RSV in infants with severe (hospitalised) versus mild (ambulatory) infections, and will follow up these subjects to examine ensuing outcomes related to atopy/asthma.

T-cell immunity to seasonal versus perennial allergens in children

A Rudin, BJ Holt, PG Holt in collaboration with C Macaubas (Division of Cell Biology, Telethon Institute for Child Health Research) and PD Sly (Division of Clinical Sciences, Telethon Institute for Child Health Research).

These studies have provided clear evidence of "bystander" stimulation of T-cell immunity to a seasonal allergen (Rye grass) via ongoing responses to the perennial allergen House Dust Mite (HDM). The key finding is that in a large group of children who are SPT+ to Rye, circulating Rye-responsive Th2 cells are only present in the blood "out of pollen season" in children who are concomitantly sensitised to a perennial allergen such as HDM. These findings are important from several viewpoints, but in particular provide a potential mechanism to explain clinical findings which suggest that desensitisation of children to individual "major" allergens can also provide protection against sensitisation to other allergens present in the environment.

Differential gene expression studies in peanut allergy

A Bosco, A Rate, BJ Holt, PG Holt in collaboration with Richard Loh (Department of Clinical Immunology, Princess Margaret Hospital for Children), SL Prescott (Department of Paediatrics, University of Western Australia), D Mallon (Princess Margaret Hospital for Children and Fremantle Hospital), WR Thomas (Division of Molecular Biology, Telethon Institute for Child Health Research).

We are in the process of initiating a program aimed at identification of specific, covert elements in the T-cell responses of peanut-allergic subjects that are associated with susceptibility to potentially fatal anaphylaxis. Peanut allergy is unique with respect to high risk for anaphylaxis, and is increasing in frequency in developed countries such as Australia. Our approach involves screening allergen-specific T-cell responses in peanut responsive atopics employing Affymetrix microarrays, to identify a panel of genes associated with disease severity. The expression of these genes will then be further explored employing more quantitative Taqman PCR methodology, in panels of subjects spanning a broad range of clinical phenotypes.

As well as generating primary data of relevance to peanut allergy, these studies will assist in establishing a methodological base for more comprehensive Affymetrix gene array studies in asthma and allergic rhinitis, which are programmed to start in mid 2002.

Studies on fetal allergen-reactive T cells

CA Jones, BJ Holt, PG Holt.

While numerous groups have demonstrated that umbilical cord blood mononuclear cells from term and preterm neonates respond to a variety of antigens, the nature of this response has not been characterised in any detail. We have demonstrated that the response is dependent on the presence of MHC Class II-positive antigen presenting cells and have focused on further characterising the response of the CD4+ subset of T cells. Of particular interest, we have observed that programmed cell death (apoptosis) after allergen stimulation is more predominant amongst cord blood T cells compared to adult peripheral blood T cells. Preliminary findings suggest that the population of T cells that survive in these cultures are regulatory T cells as they co-

express CD25 and CTLA4, are anergic, and are suppressive in a mixed leukocyte reaction. Further investigations of the features of these cells that favour survival are under way. As a result of these findings we have postulated that antigen-induced proliferation by umbilical cord blood mononuclear cells is a marker of peripheral deletion rather than a specific recall response. This is further supported by the finding that allergen-specific reactivity in cord blood remains demonstrable upon the removal of CD45RO+ "memory" T cells. The biological significance of these findings remains to be established, in particular the relationship of these putative regulatory cells to the subsequent development of conventional CD4+ T-cell memory.

Vaccine immunity in early childhood

Longitudinal studies on vaccine-specific immunity during the preschool years

J Rowe, D Suriyaarachchi, BJ Holt, PG Holt in collaboration with PD Sly (Division of Clinical Sciences, Telethon Institute for Child Health Research), R Loh and P Richmond (Department of Clinical Immunology, Princess Margaret Hospital for Children).

We have recently published (J Infect Dis 184:80-88, 2001) our results on postnatal maturation of DTPa-specific T-cell immunity in infants up to the age of 18 months. This study on a large cohort of 130 children provided a range of new information, including comprehensive data on the ontogeny of Th1 (IFN γ) function in the population, the nature of the Th1/Th2 balance in vaccine-specific responses and their relationship to risk for atopy, and relationship between the kinetics of postnatal maturation of Th1 function and susceptibility to RSV infection. Additionally, it provided the first indication that capacity to express Th1 immunity to vaccine antigens subsequent to priming may be limited by developmental processes extrinsic to vaccine-specific immune responses, but intrinsic to the immune system at large. The limitation of this study was that for logistical reasons, it was terminated at 18 months, leaving unanswered key questions relating to the stability of Th1 memory between infancy and the booster vaccination given at age 5 yrs. To address this issue we have initiated studies on another birth cohort of 240 subjects which are being monitored to age 5, including annual blood collections which are being cryobanked for assessment of

immune function, and DTPa-specific immunity will be included within the protocol. In addition, we are recalling a subset of the original cohort employed in the published study cited above, with the aim of assessing their responses to Tetanus Toxoid before/after their 5 yr booster vaccine.

Immunostimulatory potential of vaccines during infancy

J Rowe, D Suriyaarachchi, P Richmond and PG Holt in collaboration with PD Sly (Division of Clinical Sciences, Telethon Institute for Child Health Research), R Loh and P Richmond (Department of Clinical Immunology, Princess Margaret Hospital for Children).

One of the long-term goals of vaccine research is the development of multivalent vaccines that will provide long-lasting protection against the widest possible range of pathogens from as early as possible during infancy. However, with the introduction of these potent vaccines, it is important to gain a clear understanding on what non-specific effects they may have on the developing infant immune system, and virtually no published information is available relating to this question. We have recently completed a pilot study on a cohort of 48 children before and after the administration of a Measles-Mumps-Rubella-Varicella combination vaccine, given at 12 months. In this study, we have examined the response to allergens (HDM and cat), a vaccine antigen (tetanus toxoid), and a polyclonal stimulus (PHA). Peripheral blood mononuclear cells were isolated and cultured either alone, or together with the allergens, vaccine antigen or polyclonal mitogens, and cytokine production determined. At the population level, there were no significant differences when comparing pre and post vaccination PBMC responses to the allergens, the unrelated vaccine antigen, or the polyclonal mitogen. However, preliminary data suggests that polyclonal responses are heterogeneous within the population, with a subset of the children exhibiting increased polyclonal responses 6 weeks after the vaccination, when compared to pre-vaccination responses. Further studies are required to determine whether the fluctuations observed in overall immune function in this study group is due to the boosting effects of the vaccine, or simply a reflection of cyclical changes during the natural development of immune function, and a follow-up to clarify this issue is in the planning stages.

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Invited Presentations

PG Holt. Plenary Speaker: Programming of allergen-specific immunological memory during early life - 30th Anniversary Meeting, Finish Paediatric Association, Division of Allergy & Clinical Immunology, Helsinki, 2001.

PG Holt. Workshop Participant: The Allergic March - UCB Institute of Allergy, Berlin, 2001.

PG Holt. Plenary Speaker: The early life origins of asthma and allergy - European Academy of Allergy & Clinical Immunology, Berlin, 2001.

PG Holt. Workshop Participant: Peanut Allergy - US Food Allergy Institute, New York, 2001.

PG Holt. Symposium Speaker: The development of the immune system during early life - potential modulation by early infections - American Thoracic Society, San Francisco, 2001.

PG Holt. Symposium Speaker: Implications of the "Hygiene Hypothesis" for primary prevention of allergy and asthma - American Thoracic Society, San Francisco, 2001.

PG Holt. Plenary Speaker: Selective maturational deficiency in Th1 function as a risk factor for early life infections - International Workshop on Immunomodulation in Infancy, Geneva, 2001.

PG Holt. Plenary Speaker: Biology of airway inflammation: Initiator cells - 3rd Triennial World Asthma Meeting, American Thoracic Society/American Academy of Allergy, Asthma & Immunology, Chicago, 2001.

PG Holt. Plenary Speaker: Functionally distinct Dendritic Cell populations in the respiratory tract regulate immune responses to inhaled antigens - 13th International Congress, International Society for Aerosols in Medicine, Interlaken, 2001.

PG Holt. Symposium Speaker/Chair: Regulation of the induction and expression of allergic inflammation in the respiratory tract - 5th World Congress on Inflammation, Edinburgh, 2001.

PG Holt. Symposium Speaker: Primary allergy prevention: targeting immunological events in early childhood - 4th International Symposium on Specific Allergy, Copenhagen, 2001.

PG Holt. Symposium Speaker: Immunopathogenesis of Airway Inflammation in Asthma - Annual Meeting of American College of Allergy, Asthma & Immunology, Orlando, 2001.

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.

Children's Leukaemia and Other Cancers

Overview

Leukaemia is the most common form of cancer in children, accounting for a third of cases, followed by brain tumours that are diagnosed in 25% of patients. The Oncology Total Care Unit at the Princess Margaret Hospital (PMH) and our research laboratory maintain a close relationship and we are members of the largest study group into childhood cancers, the US-based Children's Oncology Group (COG).

Despite tremendous improvements in therapy, some patients still experience relapse. Our research focuses on the genetic events that lead to cancer formation and on the development of more effective and less toxic anti-cancer drugs. Both areas depend on a better understanding of the genetic differences between normal and cancer cells. This in turn will change our approach to the diagnosis, classification and treatment of childhood cancer.

The research program of the division comprises four areas:

- First, gene expression profiles in childhood acute lymphoblastic leukaemia (ALL), where we make use of the novel microarray technology. The HOX11 oncogene is of particular interest. It was discovered at a chromosomal breakpoint in one of our cell lines from a patient diagnosed with ALL of T-cell type (T-ALL). HOX11 is a DNA-binding oncoprotein aberrantly expressed in a significant proportion of T-ALL patients. Our recent studies confirm that HOX11 deregulation occurs in the absence of any translocation, hence other mechanisms must cause gene activation and these are currently under investigation. We have employed various approaches to identify genes whose expression is altered by HOX11.
- Second, in previous work we showed that deletion of the tumour suppressor gene p16 is associated with unfavourable outcome in paediatric ALL. Our most recent studies were conducted using real-time polymerase chain reaction (PCR) which confirmed and extended these findings. This method is a precise high-throughput assay with applications in a wide range of cancers.
- Third, a new research program focuses on paediatric brain tumours. The major aim is to identify tumour suppressor genes by using representational difference analysis in combination with array technology.
- Fourth, our research is targeted at the development of new approaches to find anti-cancer drugs. The current project aims at developing a platform technology for isolating specific peptide inhibitors of oncoprotein interactions. The study provides the groundwork for a high-throughput screening system for novel peptide-based drugs.

Key words: childhood cancer, leukaemia, brain tumours, oncogenes, tumour suppressor genes, anti-cancer drug screening.

Gene expression in paediatric ALL

Gene expression profiles in childhood leukaemia cell lines

UR Kees and J Ford in collaboration with RL Walker and P Meltzer, National Human Genome Research Institute, NIH, Bethesda, MD, USA.

The prognosis for children with ALL has dramatically improved over the past decades and survival has

reached 70-75%. Up to 85% of patients classified as standard risk are projected to be long-term survivors. Current treatment protocols make use of risk-directed therapy with the aim of administering intensified therapy to those patients with a high likelihood of relapse and decreasing toxicity for those patients with a lower risk of relapse, while maintaining high cure rates. The risk stratification for appropriate therapy is based on clinical and laboratory features, including age, white blood count, immunopheno-

type, DNA index, cytogenetics and early response to induction chemotherapy. However, a substantial number of patients currently classified and treated as standard risk patients do continue to relapse. The novel microarray technology has the capacity to identify gene expression patterns that can predict outcome in cancer patients. Since cell lines provide ideal models for the assessment of potential novel therapies, we analysed gene expression in our panel of established paediatric leukaemia cell lines, comprising ALL of B-lineage and T-ALL phenotype. Hybridisations were performed using microarrays containing 13,825 cDNA clones. There was excellent correlation between gene expression and independently assessed genetic features, as well as protein expression determined by cell surface marker analysis. The cluster analysis showed clear segregation between B-lineage and T-ALL. The genes that best separated the two diseases were ranked by weight. This weighted list identified several hundred candidate genes potentially related to lineage specific leukaemogenesis. Furthermore, the results revealed considerable heterogeneity among T-ALLs, suggesting that this entity may be composed of distinct subgroups. Further investigation will explore the biological and clinical implications of these disease gene expression patterns.

The HOX11 oncogene is expressed in half the cases of paediatric T-ALL and occurs in the absence of cytogenetically detectable aberration

UR Kees, R Kumar and PM Watt in collaboration with DL Baker, Princess Margaret Hospital and NA Heerema, F Uckun, M La and H Sather, Children's Oncology Group, Arcadia, USA.

The HOX11 is a proto-oncogene that plays a role in T-ALL. The major objective of this project was to measure HOX11 gene expression in paediatric T-ALL patients in order to test the hypothesis that HOX11 expression is associated with clinical outcome. The second aim was to correlate transcriptional activation of the HOX11 gene with cytogenetic findings for each patient. Expression of HOX11 was studied in 76 bone marrow specimens of T-ALL patients obtained from the COG ALL Biology Reference Laboratory. The real-time quantitative RT-PCR method was used to determine HOX11 and b-actin expression, the latter gene as reference in a multiplex reaction. The patients whose leukemia cells showed expression of HOX11 were also stud-

ied with respect to cytogenetic aberrations occurring at the HOX11 locus in chromosomal band 10q24. Aberrant HOX11 expression was present in 37 of 76 patient specimens (49%), yet was never found in B-cell lineage ALL. Direct cytogenetic analysis of the T-ALL specimens showing HOX11 expression revealed that only two of 16 exhibited abnormalities of the HOX11 locus at 10q24. These results confirm and extend our previously published findings and implicate mechanisms other than chromosomal translocations for the deregulation of HOX11. Analysis of clinical outcome for the whole study group showed a trend for better outcome for patients with leukaemia cells expressing HOX11 at high or intermediate levels compared to the other patients.

Transcriptional control by HOX11

Gene expression profile induced by the HOX11 T-ALL oncogene

K Hoffmann, J Ford and UR Kees in collaboration with N de Klerk, Division of Biostatistics, ICHR.

The T-cell oncogene HOX11 is activated in T-ALL by specific chromosomal translocations involving the T-cell receptor genes. HOX11 activation is implicated as a crucial step in the pathogenesis of T-ALL, based on evidence that as many as 49% of T-ALLs exhibit deregulated HOX11 expression, often in the absence of chromosomal translocations. Targeted genes disruption has revealed this homeoprotein to be essential for spleen development. In order to identify downstream functions of HOX11 we generated stable transfectants of the human cell line PER-117 and assessed their expression patterns by oligonucleotide microarray analysis (Affymetrix HG-U95A). Enforced expression of HOX11 revealed transcriptional stimulation and repression of a large number of genes. We focused on genes highly deregulated and giving concordant results in independent stable clones. We identified those yielding a more than fourfold difference in expression compared to the untransfected PER-117 cells. 31 genes were found to be upregulated and 11 downregulated. Comparison with cell line PER-255 which expresses HOX11 due to a chromosomal translocation of the HOX11 locus revealed 12 of the upregulated genes to be highly expressed and 6 of the downregulated genes to be expressed at low levels. Some of the differentially expressed genes are implicated in cell proliferation, cell adhesion or have

enzymatic activity. The identified genes also included many whose correlation with cancer development had not been recognized before. Our data should contribute to a greater understanding of the mechanisms by which HOX11 activation leads to human T-cell tumours. The further investigation of the genes will explore the biological implications of the identified gene expression patterns.

The search for genes regulated by HOX11

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Abundant evidence suggests that the immortalising ability of HOX11 is not restricted to T-cells. We therefore examined its effect in the erythroid lineage using the J2E cell line, which is capable of terminal differentiation. J2E cells were profoundly altered by HOX11 in terms of cellular morphology and differentiation status. This result lends support to the notion that HOX11 function to immortalise cells by disrupting haematopoietic differentiation. This is likely to be due to HOX11 directly or indirectly changing the expression of regulatory and structural genes associated with cell lineage commitment. Thus, we have employed representational difference analysis and cDNA array technology to find genes whose expression is altered by HOX11. A number of candidates have been identified, which intriguingly, encode proteins involved in the regulation of the cell cycle, or play a role in cell differentiation or cell adhesion. Notably, a gene encoding guanine nucleotide dissociation inhibitor beta (GDIb) was identified as a potential HOX11 target gene. By Northern blot analysis, GDIb was found to be upregulated in J2E cells as a result of enforced HOX11 expression. More significantly, GDIb was aberrantly expressed in three of three HOX11 positive T-ALL cell lines, but was not detectable in seven HOX11 negative T-ALL cell lines tested, nor in normal human thymocytes. GDIb functions as a signalling factor to regulate vesicle membrane transport and is known to facilitate expression of adhesion molecules. Future studies will seek to determine whether this target is oncogenically relevant in a bid to piece together the mechanism by which this homeoprotein immortalises cells.

Regulation of ALDH1A1 Gene Expression by HOX11

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

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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, aldehyde dehydrogenase 1a1 and Slim1 (Greene et al, 1998). The former is intriguing because of its demonstrated role in synthesizing retinoic acid, a key modulator of several cellular process including differentiation. We have now begun to use 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. Our preliminary data has demonstrated that the ALDH1A1 promoter can be transactivated approximately 12-fold in HEL cells in a HOX11-dependent manner.

The available evidence suggests that HOX11 binds to a core sequence consisting of TAAGTG. This site, however, was determined 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 sites on the ALDH1A1 promoter, various deletions of the promoter have been generated by PCR and cloned into pGL3-Basic. Reporter gene activity of each construct will be measured and responsive sites will be further delineated by synthesizing oligonucleotides 20-40 base pairs in length and cloning these upstream of a minimal promoter fragment in pGL3-Basic. Resultant transactivation of the luciferase gene above background would indicate the presence of a HOX11-responsive site.

In parallel experiments on the same cell lines, luciferase reporter gene assays will also be performed using a retinoic acid response element introduced into pGL3-Basic. A significant difference between HOX11 and non-HOX11 expressing cells would be consistent with altered ALDH1A1 expression leading to a change in retinoic acid levels.

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 that is silent in normal mature T-cells while being aberrantly activated in T-cell acute lymphoblastic leukemia (T-ALL) by translocation. Many oncogenes are expressed in alternate forms in cancer, however to date only one form of the human HOX11 transcript has been reported. We identified and characterised 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 that 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 characterised 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.

Expression of HOX11 as a GST Fusion Protein

M Heidari*, KL Rice*, UR Kees and WK Greene.
*WABRI, DVBS, Murdoch University

HOX11 is a transcription factor belonging to the homeodomain family that is essential for spleen development during embryogenesis. It is also tumorigenic, being associated with T-ALL in children. In order to understand the functional role of HOX11 in both normal development and malignancy, protein-DNA and protein-protein interaction studies involving this factor are required. Such investigations would be facilitated by the availability of significant amounts of purified HOX11 protein. However, expression of full-length HOX11 in bacteria has been reported to be problematic owing to fusion protein instability. We have purified human HOX11 expressed in *E. coli* as a soluble and functional glutathione S-transferase (GST) fusion protein. In addition, a mutant version of HOX11 was produced (HOX11DH3) which lacked the DNA-recognition helix (helix 3) of the homeodomain. Through a single purification procedure using glutathione-Sepharose, 2 mg of the recombinant proteins were obtained per litre of bacterial culture. Notably, recombinant GST-HOX11 fusion proteins had a markedly higher stability when purified at low temperature (4°C). Purification to near-homogeneity was achieved as judged by SDS-PAGE and the purified proteins were recognized by anti-HOX11 antibodies. The biological activity of the recombinant protein was verified by the specific binding of GST-HOX11, but not GST-HOX11DH3, to DNA containing consensus HOX11 recognition sites.

Tumour suppressor genes in paediatric leukaemia

Deletions at the INK4/ARF locus in paediatric acute lymphoblastic leukaemia

UR Kees and P Terry in collaboration with N de Klerk, Division of Biostatistics, TVW Telethon Institute for Child Health Research, DL Baker, Princess

Margaret Hospital, H Sather and M. La, Children's Oncology Group, Arcadia, USA.

The genes at the INK4/ARF locus at 9p21 are frequently involved in human cancer. Virtually all p16INK4A exon 2 (henceforth called p16 E2) inactivation in paediatric ALL occurs by gene deletion. We pioneered the use of real-time quantitative polymerase chain reaction (PCR) for the detection of gene deletion in primary patient specimens and achieved a precision not previously obtained by conventional methods. We were able to demonstrate that childhood ALL patients who showed deletion of the p16 E2 in their leukaemia cells had an increased risk of relapse. Compared to patients with no deletion, patients with hemizygous (D/G) deletion have a 6.6-fold risk of relapse and patients with homozygous (D/D) deletion an 11.6-fold risk. This clearly indicated that p16 E2 deletion was an independent prognostic indicator of outcome from therapy. In order to confirm this finding we studied a much larger patient group, comprising 307 patients who were treated on three recently completed studies, CCG-1882, CCG-1901 and CCG-1922. The study had two aims, first, to examine the prognostic significance of deletion of the p14, p15 and p16 genes at the INK4A/ARF and INK4B loci and second, to test the hypothesis that the incidence of specific nonrandom combinations of molecular genetic lesions is correlated with clinical outcome. Multiplex reactions were established to assess the status of four additional exons, including p15E1, p15E2, p14E1b and p16E1a. Based on the results for all patient specimens, the deletion status was assigned for the three genes, taking into consideration that p16E2 is shared between p14ARF and p16. Of the 307 patients in the study, 198 were diagnosed as standard risk (SR) and 109 were high risk (HR) patients based on conventional criteria (age and WBC). The DNA status was determined for all three genes at the locus. Interestingly, for all genes the frequencies were clearly different for SR patients compared to HR patients, showing a markedly higher rate of D/D patients in the HR group, compared to the SR group. For the three genes at the INK4 locus a RT-PCR method was developed to determine the incidence of gene expression in 109 specimens. A small group of patient specimens expressed p15, while the other two genes were expressed in 30-60% of cases. The correlation analysis with clinical outcome of patients is in progress. This is the first study on a large cohort of paediatric ALL patients

that comprehensively assessed the genotype and gene expression patterns for the three genes at the INK4 locus which contains two well characterised tumour suppressor genes, p14ARF and p16.

INK4a/ARF deletions are acquired at relapse in childhood ALL: a paired study on 25 patients

TL Carter and UR Kees in collaboration with GH Reaman, Children's National Medical Centre, Washington, DC, USA.

Current risk adjusted intensive therapies for childhood ALL are expected to result in an event free survival of greater than 75%. In sharp contrast, relapsed paediatric ALL is a difficult disease to treat. In this study, 25 paediatric patients with ALL were analysed at diagnosis and relapse for their p16 E2 status using the most accurate method of detection, real-time PCR. The median time to relapse for the group was 27 months. At diagnosis the incidence of p16 E2 homozygous and hemizygous deletion in this group was 32% and 20%, respectively. The incidence of homozygous p16 E2 deletion at relapse was 64%. A large number of patients, eight of 16 (50%), developed p16 E2 homozygous deletion at relapse. Of those 8 patients 4 were hemizygous and 4 were germline at diagnosis. At diagnosis those patients with a homozygous or hemizygous p16 E2 deletion relapsed sooner than those germline for p16 E2. We have shown that p16 E2 alterations are frequently present in relapsed lymphoblastic leukaemia in children.

Paediatric brain cancers

The identification of tumour suppressor genes involved in the growth and development of primitive neuroectodermal tumours

PB Dallas and UR Kees in collaboration with D Stephan, Children's National Medical Center, Washington, DC.

Primitive neuroectodermal tumours (PNETs) are the most common form of childhood brain cancer. Unfortunately, the 5 year survival rate is relatively poor, post-operative sequelae are often serious, and better therapeutic strategies are urgently required. To address these issues we are investigating the molecular genetic basis of PNET growth and development. Our current emphasis is towards the identi-

fication of tumour suppressor genes that are inactivated or deleted in PNETs. A few tumour suppressor genes, including PTEN/MMAC1, DMBT1, and PTCH are mutated in a small percentage of PNETs.

However, it is clear from the molecular and cytogenetic data that other tumour suppressor genes are involved in PNET pathogenesis. We are searching for these genes using a novel correlative approach combining representational difference analysis (RDA) with microarray expression analysis.

We have generated 71 DNA clones by applying a RDA procedure designed to detect DNA sequences that are deleted in our three PNET cell lines. The DNA sequences of these clones have been determined, and we have taken advantage of the recently completed draft version of the human genome to identify the chromosomal origins of our clones. So far, we have identified one homozygously deleted and 6 hemizyously deleted regions in our PNET cell lines pointing to 7 chromosomal locations of interest (Xq, 10q, 16q, 14q, 7p, 3q, and 1p). Some of these regions (in particular 10q and 16q) have been consistently implicated in PNET pathogenesis based on previous studies. These findings are encouraging, and we are currently mining the human genome databases for candidate tumour suppressor genes mapping to these high priority chromosomal regions. We have access to PNET specimens in local and international tumour banks that will allow us to assess the frequency of deletion of targeted sequences in a large number of PNET specimens.

Having completed our RDA experiments we are now moving on to the next phase of our research plan, microarray expression analysis. In collaboration with Dr Dietrich Stephan at The Children's National Medical Centre in Washington DC, we have undertaken a survey of the expression levels of nearly 2000 cancer related genes in our three cell lines. We are currently assessing these and other recently released PNET microarray data in relation to our RDA results. We intend to expand our analysis to investigate the transcript levels of 14,000 genes in each of our cell lines and primary tumour specimens.

We anticipate that our RDA data, when combined with data obtained from expression microarray experiments proposed for the near future, will expedite our search for PNET tumour suppressor genes

and ultimately lead to a clearer understanding of the molecular pathways involved in PNET growth and development.

Leukaemia drug discovery program

Development of a new cancer drug discovery platform using yeast reverse two hybrid screening

PM Watt, R Hopkins and T Johanssen in collaboration with E Golemis, Fox Chase Cancer Centre, Philadelphia USA.

This project has focused on the development of a genetic system for isolating specific peptide inhibitors of oncoprotein interactions, which is sufficiently robust for routine industrial application. Our model system makes use of 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. Using homologous recombination we constructed a panel of eight different dual-reporter yeast strains that are conditionally resistant to the drugs cycloheximide and 5-FOA. These strains all contain the counter-selectable reporter genes URA3 and CYH2 that are activated in the presence of the interaction of interest, causing the yeast to die in the presence of drug. We have constructed vector constructs for expression of interacting proteins using a low copy-number inducible expression vector. These vectors possess the advantage of enabling adjustment of expression level by titration of the amount of galactose present in the yeast media. This inducible system was devised to overcome background 5-FOA toxicity problems that arose due to autoactivation of bait constructs. In this system, we constructed multiple expression plasmids encoding the interacting oncoprotein pairs: FOS/JUN, JUN/JNK, LMO2/SCL and E47/SCL. We have also shown that the galactose titration approach is sufficient to overcome the auto-activation problem and enables powerful control of stringency. The interacting oncoprotein pairs were shown to activate transcription of the counterselectable reporter genes in our selection system and to cause the death of the host yeast strain under the restrictive selection condition. In addition, our system has confirmed the interaction of a model peptide that can interact with JNK, inhibiting its interaction with JUN. The work of our collaborator, Dr

Bogoyovich at the UWA Biochemistry department has established that this peptide can inhibit the enzymatic activity of JNK.

We have also constructed a range of reverse two hybrid vectors for high-throughput screening which are suitable for mammalian, bacterial and yeast expression and contain an alternative selectable marker conferring resistance to blasticidin (Bsd). Clones isolated from libraries constructed in these vectors can be shuttled directly into leukaemia cell growth inhibition assays. A library of 700,000 potential peptide drug leads has been constructed using one such shuttle vector. In this project we have established a practical, high-throughput screening system which will provide a useful platform technology for the identification of novel peptide-based anticancer drugs. 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 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.

Staff and Students

Head of Division

Ursula R Kees, PhD, Adj. Associate Professor UWA

Research Staff

Peter B Dallas, PhD

Darryl D'Souza, BSc (Hons)

Jette Ford, BAppl Sc, GradDipComp

Wayne K Greene, PhD (at Murdoch University)

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Richard Hopkins, PhD

Timothy Johanssen, BSc (Hons)

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Philippa Terry, BSc (Hons)

Paul M Watt, D.Phil

Support Staff

Stewart Cattach

Students

Rachael Brake, BSc (Hons), PhD candidate

Tina L Carter, MBBS, FRACP, PhD candidate

Nadia M Milech, BSc (Hons), PhD candidate

Violet Peeva, BSc Honours candidate

Thesis passed

Violet Peeva. A search for tumour suppressor genes in primitive neuroectodermal tumours. Honours thesis. University of Western Australia.

External Committees

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

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

PM. Watt. Committee Member of the Combined Biological Sciences Association of Western Australia

WK Greene. Committee Member of the Lotteries State Microarray Facility Implementation Committee

WK Greene. Member of the Western Australian Biomedical Research Institute Senior Management Group

Invited Presentations

UR Kees. Molecular pathology of childhood leukemia. St. Jude Children's Research Hospital. Memphis, USA.

UR Kees. Molecular pathology of childhood leukemia. Children's National Medical Center, Washington, USA.

UR Kees. Molecular genetics of childhood leukaemia. University of Zurich, Switzerland.

UR Kees, J Ford, RL Walker and P Meltzer. Gene expression profiles in childhood leukaemia cell lines. International Symposium "Childhood Cancer Conference", Sydney.

PM Watt. Protein Workshop. Western Australian Institute for Medical Research.

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, Australia, New Zealand and UK. Applicant: TVW Telethon Institute for Child Health Research

UR Kees and PM Watt (1999). Inventors on patent entitled: "Method of detecting the presence or absence of specific genes" filed internationally under the PCT. Priority date November 3, 1999 claimed from US provisional application number 60/163252.

Applicant: TVW Telethon Institute for Child Health Research

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

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.

Acknowledgments

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Division of Clinical Sciences

Overview

2001 has been a year of considerable change and achievement for the Division of Clinical Sciences. The major achievement was the successful application to the NH&MRC for a five-year program grant. This application, titled "Developmental aspects of respiratory inflammation, allergy and asthma" was submitted on behalf of 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). This grant, due to commence in January 2002, gives the asthma program secure funding for the next five years and will act as a platform for major advances in our research in this area.

We also saw some major changes in staff and research directions during 2001. Ann Callaghan retired and we will miss her calming and mature influence. Karen Willet also left us to pursue a career in State Government public service. With Karen's departure Clinical Sciences is no longer actively pursuing Lung Growth and Development as a major research theme. Debra Turner has joined our group as the Program Co-ordinator of the Respiratory Physiology Group. We are extremely pleased to have Debra join our group and she strengthens the physiological expertise of Clinical Sciences considerably.

The Division of Clinical Sciences aims to conduct high quality, clinically oriented research that focuses on paediatric respiratory diseases, especially asthma, cystic fibrosis and vaccine preventable disease. The division consists of five subgroups loosely based around different methodologies. There is close interaction and some overlap of staff between the groups.

The Infant Lung Function Group works on the development and application of new techniques for measuring lung function in infants up to the age of two years. These new techniques are used to measure the growth and development of airways and lung tissues, determine the site of action within the lungs of asthma drugs and determine whether lung function abnormalities predict which infants will have persistent asthma.

The Respiratory Physiology Group uses sophisticated measurements of lung function in small animals to investigate: structure-function correlates in animal models; mechanisms of atopic sensitisation, bronchial responsiveness and asthma; the role of virus infections in altering lung structure and function; and mechanisms of inflammatory damage to lungs in animal models of human lung diseases.

The Cystic Fibrosis Group studies the mechanisms underlying the host inflammatory response in cystic fibrosis as well as issues related to diet, growth and nutritional requirements. The group also develops strategies to prevent progressive lung damage in this condition. Laboratory-based techniques, animal models and clinical methodologies are used to conduct multi-disciplinary research in close collaboration with the Cystic Fibrosis Clinic at Princess Margaret Hospital for Children.

The Clinical Asthma Research Group conducts projects involving infants and children with asthma. Projects focus on the mechanisms underlying the development of asthma and on better methods for managing, monitoring and treating asthma. This group runs a number of cohort studies looking at the antenatal antecedents of asthma, the influence of infections on the development of allergic sensitisation and the genetic basis of asthma.

The Vaccine Trials Group is a collaborative venture between the Division of Clinical Sciences, Princess Margaret Hospital for Children and the Department of Paediatrics, University of WA. The group performs Phase 1, 2 and 3 trials with new vaccines, conducts trials into non-vaccine treatments of vaccine-preventable diseases and conducts research into the development of immunity to vaccines and vaccine-preventable diseases.

Infant Lung Function

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

S Stick, P Franklin, S Turner, R Mutch

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

S Stick, P Franklin, S Turner, R Mutch

A double blind, randomised-controlled study of inhaled Fluticasone Dipropionate is in progress. Outcome measures are lung function using the RVRTC method, exhaled nitric oxide 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 aim to recruit 40 children to this study and hope that the final 5 subjects will complete the study this winter. Funding: Glaxo-Smith-Kline

Epithelial Nitric Oxide Synthase expression in asthma and atopy

S Stick, J Legg, A Moeller, C Lane, Darryl Knight

Epithelial cells have been collected from 60 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. This strategy has been used as proof of concept for further analyses focussing on genes involved in inflammation and airway remodelling. NHMRC Program Grant.

Epithelial Nitric Oxide Synthase expression in cystic fibrosis

S Stick, A Moeller, C Lane, Darryl 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

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. Funding: NHMRC Program Grant.

Breath condensate analysis in infants and children.

A Moeller, P Franklin, S 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, that is, 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 nitrite/nitrate concentrations in infants with a variety of respiratory conditions.

Personal exposure of infants to environmental pollutants

P Franklin, J Jones, S 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 will be developed that can be used to assess the exposure of infants to formaldehyde, volatile organic compounds and particulates. 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 serendipitous 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. Funding: NHMRC/JDF Program Grant.

Respiratory Physiology

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

J Kovar, KE Willett, PD Sly

The use of inhaled steroids in children under the age of 2 has posed great concern given that the human lung is thought to be developing during this time. Rat studies have shown that high systemic exposure to glucocorticoids during the period of septation (alveolar formation) causes marked adverse changes in lung development. This study aims to examine the effects of chronic exposure to inhaled glucocorticoids on lung structure and function during the early postnatal period using the rabbit as a model. We measured lung function using the forced oscillation technique and examined the lungs for alveolar number, volume, wall thickness and overall surface area within the right lung. Our data thus far indicates that inhaled glucocorticoids do not alter lung function parameters in rabbits treated twice daily for a period of 4 weeks between 1-5 weeks of age nor lung structural parameters.

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

RA Collins, DJ Turner, PD Sly, S Dunsmore, G Laurenta (aUniversity College, London)

Pulmonary fibrosis 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. This study examines the underlying susceptibility to pulmonary fibrosis using a bleomycin-induced lung fibrosis model in three types of trans-

genic mice and the wild type control. Mice with the gene deleted for neutrophil elastase, cathepsin G or both have been produced on a 129 background. These knock-out mice are expected to alter the susceptibility to bleomycin-induced lung fibrosis and to bacteria-induced emphysema. Bleomycin is a currently used anticancer drug that causes lung injury and fibrosis in up to 5% of patients and induces lung fibrosis in animal models. An understanding of how and why fibrosis (scarring of the lungs) occurs will potentially allow new treatment to be developed which prevents this scarring. Throughout 2001, a colony of all four genotype mice has been bred here at ICHR and we have begun to characterize the lung function of these mice 30 days post bleomycin administration. The project is a three year 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.

Characterisation of a new model of airway-specific inflammation

R Kumara, C Herberta, RA Collins, DJ Turner, PD Sly (aUniversity of New South Wales, Sydney, Australia)

Our collaborators, Rakesh Kumar & Cristian Herbert, Sydney, have recently described an improved mouse model of allergic inflammation capable of inducing airway-specific chronic inflammatory changes and remodelling, features of key importance in human asthma, and commonly missing in conventional animal models. The greater degree of similarity between this model and human asthma suggests that it may yield more significant insight into the pathophysiology of the human disease than other models. Our aim was therefore to validate the model by site-specific physiological evaluation of hyperresponsiveness. We measured respiratory input impedance (Zrs) in response to bronchoconstrictor challenge in sensitised mice receiving either short-term uncontrolled (sens-acute) or long-term controlled low-level (sens-chronic) exposures to aerosolised ovalbumin. The constant phase model was fitted to Zrs data to partition airway and parenchymal mechanics to determine the specific site of hyperresponsiveness. Sens-acute mice had significantly increased tissue responses to methacholine, but no significant increase in airway resistance, indicating tissue-specific hyperresponsiveness. In contrast, sens-chronic mice had significantly elevated airway responses but no increases

in tissue parameters, indicating airway-specific hyperresponsiveness. The data from this study have shown a direct correlation between the site of inflammation in the lungs and the physiological consequences. These data demonstrate that extreme care must be taken with animal models of allergic inflammation and that the site of the inflammation induced must be carefully assessed and reported. They also show the development and assessment of animal models of asthma must be accompanied by the use of appropriate measurement techniques capable of partitioning the mechanical properties of the lungs into components representing the airways and lung parenchyma.

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

RA Collins, DJ Turner, P McMinn, 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 that commenced in the latter part of 2001. To date, characterization of the acute phase of RSV infection in adult Balb/c mice has begun. Further work in 2002 will concentrate on the effect of age of infection (neonates versus juveniles versus adults) and the long term effects of virus exposure, that is whether or not lung function is altered at 4, 8 and 24 weeks post infection.

Determining airway tone and tissue properties in mice

DJ Turner, RA Collins, 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 measure-

ments 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. This 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.

Volume dependence

RA Collins, DJ Turner, Z Hantos, PD Sly

Diseases that cause chronic inflammatory changes in the lungs are likely to result in changes in lung volume and altered respiratory mechanics. Studies assessing the volume-dependence of airways and lung tissues separately have shown that airway resistance decreases with increasing lung volume while lung tissues become stiffer and tissue damping increases. Similar data are not available for mice, but are needed to accurately interpret changes in lung function induced in chronic disease models. The present study was conducted in mice to determine the changes occurring in lung function, partitioned into components representing the airway and lung tissues, with changes in lung volume. Recent technical developments have seen sophisticated measurements of lung function used in mice, in which input impedance (Z) is measured over a frequency range from 0.5 to 20 Hz. A model including an airway compartment comprising a frequency-independent Raw and airway inertance (Iaw) and a constant-phase tissue compartment comprising coefficients of tissue damping (G) and tissue elastance (H) can then be fitted to Z, allowing the partitioning of lung function into components representing the mechanical properties of the airways and lung tissue. The tissue mechanical properties have

also been represented as hysteresivity (h), an expression of the coupling of the elastic and energy dissipative properties of lung tissue. Within a particular animal, h has been shown to be relative constant, changing little with changes in tidal volume or ventilation frequency. In normal mice, we demonstrated the expected changes in R_{aw} , G and H with lung volume, however a marked decrease in h with increasing lung volume was seen. Studies were then undertaken to investigate the mechanisms responsible for changes in h with lung volume. Further studies are underway to characterize the changes in h with lung volume in lung disease using mice with bleomycin-induced fibrosis.

Cystic Fibrosis

Early detection of inflammation in cystic fibrosis

S Brennan, K Winfield, PD Sly

In 2001 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 characterise the ability of products secreted by *Staphylococcus aureus*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa* to stimulate inflammatory cytokine production by epithelial cells and to determine the ability of various antibiotics to inhibit this process.
3. To establish 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:

- One hundred and three broncho-alveolar lavage fluid samples have been collected from 53 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. There appears to be no difference in the age of acquisition in *Pseudomonas* in our clinic compared with the other national CF centres.

National Hypotonic 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 is due to finish by June 2002. Children with CF aged 6-18 years have been approached and to date we have enrolled five children through PMH.

Inflammation in cystic fibrosis: Friend or Foe?

PD Sly, S Brennan, K Winfield, N Kent, D Randall

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. We have already begun to collect samples from patients and currently have samples from over 50 patients locally and another 50 – 70 patients nationally.

This study has received funding from the National Cystic Fibrosis Association for 2002.

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

PD Sly, S Brennan, K Winfield, G Ryan, P Robinson

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

Macrolides are a class of antibiotics, which are not routinely used in cystic fibrosis. The macrolide clarithromycin is being trialed in 90 subjects in total in this study. There are 30 subjects recruited in the US arm of the study and a further 60 from Australia. Clarithromycin is being tested for its ability to reduce inflammation and improve lung function when used in conjunction with current antibiotic therapies.

Recruitment of subjects into this study began in September 2001, runs until September 2002 and the study is expected to reach completion by the end of 2003.

Clinical Research Projects

Family Asthma Study

PD Sly, RC Mutch, AM Callaghan, GE Kendall

The Family Asthma Study is part of an international collaborative effort funded by Glaxo Smith Kline investigating the genetic basis of asthma and allergy. Begun in 1999, the work at our site was completed in 2001 with testing complete on 100 families. We were one of 11 sites world-wide and over 1000 families were recruited, making this one of the largest asthma genetic studies undertaken. The long

process of analysing the data has begun with an emphasis on determining whether different clinical patterns of asthma are due to different combinations of asthma susceptibility genes.

Role of early, repeated viral respiratory infections and the development of atopy in childhood (The

Childhood Asthma Study

M Kusel, PD Sly, P Holt, R Loh

This prospective study which commenced in 1996 recruited a total of 263 families. Over 3,500 thousand 'infectious' mucous specimens have been collected over the past 5 years. The specimens collected in the children's infancy are currently being analysed.

The prevalence of eczema in the cohort was found to be high at 56% in infancy, but this rate dropped to a third at 3 and 4 years of age. We are now following up the children who have turned 5 years of age, and of those seen, a quarter of them have still been found to have eczema.

This study is coming to the conclusion of the first phase with the children undergoing lung function tests, skin prick test and blood test as they turn 5 years of age.

The retention rate has remained very high at 81% and it is certainly a privilege for the study team to be able to work with this group of extremely enthusiastic, committed and supportive families.

Vaccine Trials

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, broadening immunisation and the continuation of the vaccine trials.

In 2001 we conducted or commenced several international studies. These included:

- A randomised, double-blind phase 2 study comparing the immunogenicity and safety of a thiomersal-free inactivated split-virion influenza vaccine to the reference (Thiomersal-containing) influenza vaccine.
- Booster phase at age 18 months for: A phase 3 randomised study to compare the safety and immunogenicity of three unique lots of the 9-Valent Pneumococcal Capsular Polysaccharide-CRM197 conjugate vaccine in healthy infants.
- A phase 1 multicentre study of the safety, tolerability, viral shedding profile, and immunogenicity of a recombinant, live, attenuated Respiratory Syncytial Virus subgroup A strain virus rA2cp530/1009DNS2 in adults, RSV-seropositive 15 to 59 month old children and RSV-susceptible 4 to 24 month old infants.
- A phase 3 randomised, controlled primary vaccination study to assess the consistency of three production lots of SmithKline Beecham Biologicals combined measles-mumps-rubella-varicella vaccine (MeMuRu-OKA) in terms of immunogenicity and safety, compared to the administration of measles-mumps-rubella vaccine (Priorix[®]) and varicella vaccine (Varilrix) (either concomitantly or 6 weeks apart) in healthy children in their second year of life.
- 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 4 doses of DTPa, and polio vaccine, and co-administered with GSK Biologicals' MMR Vaccine (Priorix[™]).

Staff and Students

Head of Division

Peter D Sly MD FRACP

Professorial Fellow, Department of Paediatrics,
The University of Western Australia

Research Staff

Siobhain Brennan PhD

Rachel A Collins BSc (Hons)

Felicity S Flack PhD

Zoltan Hantos PhD (Perpetual Visiting Professor)

Jennifer Kent RN

Renata Liberatore

TuPhuong Quach BSc(Hons)

Stephen Stick MBBS PhD FRACP

Debra J Turner PhD

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Miranda Odam RN MPH

Maria Pradhan RN

Peter Richmond MBBS MRCP FRACP

Melanie Trainor RN

Kathleen White RN BSc

Megan Wright RN

Post Graduate Students

Jacqui Joseph-Bowen BscOT PgradDip(HlthAdmin)

MSc(Addiction) PhD candidate

Jana Kovar BSc PhD Candidate

Merci Kusel MBBS PhD Candidate

Raewyn Mutch MBChB DipRACOG FRACP PhD

Candidate

Support Staff

Lauren Bamberger BA (Hons)

Sue Davies BSc (Hons)

David Sly

Theses Passed

Meri Tulic PhD University of Western Australia, 2001.
Modification of allergic inflammation with bacterial lipopolysaccharide.

Denise Stapleton PhD Curtin University of Technology, 2001. Development, implementation and evaluation of a nutrition education and behaviour program for children with cystic fibrosis.

Awards

R A Collins. Thoracic Society of Australia and New Zealand (WA) Travel Award 2001.

J Kovar. Thoracic Society of Australia and New Zealand (WA) Travel Award 2001.

J Kovar. Allen and Hanbury's Travel Award 2001.

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-)

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 in 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. Asthma Consultative Group, Australian Lung Foundation (1999-2001)

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

PD Sly. NH&MRC Discipline Panel (2001)

Local

PD Sly. Asthma Foundation of Western Australia Research Sub-committee, Member (1993-95), Chairman (1998-2001)

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

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

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

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-)

Invited Presentations

PD Sly. Consequences of Therapeutic Interventions in the First Year. 10th Greek Paediatric Respiratory Society Meeting. Athens. May 2001.

PD Sly. Surfactant and the Lung. 10th Greek Paediatric Respiratory Society Meeting. Athens. May 2001.

PD Sly. Update on Asthma Treatment. IX Brazilian Congress of Paediatric Pneumology, X Latin American Congress of Cystic Fibrosis, V Congress of the Latin American Society of Paediatric Pneumology, IX Brazilian Conference of Cystic Fibrosis, II Brazilian Conference of Respiratory Physiotherapy in Paediatrics. Florianopolis. September 2001.

PD Sly. Mechanisms on the Development of Asthma. IX Brazilian Congress of Paediatric Pneumology, X Latin American Congress of Cystic Fibrosis, V Congress of the Latin American Society of Paediatric Pneumology, IX Brazilian Conference of Cystic Fibrosis, II Brazilian Conference of Respiratory Physiotherapy in Paediatrics. Florianopolis. September 2001.

PD Sly. Adapting the inhalatory technique to the Child. Argentinean Pulmonary Congress. Buenos Aires, November 2001.

PD Sly. Respiratory function testing in preschool children. Argentinean Pulmonary Congress. Buenos Aires, November 2001.

PD Sly. Wheezing after acute lower respiratory tract infections. Argentinean Pulmonary Congress. Buenos Aires, November 2001.

PD Sly. Weighing the evidence for preventive environmental control strategies for asthma – Breastfeeding reduces the risk of developing asthma. American Thoracic Society Postgraduate Course, San Francisco, May 2001.

PD Sly. Viruses, Asthma and COPD. Wyeth-Ayerst Research. New York, May 2001.

PD Sly. Problems with asthma diagnosis in children. Pzfier Sandwich. London. June 2001.

PD Sly. Long-acting Beta agonists and inhaled corticosteroids. IX Brazilian Congress of Paediatric Pneumology, X Latin American Congress of Cystic Fibrosis, V Congress of the Latin American Society of Paediatric Pneumology, IX Brazilian Conference

of Cystic Fibrosis, II Brazilian Conference of Respiratory Physiotherapy in Paediatrics. Florianopolis. September 2001.

PD Sly. Volume dependence of lung function in mice. Vermont Lung Center, University of Vermont. October 2001.

PD Sly. American Thoracic Society, Annual Scientific Meeting 2001. Advances in assessing infant lung function. San Francisco.

Division of Molecular Biology

Overview

The research of the division concentrates on the molecules that cause allergic sensitisation and the development of immunotherapy to prevent and treat allergic diseases, especially asthma. The scope of the investigations extends from the molecular characterisation of allergens and allergic responses to the production of modified allergens and molecular mimics for new therapies. Our study of indoor allergens has broadened from the house dust mite, to the next most prevalent source, the cat. This new direction has been driven by critical developments in the study of cat allergy especially the implementation of clinical trials with peptide-based immunotherapy and the discovery that tolerance to cat allergens can be induced by high dose exposure during infancy. The spectrum of allergenic specificities produced by the cat, and how the responses to individual allergens interact, has however remained virtually unexplored. Our studies with molecular cloning have identified several unrecognised allergens that are under investigation in the contexts above. One of the allergens is a major IgE-binding specificity. The study of house dust mite allergens has continued with the main aim of identifying major allergens and antigens that induce sensitisation, and, for non-allergic subjects, protection. The measurement of T-cell cytokine responses to a panel of recombinant allergens has shown the importance of the group 1 and 2 allergens for inducing Th2 cytokines and suggest that immunotherapy should be based on these molecules. Other allergens which have been difficult to clone or produce, or have only recently been identified, are however being studied further. One of these that have been studied this year is the amylase allergen which has isoforms encoded by two divergent genes. While the major group 1 and 2 house dust mite allergens are products of single genes a feature is their high degree of allelic polymorphism. It is possible that the sequence variation may be a factor that enhances allergenicity and, less speculatively, information on the variants is required to develop genetically engineered allergens. Recombinant proteins made from different alleles of Der p 2 have now been examined for antibody binding and their ability to stimulate T-cell cytokine release. While some interesting effects of amino acid substitutions were evident, a very practical result was to identify that the combination of the two most divergent alleles can represent the allergen for therapeutic formulations based on recombinant allergens.

The structural information and recombinant allergens are being obtained principally to develop new types of immunotherapy. As well as providing critical information and reagents for other research laboratories and collaboration with pharmaceutical companies the division has been investigating several potential therapies. The intranasal administration of peptides containing T-cell epitopes of allergens has been shown to protect mice from making IgE hypersensitivity responses to the whole allergen. This has potential for immunotherapy, but follow up studies have shown that regimens that block allergic reactions to the injection of allergen do not inhibit responses to inhaled allergen. Indeed they can even enhance pulmonary inflammation. A critical strategy has therefore been to study animal models that induce allergies by inhalation rather than the more convenient models used in other laboratories that inject the allergens. In addition to therapy based on genetically engineered allergens, a new strategy is to use of unrelated peptides that mimic the shape of the allergens. This "mimotope technology" is being studied with an allergen in a mouse model using mimetics isolated with monoclonal antibodies shown to be able to inhibit sensitisation. The studies are examining mimetics derived from the classical random combinations of amino acids and a new mimetic strategy based on a phylogenetically diverse biological source of peptides. A combination of genetically engineered T and B-cell epitopes is thus being studied to develop more efficient and safe methods of improving immunotherapy.

Highlight

Genetic diversity of house dust mite allergen - polymorphisms rationalised.

Recombinant allergens provide a gateway to the development of new types of immunotherapy for asthma. The sequence analyses of the major house dust mite allergens have however revealed a great deal of genetic diversity or polymorphism, even within mites in the same home. This has raised the possibility that a complex mixture or recombinant molecules may be required to adequately represent the natural allergens. The division has shown that the major Der p 2 allergen of house dust mites has diversified along two genetic pathways involving changes in a cluster of residues which affect the antigenicity. It has now been shown that only two recombinant allergens that contain all the most abundant genetic changes are required to represent the diversity of Der p 2 for therapeutic preparations.

IgE and T-cell responses to polymorphic house dust mite allergens

BJ Hales, LA Hazell, W Smith and WR Thomas

The major allergens of the house dust mite have a high degree of allelic polymorphism which may be important for their allergenicity and for developing new immunotherapeutic agents. For the group 2 allergen it has previously been shown that the polymorphism follows a pattern of evolution which centres on the divergence to 2 major alleles, Der p 2. 0101 and Der p 2. 0104. The 2 divergent alleles have amino acid substitutions in positions 40, 47, 111 and 114 of the 129 amino acid protein. All of these substitutions are proximal in or adjacent to the loops, at one end of the immunoglobulin fold that forms the major structural element of this molecule. The immune responses of human peripheral blood T cells to 4 polymorphic forms of recombinant Der p 2 were tested to ascertain which molecule, or mixture of molecules, would best represent natural Der p 2. This information is important for selecting recombinant proteins and sequences which can be used as a basis for new types of immunotherapy. The two alleles Der p 2. 0101 and Der p 2. 0104 representing the two evolutionary arms induced strong and similar T-cells responses as measured by proliferation assays and cytokine release. A mixture of these two alleles would therefore be suitable for a preparation of recombinant Der p 2. Two other less frequent allelic variants tested showed variations in T-cell responses showing that the different epitopes presented had an effect on the responses, and that this interestingly was found for most subjects. One variant consistently induced a high level of IL-10 release and low levels of IL-5 and interferon-g as well as proliferation. Responses that induce high levels of the regulatory cytokine IL-10 can possibly

be developed for therapeutic potential. IgE antibody responses to the 4 different alleles showed that the most abundant allele Der p 2. 0101 had reduced IgE binding activity compared to proteins representing the other alleles. All of the other alleles have a substitution in position 47 which is found in the region of Der p 2 molecule shown to be antigenically variable by monoclonal antibody-binding studies. It should however be noted that although the IgE binding to Der p 2. 0101 was less the binding was still large and was highly correlated with that of the other alleles. The variants with reduced cytokine production had high IgE binding activities which shows that the changes in T-cell responses were not due to altered presentation caused by natural or artefactual structural changes in the molecule.

T-cell responses to the mite allergen Der p 3

BJ Hales, W Smith, LA Hazell and WR Thomas

The IgE binding data for Der p 3 suggest it may be a major determinant in the development of house dust mite allergy and be a necessary component in formulation of recombinant allergens for immunotherapy. In order to corroborate this with T-cell data, *in vitro* stimulations were conducted with natural Der p 3 isolated by chromatography and with overlapping peptides spanning the length of Der p 3. The peptides incorporated a series of polymorphic substitutions clustered in a variable region of the molecule. These were previously identified to be allelic variants frequently found in mites from homes in the Perth region but rare in the Der p 3 cDNA analysed from a commercial culture of mites. Stimulation with natural Der p 3 was complicated because the protein (a trypsin) was toxic to cells. Experiments with the inclusion of a serine protease inhibitor showed cells from about 50% of both aller-

gic and non-allergic subjects were significantly stimulated, a frequency significantly less than the 80% found for the Der p 1 allergen. This was in agreement with the responses induced by peptides that were also less frequent than responses previously found to Der p 1 peptides. The regions of the Der p 3 sequence which were polymorphic did not contain frequently-stimulatory peptides and the peptide variants containing the polymorphisms were not stimulatory. The results show that, despite the allelic variation, the Der p 3 sequence which predominates in mite extracts is suitable for immunotherapy and that Der p 3 is less stimulatory than the major Der p 1 allergen. Studies with cytokine release are continuing for a more complete evaluation of the potency of this allergen.

Allergenic amylases of house dust mite

KL Mills, LA Hazell, WR Thomas and W Smith

An allergenic protein of the house dust mite was previously shown by N-terminal sequence similarity to correspond to an alpha amylase. Evidence was presented to show it bound IgE in 46% of subjects allergic to house dust mites but definitive analyses could not be conducted because of the low yield of purified allergen. As previously described cDNA encoding an alpha-amylase was isolated from the house mite and produced as a recombinant polypeptide by a *Pichia pastoris* expression system. The secreted polypeptide was highly active as an amylase enzyme and bound IgE antibody from 40% of allergic subjects. The possibility of other amylase allergens is now being investigated because most species have at least two genes encoding amylase and the IgE-binding activity of most people to the cloned amylase was low. A second amylase gene close to the known gene was found by screening a genomic library. The sequence was 70% identical to the known gene. This is lower than the identity between amylases of different mite species and lacked critical conserved regions found in amylases as well as cysteines found in conserved disulphide bonds. It also had a short leader peptide suggesting it may not be secreted. RT-PCR of RNA from mite preparations however showed the gene was transcribed. It has not been possible to produce a recombinant polypeptide from the cDNA encoding this gene cloned in *P. pastoris* or in many *E. coli* systems. Polypeptides have however been produced as a fusion with the thioredoxin in the tightly regulated pBAD system in *E. coli* and less efficiently

by pET vector with *E. coli* BL21 codon plus to eliminate codon bias. Antigenic cross reactivity between the products of the two genes (AMY1 and AMY2) has shown with mouse antiserum but IgE binding to the AMY2 product has been very low possibly because of a poor conformation of the recombinant polypeptide. Studies are in progress to detect a natural product of the AMY2 gene.

Cat allergens

W Smith, AJ Butler, LA Hazell and WR Thomas in collaboration with Dr MD Chapman Virginia, USA

Although studies such as western blotting have shown that cats produce a variety of IgE-binding proteins, work to date has concentrated on the allergen Fel d 1. This is a potent allergen that is abundant in cat dander extracts and the air. Many cat-allergic subjects however have small responses to this allergen and considerable data exist for the importance of other allergens. Moreover clinical trials using immunotherapy for cat allergy with peptides representing only Fel d 1 have raised some questions. Would immunotherapy with a large spectrum of allergens be more efficient and do alterations in the response to Fel d 1 induced by therapy affect the responses to other allergens? For a comprehensive study of cat allergens, cDNA expression libraries have been constructed from tissues that are potential sources of allergens - skin, parotid gland, anal gland, liver (for exported proteins) and tongue. The cDNA has been cloned in a lambda ZAP vector and screened with allergic sera for the production of IgE-binding peptides. To date non-Fel d 1 allergens have been isolated from the liver, the tongue and the parotid library as well as the previously described cystatin allergen from the skin. One of the proteins binds IgE in 80% of sera and is thus a major allergen. The allergens, which have been expressed as recombinant polypeptides in *E. coli* or in *P. pastoris*, are under extensive investigation.

Spectrum of mite allergens

N Malainual, BJ Hales, W Smith and LA Hazell

One of the key problems in house dust mite allergy is to define the important allergens so balance therapeutic formulations can be devised. A comparative study of T-cell and antibody responses has been conducted with the allergens Der p 1, 2, 5, 7, 8 and 10. These were chosen because they could readily be produced as purified and serologically reactive

recombinant polypeptides or in the case of Der p 1 can be readily isolated as a natural protein. Natural Der p 3 was included in the study of antibody responses. The T-cell responses showed that Der p 1 and Der p 2 induced the highest proliferation and IL-5 production which was higher from cells of allergic subjects than cells from non-allergic subjects. The proliferation induced by Der p 5 was also higher in the allergic subjects when considered as a group and many but not all allergic subjects had high responses to the group 7 and 8 allergens which were greater than the responses of non-allergic subjects. Similar results were obtained for IL-13 while interferon-gamma release was similar when compared between allergens and between allergic and non-allergic subjects. The comparative IgE binding analysis was conducted with a europium-based time resolved fluorescence that included a human-mouse chimeric anti Der p 2 antibody for standardisation. The IgE binding to Der p 1, 2 and 3 allergens was high for most allergic subjects while the binding to the other allergens was found in about 50% of sera, usually at much lower levels. The results emphasise the importance of the group 1 and 2 allergens and the need for further study on the group 3 allergens.

Allergen mimotopes

SR Gunn, TK Heinrich, AG Jarnicki and WR Thomas in collaboration with PM Watt, Division of Children's Leukaemia and Cancer Research, Telethon Institute for Child Health Research

A mimotope is a peptide that mimics the shape of an epitope on an antigen. Typically mimotopes are derived by selecting peptides that bind monoclonal antibodies using phage display technology. The studies here have used monoclonal antibodies to allergens to derive mimotopes representing the epitopes. The emphasis has been to use a model allergen to aid the testing of the therapeutic efficacy of mimotopes in an animal system and to test the potential of biologically derived peptides for a source of mimotopes. The non-random nature of peptides from biological sources may stabilise secondary structures and peptide from homologous proteins of diverse species can be tested for mimotope activity. Monoclonal antibodies have been prepared against the cysteine protease allergen from papaya. This is because a mouse model has been developed where respiratory sensitisation can be induced by intranasal administration of the allergen

without adjuvant. The monoclonal antibodies have been shown to be able to inhibit the development of IgG antibodies induced by papain and regimens to block IgE are under investigation. Mimotopes have been isolated by selection from phage display of conventional combinatorial random peptide libraries. The peptides have a consensus sequence and the binding in ELISA assays is specific for the monoclonal antibody and is blocked by free allergen. Experiments to immunise mice with the mimotope and to modify immune responses are in progress.

To test a biological source of peptides gene fragments from a collection of 18 genomes from a diverse range eubacteria and archaeobacteria have been prepared. The fragments were generated by a combination of random PCR and restriction enzyme digestion in strategy designed to produce overlapping 100-200 bp fragments that have the potential to encode peptides of 30-60 amino acids. 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 5×10^7 has been constructed and a sample of 50 clones has been sequenced to verify the size of the inserts and the random representation. Panning to isolate clones binding to anti-allergen monoclonal antibodies is being performed.

Respiratory sensitisation

AG Jarnicki and WR Thomas in collaboration with PG Holt, Division of Cell Biology, Telethon Institute for Child Health Research

One of the difficulties in studying allergic sensitisation to aeroallergens is that laboratory rodents do not become sensitised by the inhalation of aeroallergens without adjuvant. To the contrary, chronic inhalation over a large range of doses has 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 without adjuvant it was found that intranasal administration of the cysteine protease from papaya, an 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 that the enzymatic

activity is not required for the sensitisation. 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 is now being used to study the efficacy of new types of immunotherapy with peptides and mimotopes.

Hypersensitivity to intranasal peptides

AG Jarnicki and WR Thomas

The intranasal administration of peptides containing T-cell epitopes of allergens has been shown to induce a form of immunological tolerance so that mice cannot be subsequently immunised by the injection of allergen. The tolerance has considerable potential for immunotherapy of allergy because the peptide will not bind IgE and elicit immediate side effects and because one peptide can block the response to the whole allergen and even bystander molecules. A feature of the intranasal administration is that during the induction of tolerance the peptides activate T cells that can be reactivated on further intranasal administration of peptide at a time when the mice are tolerant to the injection of peptide. It has now been shown that while peptide containing the major T-cell epitope of the allergen Der p 1 inhibits responses to the allergen when it is injected in alum or complete Freund's adjuvant it enhances the inflammatory response produced by the intranasal administration of allergens. Moreover mice administered only with intranasal peptide produce delayed hypersensitivity responses to allergen injected subcutaneously in the ear, and the peptide does not inhibit the delayed hypersensitivity response induced by the intranasal administration of Der p 1. The intranasal administration of peptide therefore has the potential to produce unwanted hypersensitivity responses and its ability to inhibit responses to inhaled allergen is less than its ability to inhibit responses to injected antigen. Further investigations are required to determine the suitability of this form of immunotherapy to ameliorate allergic respiratory sensitisation.

Staff and Students

Head of Division

Wayne R Thomas BSc Hons PhD

Research Staff

Amanda J Butler BSc Hons

Belinda J Hales BSc Hons PhD

Lee A Hazell Dip Appl Sci

Tatjana K Heinrich PhD

Andrew G Jarnicki BSc Hons PhD

Wendy-Anne Smith BSc Hons PhD

Students

Stephanie R Gunn BSc Hons PhD candidate joint Children's Leukaemia and Cancer

Nat Malainual BSc MSc PhD candidate

Kristina L Mills BSc Hons PhD candidate

Theses Passed

Michael J Epton. Human immune responses to house dust mite allergens, non-allergens and nasopharyngeal colonising bacteria. PhD (Distinction) University of Western Australia.

Andrew G Jarnicki. Effect of intranasal peptides containing T-cell epitopes on immune responses in mucosal tolerance. PhD University of Western Australia.

Awards

Kristina Mills. Friends of the Institute travel Award.

External Committees

WR Thomas. Deputy Chairman NMMRC 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 Orleans, USA

WR Thomas. Role of early allergen exposure in disease prevention. Symposium. Australasian Society for Allergy and Clinical Immunology, Perth

Division of Population Sciences

Overview

The Division of Population Science comprises 132 staff and students engaged in a diverse range of scientific activities. Our research 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
- assessing the significance of these findings for the prevention of disease and/or the promotion of health.

The research 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 disease				
otitis media	✓	✓	✓	
meningitis	✓	✓	✓	✓
Developmental disorders				
autism	✓	✓		
birth defects	✓	✓	✓	✓
cerebral palsy	✓	✓		✓
intellectual disability	✓	✓		
mental health disorders	✓	✓	✓	✓
newborn encephalopathy	✓	✓		
newborn hearing screening	✓			✓
Rett syndrome	✓	✓		
Innovative methodologies				
Aboriginal health research	✓	✓	✓	✓
genetic epidemiology	✓	✓		
geocoding/mapping	✓	✓	✓	✓
population databases	✓	✓	✓	✓
population surveys				

During 2001 several achievements should be noted.

- The division hosted Professor Martha Werler, Fogarty Senior International Visiting Fellow, from the Boston University Slone Epidemiology Unit during her sabbatical. Professor Werler, an epidemiologist, is well known for her research on birth defects and has a particular interest in the effect of medication use in pregnancy.
- Dr Deborah Lehmann and her team launched their findings on a study of the relationship between the provision of public swimming pools in remote Aboriginal communities and the occurrence of otitis media in Aboriginal children. Preliminary findings are encouraging and have attracted considerable national interest.

- Division scientists commenced a new area of epidemiological research into childhood cancer initiated by the unexpected finding of a reduction in the risks of acute lymphoblastic leukaemia in association with maternal folate intake.
- In collaboration with the University of Western Australia Department of Public Health, the Institute hosted a five day course on data linkage. The course was organised and presented by Professor D'Arcy Holman, a leading expert and proponent of the use of data linkage in addressing key questions in population health, genetics and preventive medicine. The course attracted participants both locally and from across the country.
- Drs Carol Bower, Jennifer Kurinczuk, Sandy Webb and Michèle Hansen published a new Australian study showing that babies conceived through assisted conception procedures are more than twice as likely as naturally conceived infants to be diagnosed with major birth defects in their first year of life.
- The Institute and Curtin University of Technology entered into a joint venture to create the Centre for Development Health. Professor Sven Silburn has been appointed as the Director of the Centre. Based at the Institute, the Centre for Developmental Health seeks to progress multidisciplinary research into the causal pathways and mechanisms that underpin developmental health and wellbeing by investigating the way in which human development interacts with social circumstances across the lifespan.
- The division established a contract management team to assist in developing and maintaining the contract environment through which many of the division's research services and outputs are purchased. Operating under the title of the Collaboration for Applied Research and Evaluation (CARE), the CARE team insures an accountable contract environment with State, Commonwealth and private sector purchasers that is in line with the Institute's mission and aims.

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

The year 2001 culminated in the quinquennial International Scientific Review in which division staff played a major role. As with the rest of the divisions in the Institute, Population Science research programs were scrutinised, data and findings presented, operational and support infrastructures examined and future directions for the group were assessed. The review process was open to all staff and students. This proved to be a demanding but highly rewarding process that affirmed the productivity of the division's scientific effort and its general impact on the community that it serves.

Aboriginal Health Research

Kulunga Research Network

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

Kulunga is a collaborative maternal and child health research, information and training network project involving the Institute for Child Health Research and member services of the Western Australian Aboriginal Community Controlled Health Organisation. The primary philosophy of the Network is to advocate for Aboriginal children and families in Western Australia. It aims to ensure that community-based and culturally relevant research benefits them by influencing the policy and planning of government and other key agencies, and by ensuring Aboriginal people are involved in all areas of research and implementation of outcomes. We believe that this philosophy is the key to the model of success in realising improved Aboriginal health outcomes.

Network vision statement

The Network will enable Aboriginal people to conduct research and training to form a basis for an improvement in the health and whole of life expectation for Aboriginal children and families in Western Australia. This network respects the right of Aboriginal people to control research activities in keeping with the principle of Aboriginal self-determination.

Training aims

- Train Aboriginal people in all areas of research.
- To increase individual Aboriginal Community Controlled Health Organisation's (ACCHO) knowledge and use of research in ongoing work.

Research aims

- Conduct research in Aboriginal maternal and child health that is community-based and culturally relevant.
- Ensure research is conducted in such a way that it can be used to influence health policy.

Information aims

- To provide open community forums for discussion of maternal and child health issues, bringing in high profile Aboriginal speakers, both national and international.
- To provide awareness, knowledge and information to the Aboriginal community.
- To provide awareness, knowledge and information to government and other key agencies.

The Network will implement this philosophy by stimulating new projects in close collaboration with ACCHO and focusing on existing Aboriginal projects to assist them in the areas of research, information and training.

An Executive Committee made of representatives from the current interested partners who meet quarterly has directed the Network. These include the Child Health Research Foundation, Western Australian Community Controlled Health Organisation, the Derbarl Yerrigan Health Service and the Institute. There is also an internal 'management type' arrangement where projects within the Institute under the scope of the Network are able to raise and discuss issues and provide updates on how the projects are progressing.

Current research projects falling within the scope of the network

The Network encompasses all of the Aboriginal health projects conducted by the Institute and collaborative partners. These include Bibbulung Gnarneep (Solid Kid) Phase 2, the Western Australian Aboriginal Child Health Survey, Kalgoorlie Otitis Media Study and the Swimming Pool Study.

The Bega Garnbirringu Health Services in Kalgoorlie has completed an analysis of the community needs and perceptions of early postnatal and antenatal issues in the Kalgoorlie/Boulder area. This will be beneficial in planning and modifying antenatal and postnatal services for this area.

Future research project/activities

- Develop and evaluate health information pamphlets created from the findings of the Bibbulung Gnarneep study
- Analyse community perceptions of and barriers to dental care of the South West Aboriginal Medical Service clients.
- Develop a resource kit of health promotion materi-

al for specific Aboriginal child health issues.

- Develop a resources kit of Aboriginal specific education course in TAFE and higher education settings, including scholarships and other sources of financial assistance.
- Develop a resource list of current Aboriginal child and maternal health research projects and the Institutions involved.
- Engage a consultant to assist the Network to develop a five year business plan and business case to attract core funding from corporate bodies and Commonwealth and State Government.

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

D Lehmann, M Tennant, D Silva, D McAullay, K Sivwright, F Stanley, A Read 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 and KS Sriprakash (Menziess 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, eye and skin disease. The communities adopted a policy of "no school no pool" or "school means pool" and so we have been monitoring school and pool 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 (winter

2000) and at 6 monthly intervals. A hearing test and tympanometry was done on the first visit and will be repeated on the fourth visit in March 2002.

Whenever possible, pictures of eardrums were taken using a video-otoscope and then stored on computer file. An ENT Specialist, who also reviewed the pictures of eardrums taken with the video-otoscope, examined those children with severe ear disease on the first visit.

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. For all children in the study, we have recorded information about health clinic visits 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. In Burringurrah 31% of children had perforated eardrums during the winter before the pool was opened compared to 21% one year later. In the same community 63% of children had skin sores before the pool was opened compared to 22% one year later. Despite the fact that the Jigalong pool was closed for a substantial period over the summer of 2000 children still had some improvement in their health. In that community the proportion of children with bad skin sores fell from 28% to 5% one year later; the proportion of children with runny ears was 16% on the first visit compared to 11% on the third visit.

Aboriginal Child Health Survey

SR Zubrick, S Eades and SR Silburn.

The first fully representative community survey of Aboriginal child health and well-being has been underway throughout Western Australia since April 2000. The project is being conducted under the auspices of the Kulunga Research and Training Network with funding from Healthway, the WA Lotteries Commission, and several State and Commonwealth Departments. Rio Tinto Aboriginal Foundation has also provided funding to assist the Institute in meeting its commitment to employ and train more Aboriginal staff on the project. 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.

We have trained over 130 screeners and interviewers (60% of whom have been Aboriginal) and by December 2001 they had 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 2,015 (88.1%) of these families have consented to participate in the survey. Intensive interviews have been conducted gathering information on 5,298 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 2,183 of these children.

During 2002 intensive data screening, cleaning, editing and validation will take place. Additionally a clinical and cultural validation study will also be conducted with a sub-sample of approximately 260 of the children stratified on the basis of their mental health status as determined by the screening instruments used in the survey. 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. During 2003 the findings will be published in several formats including a monograph, which provides an epidemiological framework not previously available as a planning resource to define the burden and impact of common child disorders at the population and regional levels. This information will assist policy makers, service planners and purchasers in health, education, family and children's services and justice in estimating service needs and the potential advantages of alternate policies and programs.

Bibbulung Gnarneep Home and community visiting research project

SJ Eades, AW Read, N Pavy-Bell, K Jones, R Sipkus, HD'Antoine, E T d'Espaignet, K Hunt, J Nannup, H Lette, R Martin.

The Bibbulung Gnarneep Home and Community Visiting Research project aims to study and report on options for cost-effective models of service delivery to maximise the health and welfare of Aboriginal babies, by supporting a sample of Aboriginal

women living in the Perth Metropolitan area throughout their pregnancy until their child reaches two years of age. The inter-dependency between a woman and the rest of her family requires a holistic approach integrating the whole family and social support structure of individual Aboriginal families.

The Bibbulung Gnarneep team continues to work in close partnership with the project's Community Reference Group to design, implement and review progress of the project. The reference group is open to any member of the Perth Aboriginal community with an interest in the project. In doing this research, the Institute has also entered in a partnership with each family unit that becomes part of the study, whereby the Institute's research assistants on the project are experienced Aboriginal health professionals who provide a home and community support to the enrolled families.

The reasons for the well-documented relatively poor health status of Aboriginal children (and adults) are complex, and include a variety of structural and individual factors that reflect both inter-generational and contemporary social disadvantage. Consequently, the activities required to maximise the health of two-year old Aboriginal children must go beyond those that are focused on health (growth and physical development) and must also include action related to the family and child's physical, emotional and social environments. These include actions aimed at ameliorating the impact of issues as dispersed as housing, education, employment opportunities, literacy, parenting skills, transport, domestic violence, learning opportunities, respect of Aboriginal culture, social isolation, supportive networks, family functioning, access to social services, locus of control over one's life, social capital, and pervasiveness of loss and grief.

Bibbulung Gnarneep - Conceptual model for Bibbulung service development/evaluation process

The concept of a 'solid kid' was developed in a focus group session with a group of health and other professionals using a Health Benefits Group framework. The possibility of conducting a similar process with Aboriginal mothers and fathers is currently being investigated.

The research team is currently working on testing a tool and process to assess the needs of families to have and grow healthy children. This tool is used to collect a variety of qualitative (individual history taking and focus groups) and quantitative data about the family circumstances, the factors that enhance and those that act as barriers to raising successful children.

Socioeconomic risk factors and treatment-seeking behaviour for otitis media in the Aboriginal population of Kalgoorlie-Boulder region

D Lehmann, C Jeffries-Stokes (also Rural Paediatric Unit, University of Western Australia), D Elsbury, J Johnston, A Mason, J Evans, K Wood in collaboration with L Dorizzi (Bega Garribirringu Health Services Aboriginal Corporation).

As part of an NHMRC Otitis Media Strategic Research initiative, we investigated perceptions of ear disease in young children and treatment-seeking behaviour for otitis media (OM; middle ear infection) in the Aboriginal population resident in the Kalgoorlie-Boulder area as well as potential socioeconomic barriers to compliance with recommended treatment for OM. The qualitative data we have collected are essential to supplement the quantitative data being collected in the larger cohort study described above in order to develop appropriate intervention programs for prevention of OM and its serious consequences, in particular hearing loss. Through community workshops and individual interviews we collected information on people's perceptions and concerns about middle ear infections, when and why they seek treatment, and any difficulties they may encounter in receiving and completing prescribed treatment. We have found that (1) there is limited understanding of how and why OM occurs, (2) while maternal smoking has been identified as an important risk factor in this population, there is limited awareness that tobacco smoking puts children at risk of OM and (3) exclusive breastfeeding is of short duration. With a new grant from Healthway, we will now do a further detailed study of practices and attitudes related to smoking and feeding practices with a view to developing health promotion programs together with members of the local community (See below).

Qualitative and quantitative studies of otitis media to improve ear health

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

Otitis media (OM; middle ear infection) is a major health problem in all children, but particularly in the Aboriginal population in whom the resultant hearing loss can seriously affect performance at school, hence employment and social circumstances in adulthood. In the Kalgoorlie-Boulder area we are following Aboriginal and non-Aboriginal children from birth to age 24 months and investigating a demographic, socioeconomic, environmental, microbiological and immunological factors which may put some children at high risk of getting OM. 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 of parents of children who are participating in the large cohort study, 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 which will assist in developing appropriate intervention programs for the Goldfields.

Cancer

Cancer epidemiology

L Milne, P Serna, C Bower, N de Klerk, U Kees 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), 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).

A new three-year Fellowship in Childhood Cancer Epidemiology was awarded to Dr Elizabeth Milne in 2001. The function of this role is to develop the capability of the Institute's Population Sciences Division in the areas of the descriptive and aetiological epidemiology of childhood cancers.

Research activities in the following areas are planned:

- Causal pathways in childhood cancer
Causal pathways will be modelled through the collection and analysis of genetic, environmental and social data on children and their families in national, collaborative epidemiological studies. One example is a proposed multi-centre case-control study of childhood acute lymphoblastic leukaemia (ALL), which is currently the subject of applications for research funding. Extensive developmental work for this study was undertaken at the Institute during 2001. The primary hypothesis of the proposed study is that maternal folate supplementation during pregnancy protects against ALL in the offspring, with the effect modified by genetic factors in folate metabolism. This hypothesis arose from the findings of a case-control study undertaken in Western Australia by Dr Judy Thompson and Professor Bruce Armstrong between 1984 and 1992. The results of this important study were published in the *Lancet* in December 2001.
- Accessibility and cost-effectiveness of childhood cancer treatment
Existing linked data on cancer incidence and health care utilisation will be used to conduct an economic evaluation of treatment of childhood cancer. Linked data will also be used to examine social and spatial influences on access to treatment for children with cancer.

Developmental Disorders

The epidemiology of Autism in Western Australia

E Glasson, C Bower, B Petterson, in collaboration with J Hallmayer (Centre for Clinical Research Neuropsychiatry).

Autism is a developmental disorder that is characterised by significant impairment in social interaction, communication, and the presence of stereotypic behaviours. It is largely considered to be a genetic disorder, but the aetiology and causal pathways are yet to be identified. This research, undertaken as Emma Glasson's PhD, consisted of three sections. The first investigated the presence of obstetric complications amongst all known people diagnosed with an autism spectrum disorder born in Western Australia between 1980 and 1995 (n = 465). Birth records were extracted from the Maternal and Child Health Research Database, and were compared to the same data on their siblings, a control group, and the control siblings. The results showed that the autism group experienced more difficulties during pregnancy, labour, delivery, and the neonatal period compared to all other groups. The second part of the thesis investigated the head growth patterns using head circumference measurements taken during childhood. Most children who developed autism were seen to have an increased head size during childhood. The third part of the thesis reported data from the WA Register for Autism Spectrum Disorders, which is the first prospective register for people diagnosed with an autism spectrum disorder in Australia.

WA Register for Autism Spectrum Disorders

Over the last decade, the prevalence of autism and the related spectrum of autism disorders have 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

Case Control Study Of Birth Defects

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

It is now well documented that adequate maternal intake of the vitamin folate before and in early pregnancy prevents the occurrence of about 70% of neural tube defects. There is some evidence that periconceptional folate may also prevent some other birth defects (heart defects, limb defects and urogenital defects have all been considered). Data collection for this Healthway-funded study was completed in late 2000, and analysis is continuing.

The aims of the study are to:

1. Determine the effectiveness of health promotion activities in WA to promote folate for the prevention of neural tube defects (NTD).
2. Determine whether vitamin B12 helps prevent NTD independently of folate.
3. Ascertain whether periconceptional folate protects against other birth defects.
4. Collect and store buccal samples from mother, father and infant.

Most women participating in the study were aware of folate and its relationship to birth defects, although a smaller proportion of women knew this information prior to becoming pregnant. The sources of information on folate and birth defects were similar for case and control groups, and the main sources were doctors and doctors' waiting rooms, family and friends, television, books and magazines. Radio, newspapers, pharmacies and supermarkets were less frequently cited as sources of information. The characteristics of women with limited knowledge of the relationship between folate and spina bifida and/or those who with inadequate folate intake prior to pregnancy are being explored, and the results will be used to identify potential health promotion strategies for improving knowledge

and practice prior to pregnancy.

Women completed a food frequency questionnaire relating to the six-month period immediately before becoming pregnant with the index pregnancy. They also answered a series of questions relating to the intake of foods fortified with folate, for the same time period. Folate intake from unfortified sources (natural folate) was estimated using the food tables, and folate from fortified products was derived from information provided by manufacturers. Because the folate added to foods that are fortified is synthetic folic acid and is 1.7 times more bioavailable than natural folate, the fortified food folate was multiplied by 1.7 before being added to natural folate, in order to estimate total daily dietary folate intake for each woman. Only four foods fortified with folate were available in WA at the commencement of the study, however, by the completion of the study, 32 foods that were fortified with folate were available in WA. Information on the use of vitamin supplements was also collected, and the amount of folic acid in each supplement was determined, and the daily intake of folic acid from supplements was estimated.

Analyses are currently underway of the relationship of supplemental folate and total folate intake for each category of birth defects (neural tube defects, congenital heart defects, limb defects, urogenital defects and other defects) compared with controls. Similar analyses are being conducted in relation to vitamin B12 intake from diet and supplements, controlling for folate intake.

Of 1,058 women who participated in the study, 617 (58.3%) provided buccal samples, 498 (47.1%) of fathers and 515 (48.7%) of babies also provided buccal samples. The samples are stored, and will be used in the future to gain understanding about birth defects by testing for genetic markers.

Congenital hypothyroidism in Western Australia 1981-1998

C Bower, JJ Kurinczuk, in collaboration with B Lewis, G Byrne (Departments of Pathology and Endocrinology, King Edward Memorial/Princess Margaret Hospital).

Newborn screening for congenital hypothyroidism began in Western Australia in 1981, with the aim of identifying children with the condition at an early stage, when treatment can prevent the later development of intellectual disability. Over 99% of

Western Australian newborn infants are screened for hypothyroidism. Using data from population-based registers and databases in Western Australia, we identified cases of congenital hypothyroidism 1981-1998 (n=126), a random sample of controls (n=1260), and descriptive data on cases and controls. The prevalence of congenital hypothyroidism was 2.88 per 10,000 livebirths. Case infants were significantly more likely to have other birth defects (particularly heart defects), be female, have a birth-weight greater than 4500gm, and be born either preterm or after 41 weeks gestation. No cases had cerebral palsy or intellectual disability. This study demonstrated the value of population-based registers and linked databases in evaluating screening programs, and extending our knowledge of the epidemiology of congenital hypothyroidism.

Trends in neural tube defects in Western Australia

C Bower, M Miller, J Payne, P Serna, in collaboration with A Ryan, E Rudy (WA Birth Defects Registry).

Knowledge of the prevention of neural tube defects by folate is high amongst women of childbearing age, although not all take folic acid supplements at the recommended dose and time. Over 100 food products are now fortified with folic acid in Australia. This study was undertaken to report on trends in neural tube defects in Western Australia.

Data on neural tube defects from Western Australian Birth Defects Registry were examined. A 34% fall in neural tube defects was documented from 1996 to 2000. The fall was seen for anencephaly and spina bifida, and in both births and terminations of pregnancy. The sustained fall in neural tube defects is thought to be due to increased periconceptional folate intake in response to health promotion campaigns and fortification of selected foods, but there is room for further improvement.

Birth defects in infants born after assisted conception techniques

M Hansen, C Bower, JJ Kurinczuk (Leicester University, UK), S Webb (WA Department of Health), N de Klerk, H Leonard, B Petterson (Disability Services Commission).

A paper describing the study of birth defects in infants born after assisted conception was accepted for publication in the New England Journal of

Medicine in 2001 and published in March 2002. The study included children conceived by intracytoplasmic sperm injection (ICSI) and by standard IVF, and a comparison group of a random sample of naturally conceived infants delivered in Western Australia during the same time period (1993 to 1997). Infants conceived with assisted conception techniques were found to have twice the risk of a major birth defect compared to naturally conceived infants. The increase in risk remained after adjustment for maternal age, parity, sex of the infant, correlations within sibships, and when the analysis was restricted to term singleton infants.

Also in 2001, a two-year National Health and Medical Research Council grant was obtained to expand this research in order to examine other health outcomes in assisted conception infants. The new study will examine admission to hospital, cerebral palsy, intellectual disability and birth defects diagnosed up to the age of six years in a larger sample of assisted conception infants compared to the remainder of Western Australian births.

Medication use in pregnancy among women in Western Australia

M Werler (Visiting Fogarty Senior International Fellow), C Bower, J Payne, P Serna.

In this study, data for control mothers (randomly selected from all births in WA 1997-2000) collected by questionnaire in the case-control study of birth defects described above, were used to assess patterns of medication use among pregnant women in Western Australia (WA). Data on medications used during the first three months of pregnancy were available for 488 control mothers. A research pharmacist working with Dr Werler at the Slone Epidemiology Unit, Boston University, coded data on medication use, such that the component substances, indications for use, and drug class could be analysed. Almost half the women reported taking no medicines, and fewer than 4% took four or more medicines. Of the women who did report taking medicine, the vast majority of use was for the treatment of pain or symptoms of colds, flu, or allergy. Paracetamol was the most commonly taken medication.

Modelling the potential impact of population-wide periconceptional folate/multivitamin supplementation on multiple births

C Bower, in collaboration with J Lumley, L Watson (Centre for Mothers and Children's Health, La Trobe University, Victoria), M Watson (Department of Perinatal Medicine, Royal Women's Hospital, Melbourne, Victoria).

There is suggestive evidence of an increase in multiple births to women taking periconceptional vitamin supplementation. This study modelled the impact of population-wide periconceptional folate supplementation on neural tube defects and twin births in a hypothetical cohort. Pooled data on relative risks for neural tube defects and twins were applied to the hypothetical cohort and, using perinatal data from Victoria and Western Australia, the effect on outcomes of neural tube defects (terminations, deaths, surviving infants), and twin births (preterm births, deaths, birth defects, cerebral palsy), were estimated. The numbers needed to treat for the prevention of one pregnancy with a neural tube defect was estimated to be 847, and for the birth of one twin with a poor outcome (death, birth defect, cerebral palsy) was 901. It was concluded that monitoring the rates of neural tube defects and twinning is essential as supplementation and/or fortification with folate is implemented.

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 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, intel-

lectual 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 (Figure 1.). 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 is that although there has been negligible change in frequency of cerebral palsy among term births, they exhibit increasingly severe disability. Figure 2 shows the increase in the proportion of cases with scores of 11 or 12 on a 12 point scale of disability.

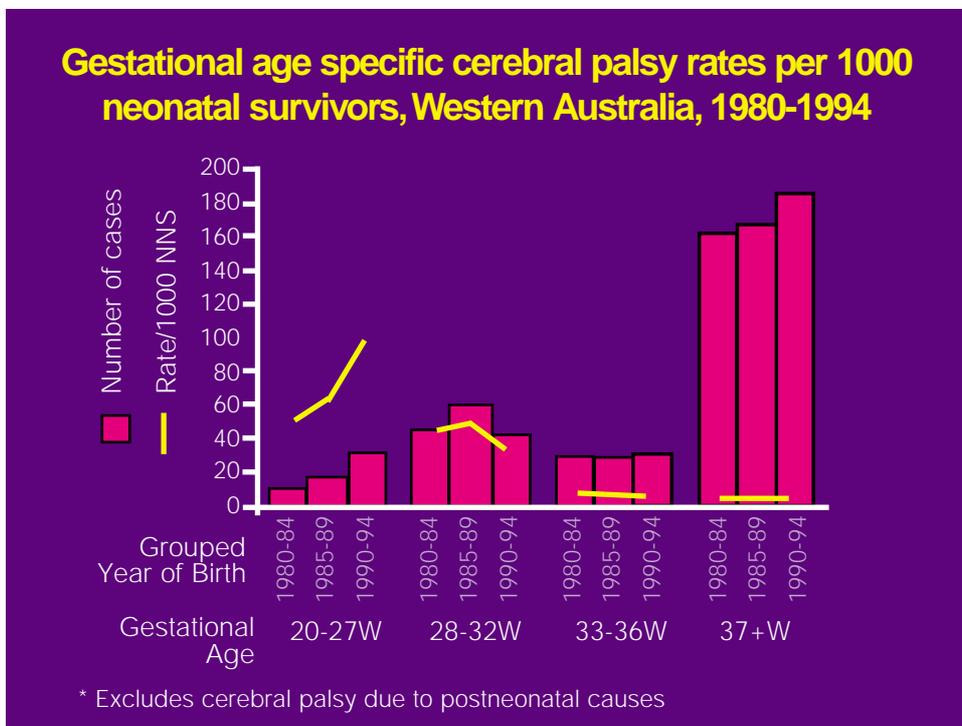


Figure 1.

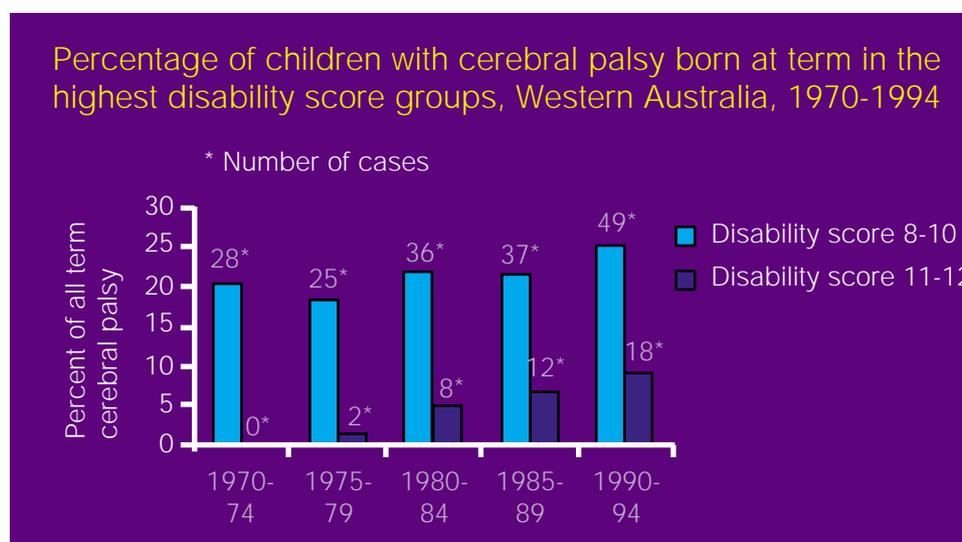


Figure 2.

How long does cerebral palsy last?

As there is no cure cerebral palsy is a lifelong condition, though functional ability and quality of life may be improved by expert management. Linking the Cerebral Palsy Register to statutory Death Registers allowed us to measure the life expectancy of people with cerebral palsy. The condition itself is neither degenerative nor fatal, but it predisposes the individual to a number of potentially fatal problems, par-

ticularly respiratory infections. For a given level of disability, life expectancy has not changed since the 1950s. Of the most severely and multiply impaired, requiring lifelong assistance in all aspects of daily living, 40% will survive to adulthood - and there are now more of these very severely impaired people. The characteristic most strongly associated with mortality in persons with cerebral palsy is their intellectual ability, as shown in Figure 3.

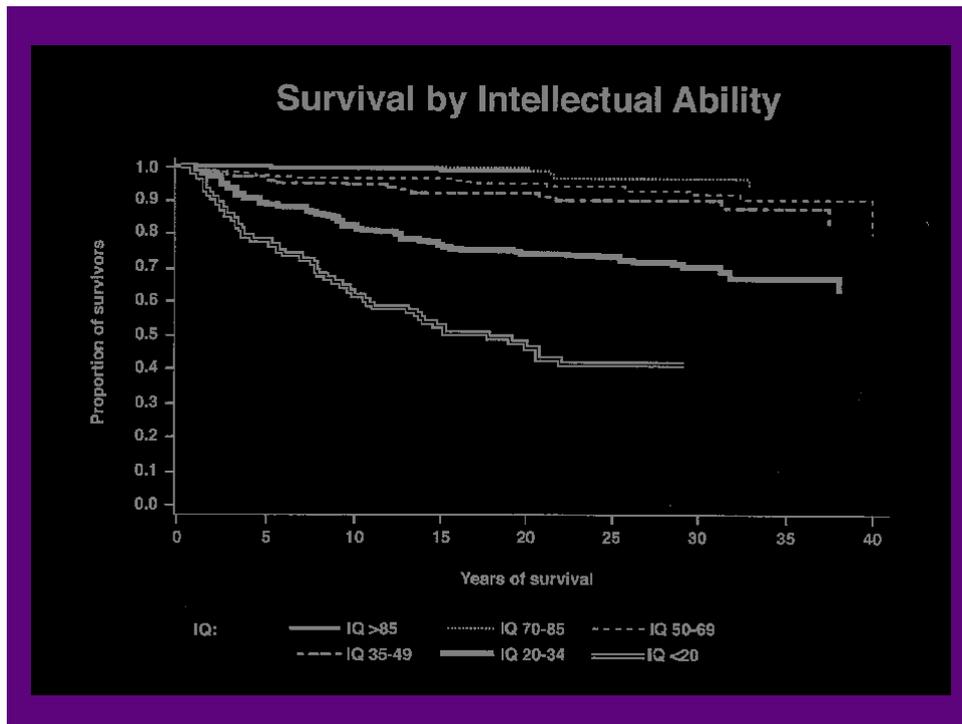


Figure 3.

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.

The causal pathway to choreoathetoid cerebral palsy and suggested preventive strategies

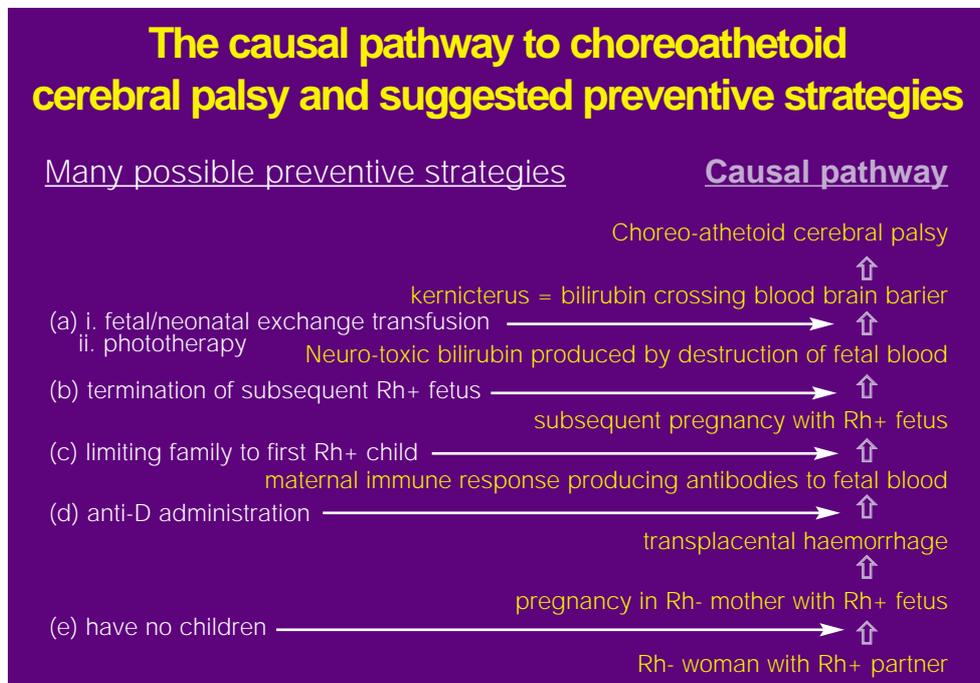


Figure 4

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 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 intrapartum stillbirths and neonatal deaths delivered 1985-1995. An exhaustive data collection was commenced in 1996 and is now almost complete for groups (a) and (b). The data collection for deaths has commenced and is expected to be complete by the end of 2002.

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 co-fetuses in a multiple pregnancy. Twins have a 4.5 fold increase in risk and triplets an 18 fold increase in risk of cerebral palsy. This provided evidence in support of the 1987 guideline for in vitro fertilisation, which limits the number of embryos that are transferred in any one cycle to 3. Legislation adopting these guidelines was passed in 1993 and the rising rate of triplet pregnancies has started to abate in WA.

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.

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%, 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 alarming rise 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: prevalence in Western Australia and challenges and opportunities for research

H Leonard, C Bower, B Petterson (Disability Services Commission), R Sanders (Department of Education), X Wen (Australian Institute of Health and Welfare).

During 2001, we presented our analysis on the prevalence of intellectual disability in Western Australian children born between 1983 and 1992. Major strengths of the study were the use of record linkage and the use of multiple sources including the state disability service, Disability Services Commission, as well as state and private educational systems to ascertain cases. The prevalence of intellectual disability was 14.6 per 1000 and was greater in males and in children of Aboriginal mothers.

During the same period we were invited to review the epidemiology of intellectual disability for an international publication. This review focussed on both the challenges and the opportunities associated with studying intellectual disability from a population perspective and drew heavily on our Western Australian experience. The challenges relate to the differences that exist in criteria for defining intellectual disability and how these may change over time, and to the difference in case ascertainment methods. These differences make it difficult to compare prevalence of intellectual disability across place and over time. The latter is particularly important as we seek to determine both the effectiveness of preventive interventions and the implications for intellectual disability of the major societal changes occurring in the Western world. In this review we examined how the prevalence of intellectual disability differs

according to age, gender, social class and ethnicity. We were able to highlight the similarities when both the Australian indigenous and African-American populations are compared with their Caucasian counterparts. We postulated that the aetiological pathways underlying these ethnic differences in the two continents might also be similar.

Other challenges relate to differences in the ways the aetiology of intellectual disability is classified in different studies. There is a need to develop a classification system that, as with prevalence, will allow comparisons over time and between countries. A further problem in this area of research is the rarity of the majority of the individual biomedical disorders causing intellectual disability. This belies the need to take advantage of new mechanisms and technologies to study these rare disorders. In Australia the Australian Paediatric Surveillance unit is an active surveillance system, which has been used to study a number of conditions associated with intellectual disability including Rett, fetal alcohol and Prader Willi syndromes. On an international scale, bioinformatic technology can be used to collect both genotype and phenotype data from cases across the world and thus provide adequate statistical power for the complex analyses involved.

Although many of the advances affecting this area such as the capacity for antenatal and neonatal screening depend on new technologies there may also be drawbacks. An increase in the incidence of birth defects has already been shown to be associated with assisted reproductive technology and some studies suggest an increase in developmental problems. On the other hand differential access to prenatal diagnosis and other technologies could even lead to an increase in inequalities with the disadvantaged at greater risk.

To implement the important "prevention-intervention-research" cycle, which surely underpins the role of epidemiology in intellectual disability, it will be necessary to address the methodological challenges. It is clear that use of multiple sources and record linkage systems such as the unique infrastructure of health databases we have in Western Australia are efficient strategies in this process. However, the next step will be to establish systems, which allow linkages across generations and jurisdictions. Such research processes will have the capacity to identify the intergenerational risk factors, which impact on child disadvantage and indicate how we can use

social policy to intervene. This sort of intersectoral collaboration is essential both in understanding the determinants of intellectual disability as well as in the provision of the most appropriate and cost effective services to these children and their families. Thus many of the research questions needing to be answered in relation to intellectual disability fit well within the scope of the new disciplines of both social and genetic epidemiology and thus provide exciting opportunities ahead to make major inroads into this field.

Mental Health Disorders

Foundations of Social and Emotional Development: a continuum

A research project commissioned by the Department of Education Students at Educational Risk/Health and Wellbeing Project (Phase 1: November 1999- December, 2000 & Phase 2: October 2001 - April, 2002)
A Williams, S Zubrick, S Silburn.

Background. In November 1999 the Department of Education identified the need for evidence-based research into the social and emotional development of children to inform developmentally appropriate policy and practice and support social and academic outcomes of schooling. The Institute was commissioned to research and articulate a social emotional developmental continuum.

Project Objectives. The specific objectives of the project were to research and articulate a continuum of social and emotional development from conception to 17 years of age; to assess the impact of positive and negative environmental exposures (risk and protective factors) across the continuum; and identify strategies and interventions to support positive learning environments across home school and community settings.

Project Outcome. A technical report on the social and emotional continuum was prepared to meet these objectives and submitted to the Department of Education in February 2000. The work is based on the findings of current research in neuroscience, early childhood education, developmental psychology and prevention science. It provides an empirical evidence base from which to guide developmentally appropriate policy and practice across government portfolios of education, health, and community services.

A second phase for the project was commissioned in October 2001. This has involved the Institute working in partnership with the Department of Education and the Centre of Excellence in Teaching to translate research findings into user friendly resources for schools to support mental health promoting schools and the implementation of Western Australian Curriculum frameworks.

Positive Parenting of Preschoolers

SR Zubrick, KA Northey, SR Silburn, D Lawrence, AA Williams, E Blair, DJ Robertson, MR Sanders.

In response to recent large scale national and regional population studies of the prevalence of child and adolescent mental health problems and illnesses, and in concert with international concerns about the global burden of mental health disorders, Australian health authorities have moved to promote an evidence-based action plan for a more comprehensive approach to promotion, prevention and early intervention in mental health. Within this broad action plan, a central focus of Australian national and regional government concern has been on serious childhood behaviour problems and on making decisions about which programs to fund and implement from an array of proffered early intervention programs.

Parent education programs – or behavioural family interventions (BFI) – are a class of early intervention strategy found to be effective in reducing the burden of serious childhood behaviour problems. One early intervention strategy is the Triple-P (Positive Parenting of Preschoolers) Program. The Triple-P has a number of versions (individual, group and self-directed) and is designed as a comprehensive system of parenting and family support. In 1995 The Health Department of WA decided to commission and implement the group version of Triple-P as a demonstration trial within one of the seven health regions in the state in response to the epidemiological findings on the health, mental health and well being of children living in the state.

This approach represents a movement within WA toward whole population approaches in preventive health services. The decision to implement a universal level of BFI was based on the recognition that while many of the risks for problems such as child mental health disorders may be quite “weak” (i.e. small odds ratios) a large proportion of the child population is exposed to these risks resulting in a

substantial burden. Just as importantly, aversive parenting as a risk exposure is likely to operate across a continuous range of intensity rather than merely as a dichotomous threshold exposure. Thus, population interventions that seek to modify the mean population exposure may result in a significant reduction in poor outcome despite rather modest effects at the individual level.

Aims and hypotheses. The primary aim of the project was to prevent the onset of serious behaviour problems (e.g. conduct disorder, oppositional defiant disorder, ADHD) in preschool children through the application of a universal behavioural family intervention delivered through primary health care systems and utilising the Triple-P. Triple-P was designed to reduce the use of aversive parenting methods; increase the use of positive parenting behaviours; reduce parental depression, anxiety and stress; and reduce the general level of marital problems. These risk exposures are likely to be present in the causal pathway to serious behaviour problems (Commonwealth Department of Health and Aged Care, 2000).

We hypothesised that following participation in Triple P parents would report lower mean levels of aversive parenting than parents in the comparison group. We further hypothesised that parents participating in Triple P would report lower mean levels of child behaviour problems in their children than parents not participating in Triple P.

Intervention program content and training. Enrolled parents participated in a two-hour training workshop in groups of 10, once a week for four weeks, followed by a 15-minute telephone support session once a week for four weeks. Each family received a copy of the text, *Every Parent* (Sanders, 1992), *Every Parent's Workbook for Groups* (Markie-Dadds, Turner, & Sanders, 1997) and a video to support their participation in the program (Sanders, 1996b). Parents were taught to apply parenting skills to a broad range of target behaviours in both home and community settings with the target child and all relevant siblings. By working through the exercises in their workbook, parents learned to set and monitor their own goals for behaviour change and enhance their skills in observing their child's and their own behaviour.

Research Design. The research design chosen to

evaluate the Triple-P intervention program was a non-random two-group concurrent prospective observational design. Participants in the intervention group were asked to complete questionnaires on four occasions: prior to the delivery of the program (pre-intervention), approximately nine weeks later immediately post-program and then at 12 and 24 months following the post-program assessment. Participants in the comparison group were asked to complete questionnaires on enrolment in the study and approximately nine weeks later and subsequently at 12 and 24 months following enrolment.

Measures. The study incorporated five outcome measures. (1) The Eyberg Child Behaviour Inventory (ECBI) which is a 36-item measure of parental perceptions of disruptive behaviour in children aged 2 to 16 years. (2) The Parenting Scale (PS) which is a 30-item questionnaire measuring dysfunctional discipline styles in parents. (3) Parent Problem Checklist (PPC) which is a 16-item questionnaire that measures conflict between partners over child rearing. It rates parents' ability to cooperate and work together in family management. (4) Abbreviated Dyadic Adjustment Scale (ADAS) which is a measure of the quality of dyadic relationship adjustment. (5) Depression Anxiety Stress Scales (DASS) which is a 42-item questionnaire that assesses symptoms of depression, anxiety and stress in adults.

Results. To assess changes in the Parenting Scale, Eyberg Intensity Score and carer response variables (i.e. PPC, ADAS and DAS) over time and assess the differences within and between the intervention and comparison groups we have used linear mixed modelling (SAS Institute Inc, 2000). This procedure was chosen because it is (1) suitable for handling correlation arising from responses measured at multiple occasions on the same subjects, (2) allows a variable pattern of missing data by utilising the full matrix of information on each subject, and (3) multivariately adjusts for the effects of covariates, allowing the effect of the intervention to be separated from the differences between the intervention and control groups and the effect of time.

The mixed model generalises ordinary multiple regression by allowing the usual assumption of independent observations to be relaxed (SAS Institute Inc, 2000; Littell, Ramon, Milliken et al., 1996). We fitted an unstructured covariance matrix that esti-

mated the correlation between measures on the same subject at each of the four time points. Separate correlation structures were fitted for the intervention and control groups to allow for the fact that the intervention itself would likely affect the correlations over time. Thus, by specifying the child's age at pre-test, the parent's qualifications, family type, and household income as covariates we simultaneously adjusted for differences between the intervention and comparison group at the beginning as well as accounted for the correlations between measures on the same individual over time. The reported means are adjusted least squares estimates and are reported with their corresponding standard errors, degrees of freedom and p values.

Parenting Style. The Triple-P intervention is targeted at parenting style to broaden the repertoire of positive parenting behaviour thereby preventing or reducing aggressive behaviour in young children. As the total score on the Arnold Parenting Scale is used as a measure of parenting style, we fitted it in a linear mixed model.

The immediate effect of the intervention was to improve parent-reported parenting style as evidenced by a 0.62 point decrease (95% ci = 0.57, 0.67) in the adjusted mean Parenting Scale total score. At 12 and 24 months post intervention, this improvement in parent-reported child behaviour, while not as large, was still significant with decreases in the adjusted mean PS score of 0.34 (95% ci = 0.29, 0.39) and 0.32 (95% ci = 0.27, 0.38) respectively.

In summary, parent-reported levels of aversive parenting behaviour declined in the comparison group as their children grew older. Adjusting for this effect in the intervention group, parent-reported aversive parenting behaviour showed a significant decline immediately post-intervention and then increased, but stayed significantly below the pre-test level for twelve and twenty-four months.

Child behaviour. The response variable was the continuous ECBI intensity score and was fitted in a linear mixed model in a manner similar to that described above. The immediate effect of the intervention was to improve parent-reported child behaviour as evidenced by a 22.4 point decrease (95% ci = 20.38, 24.48) in the adjusted mean ECBI intensity score. At 12 and 24 months post intervention, this improvement in parent-reported child behaviour,

while not as large, was still significant with decreases in the adjusted mean ECBI Intensity score of 11.3 (95% ci = 9.1, 13.5) and 12.9 (95% ci = 10.4, 15.4) respectively.

Using these mean changes in the ECBI Intensity Score and noting that the ECBI has a standard deviation of 27.0 points (Burns, G. & Patterson, D, 1990), then the immediate impact of the intervention improved child behaviour by .83 of a standard deviation which corresponds to a large effect size (Cohen, 1988). At twelve months this improvement had diminished to .41 and at 24 months it was .47 of a standard deviation corresponding to a medium effect sizes.

Additional parental outcomes. For each of the immediate post-program, 12 and 24 month observations linear mixed models were fitted for each of the parental outcome variables in the same way as for our analysis of the PS and ECBI intensity scores.

Effects on caregiver depression, anxiety and stress (DAS). Linear mixed modelling of the DAS showed that the immediate effect of the intervention lowered adjusted mean DAS scores by 7.2 points (95% ci = 5.7, 8.7). At 12 and 24 months post intervention, this improvement in the parent-reported DAS score, while not as large, was still significant with decreases in the adjusted mean DAS score of 5.5 (95% ci = 3.9, 7.1) and 4.4 (95% ci = 2.8, 6.0) respectively. Overall, there was a significant improvement in parent-rated mental health, as measured by the DAS, in the immediate post-intervention period. While this effect declines over time, it is still significant at 24 months post-intervention.

Effects on conflict between partners over child rearing (PPC). Analysis of mean PPC scores showed a similar pattern of results to that of the DAS. In the immediate post-intervention period the adjusted mean PPC score decreased by 3.5 points (95% ci = 2.4, 4.5). This effect marginally diminished at 12 months to 2.3 points (95% ci = 1.1, 3.5) and returned to 3.3 points (95% ci = 2.0, 4.7) at 24 months. In general these findings suggest that the intervention significantly decreased the level of parent reported conflict over child rearing in the immediate, 12 and 24 month time periods.

Effects on quality of marital dyadic relationship adjustment (ADAS). The results of our analysis of

the effects of the intervention on marital adjustment are in keeping with those above. Bearing in mind that increases in the mean ADAS reflect higher levels of dissatisfaction, analysis revealed a significant improvement (ie decrease) in mean ADAS scores in the immediate (-1.0, 95% ci = -1.4, -0.6), 12 month (-0.7, 95%ci = -1.2, -0.3) and 24 month (-0.7, 95% ci = -1.2, -0.3) periods.

Conclusion. This is the first study to show that a universal behavioural family intervention can be delivered on a large scale through regular services and produce clinically meaningful outcomes. These findings confirm other research into the effects of Triple P showing reduced levels of disruptive behaviour and changes in parenting practices (Sanders, Markie-Dadds, Tully, & Bor, 2000). We believe they show that a program of carefully monitored, measured and delivered behavioural family intervention is one strategy in an array of potential prevention intervention opportunities that could be effective in changing population rates of behavioural outcomes in children (Commonwealth Department of Health and Aged Care, 2000).

Finally, we are presently obtaining behavioural observations of these children from informants other than their parents. Families in both groups are being followed with measurements at 36 months post-intervention from parents as well as from Grade 1 school teachers as the children complete the first year of school.

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Antecedents and outcomes of Newborn Encephalopathy (NE) in term newborns

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There have been few long-term studies of the outcomes following newborn encephalopathy. Of those conducted, the majority were not population-based, most concentrated on encephalopathy associated with 'birth asphyxia' while others only included infants with neonatal seizures. Few studies have been concerned with outcomes other than cerebral palsy and death. With notable exceptions other disabilities such as cognitive impairment and developmental delay have not been considered or have only been reported for infants with hypoxic ischaemic encephalopathy.

We undertook a case control study of moderate and severe newborn encephalopathy with recruitment from 1993 to 1996. This was the first population-based study of newborn encephalopathy using a broad clinical definition. We have subsequently followed the cases and controls longitudinally. 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 10.1% 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.

When the children reach 3 years of age they receive a full neurological assessment performed by a paediatrician. Currently 63% of the cases and controls have undergone a neurological assessment 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 58% of eligible children to date. These assessments include receptive language, verbal and visual reasoning, 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 6 and then 7 years.

In June 2001 we commenced face-to-face assessments of the cohort as they turned 8 years old. This stage of the assessment will be complete at the end of 2004 when the youngest children turn eight. The 8-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.

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 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 8 years), we aim to:

- Estimate the cumulative mortality to 8 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 Programme

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The Western Australian Newborn Hearing Screening Programme is jointly funded by the Health Department of Western Australia, King Edward Memorial Hospital for Women and Princess Margaret Hospital for Children 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 severe to profound hearing loss is 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 is currently comparing the speech and language skills of Western Australian children with a permanent hearing loss who started intervention early to those who started intervention later.

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 program commenced at the five largest maternity hospitals in the Perth metropolitan area and was expanded to the neonatal unit at Princess Margaret Hospital in 2001. 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.

In 2001, over 10,000 babies received hearing screening. Over 99% of babies passed either the initial or repeat hearing screen. Seven babies who were referred to a paediatric audiologist were subsequently diagnosed as having severe to profound permanent bilateral hearing losses at an early age.

All aspects of the program are currently being evaluated. An important part of the evaluation has been

setting up of the Hearing Loss Prevalence Programme (HeLP). The HeLP programme 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

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During 2001, we continued to maintain the 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 are organised for mutation screening. An important component of the study is the provision and updating of information about the study to clinicians, particularly geneticists and neurologists, who are likely to come in contact with potential cases. Whilst Rett syndrome has characteristically been a disorder of females, it is now also being diagnosed, although still exceptionally rarely in boys, and, thus when diagnosed, boys are also included in the register.

A major activity during 2001 was the processing of data relating to the 2000 'Follow-up study'. During 2000 families took part in an intensive data collection by completing a follow-up questionnaire and filling in a daily calendar relating to their daughter's health and well-being. Thus we now have a unique comprehensive dataset about the day-to-day health problems and medication use of children with Rett

syndrome as well as almost 10 years of longitudinal data about various aspects of health and functioning. Information about management (including both medical and therapy services) was also been collected which will allow for evaluation of treatment strategies over time. The basic statistics now provided by our database about the presence of medical conditions are perceived as extremely valuable by both clinicians and families of newly diagnosed children. About three quarters (73.7%) of the children have epilepsy and many families (89.5%) report the presence of sleep problems. Over two thirds (71.1%) of children have scoliosis and over one third (34.9%) had suffered a fracture by the year 2000. Forty one percent of children had attended their neurologist in a 1 year period and 38% had been seen by an orthopaedic specialist.

An important aspect of the 2000 'Follow-up study' was the administration of the WeeFIM - a functional measurement tool. This is the first time that functional ability has been measured in an entire population of girls with Rett syndrome. These data on functioning can identify areas of strengths and weaknesses, which can be extremely useful to therapists and educators in devising management strategies. The mean WeeFIM score in Rett syndrome was 29 (maximum possible score=126). This compares with a mean of 106 in a similar study in Down syndrome. For Rett syndrome, performance was greatest in the mobility domain although 80.9% of children used equipment for assistance. Functioning was much poorer in the area of social cognition and 44.1% were using communication aids. Whilst 84% of families received physiotherapy, occupational or speech therapy during a 1 year period, less than half (44.1%) felt that their daughter's therapy needs were being met.

Molecular genetic studies continue in collaboration with the Neurogenetics laboratory at Royal Perth Hospital and our collaborators at the Children's Hospital, Westmead, Sydney. Mutation screening has been completed on over 174 cases (77%) and mutations identified in 70% of those cases. Thirty four pathogenic mutations have been identified. Mutations are present in 73% (68/93) of classical and 65% (53/81) of atypical cases. In addition, X-inactivation studies have also been carried out and skewing found to be present in 43% of cases tested. A publication relating to the molecular results has been submitted and another is in preparation in relation

to the extensive phenotype data we have compiled. An innovative aspect of the study has been the participation of school groups in 2001 in order to provide control data for the 'Follow-up study'. The first control group consisted of 52 girls from St Mary's Anglican Girls School who completed a weekly health and medication diary. Their participation also involved visits to the Institute to learn more about scientific research, intellectual disability and Rett syndrome, whilst we attended the school to provide parents with information about Rett syndrome and the study. At the end of the year the children visited the Institute and gave an excellent and moving presentation about their understanding of Rett syndrome. This control group will be expanded in 2002 with the inclusion of high school students who will enter their data directly into the study database using the Internet.

During 2001, a group of medical students embarked on an evaluation of the Rettnet (parent email list). The Rettnet is used by parents and other interested persons both in Australia and internationally as a source of information and support. In the light of the increasing popularity of the Internet and the complex and often conflicting nature of information about Rett syndrome, it was considered that evaluation of this forum would be a fruitful exercise. We found that 78% of (94/120) respondents found Rettnet to be a very useful source of information.

A science student also developed a video protocol to enable families to follow a clearly outlined set of instructions for recording their child's abilities and difficulties. He filmed three girls with differing levels of functional ability to provide examples of activities for parents to videotape. The video has been edited and it is now planned to trial its use in conjunction with a booklet of written instructions.

The next 'Follow-up study' to be administered in 2002 will be similar in format to the 2000 study and will allow the ongoing monitoring of functioning and health, which can be correlated with the child's genetic status. In addition, measures of family support and functioning will also be included. We have found the Internet to be an excellent mechanism for the collection of research data. Over a quarter of families in our Australian cohort now have Internet access. Following the success of the 2000 study, parents will again be able to complete the questionnaire using the Internet.

Throughout 2001 we further consolidated our international collaborative links. Drs Leonard and Fyfe attended an international meeting of Rett syndrome researchers in Baden Baden, Germany. The purpose of this meeting was to re-examine the clinical criteria and to consider different severity scales for Rett syndrome. The creation of an international phenotype database to collect information on the clinical features of Rett syndrome is currently being planned. This will complement the mutation database the development of which is now underway by our colleagues in Sydney.

Infectious Diseases

Risk factors for otitis media in Indigenous and non-Indigenous children in the Kalgoorlie-Boulder area

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

Enrolment into a study aimed at identifying the most potent factors predisposing Aboriginal and non-Aboriginal children to otitis media began in April 1999 and will continue until the end of 2002.

Funding was provided for 3 years by Healthway, who are continuing to support this study as well as an adjunct qualitative study described below.

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 are collected to investigate upper respiratory tract bacterial and viral carriage. 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 an ENT specialist on three occasions,

hearing is assessed once by an audiologist in the second year of life and tympanometry is done at each visit from age 4 months onwards. All Aboriginal babies are now offered a pneumococcal conjugate vaccine (Prevenar™) and through a donation from Wyeth we are 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. To date we have enrolled 221 children, 75 of whom are Indigenous.

Middle ear disease has been diagnosed in 50% and 26% of Aboriginal and non-Aboriginal babies aged 1-<2 months, respectively, and 58% and 33%, respectively, at age 4-5 months. At least 11 (15%) Aboriginal and 2 (1%) non-Aboriginal babies have had at least one episode of perforated ear drum. Maternal smoking and crowding have already been found to be associated with high rates of middle ear infection in young Aboriginal babies. The carriage rate of *Streptococcus pneumoniae* at age 1 month in Aboriginal babies is 50% increasing to 70% by age 4 months. In non-Aboriginal babies, pneumococcal carriage rates are 30-40% between 2 and 6 months of age. 50% of Aboriginal babies carry *Haemophilus influenzae* at age 3 months, compared to 10% of non-Aboriginal babies. 30% of Aboriginal babies carry *Moraxella catarrhalis* at age 1 month rising to 70-80% at age 4 months. In non-Aboriginal babies carriage rates of *M. catarrhalis* are 10% at age 1 month rising to 30% at age 3 months. *M. catarrhalis* and *S. pneumoniae* are found more commonly together than apart.

Findings from this study will be used to develop appropriate interventions to prevent otitis media, which can seriously affect childhood development, school performance and subsequent social and economic well-being.

Other collaborative studies of otitis media in Western Australia

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 should provide information to assist clinicians in deciding when and on whom to operate and should also assist in health services' planning regarding ear health.

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

S Couzos, I Ring, H Coates, F Lannigan, R Mueller, R Murray, D Lehmann, S Eades.

Chronic suppurative otitis media (runny ears) is extremely prevalent in Aboriginal babies from a young age and the management is often protracted and frustrating, requiring regular as well as repeated clearance of the ear discharge. Treatment may include the use of ear drops (currently Sofradex which is an aminoglycoside). This ongoing study has taken place through 9 Aboriginal Community Controlled Health Services in northern Western Australia and north Queensland and aims to compare the effectiveness of Sofradex ear drops with Ciloxan drops (0.3% ciprofloxacin, a fluoroquinolone) in clearing ear discharge, healing of the ear drum and improvement in hearing in Aboriginal children with chronic ear discharge. Isolates from ear discharge are also being tested to detect any emergence of bacterial resistance to topical ciprofloxacin. The Institute for Child Health Research provides epidemiological expertise to the project. Enrolment and follow-up is ongoing.

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

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

Following a report of increased risk of death associated with diphtheria tetanus pertussis (DTP) and oral polio vaccination of children living in rural areas of Guinea-Bissau, the World Health Organization Department of Vaccines and Biologicals sought proposals to determine the effects of routine infant immunization on survival in areas of high mortality. We were awarded a grant to investigate the impact of routine immunizations on childhood survival in Tari, Southern Highlands Province, Papua New Guinea. 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, migrations, and immunization 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. Investigators addressing this issue in six countries met in Geneva in October 2001 to discuss preliminary findings and N de Klerk reported our preliminary results to the WHO Global Advisory Committee on Vaccine Safety Committee in December 2001. A manuscript is in preparation.

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

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

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

Maternal immunization 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 immunization, 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 immunization might affect subsequent infant immunization, 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 immunization did not affect children's capacity to mount antibody responses to immunization with Pnc PS vaccine in infancy. Antibody concentrations in breastmilk of some of the immunised and unimmunised women have also been measured and data are currently being analysed to see if breast milk might afford protection against pneumococcal disease. This study together with studies in several other countries provides further support for the concept of maternal immunization as a potential intervention to reduce the very high mortality rates in non-industrialised countries.

Methodological Approach

Biostatistical analysis and support

NH de Klerk, J Hansen, M Firth, YC Liu.

This group continued collaborative work with other projects throughout the division and the Institute, as well as with the University Departments of Paediatrics, Public Health and General Practice. Particularly in areas involving the analysis of complex longitudinal data and survival analysis.

Breast feeding and atopy

W Oddy, J Sherriff (Curtin University), JK Peat (Institute of Respiratory Medicine, Sydney), NH de Klerk.

Wendy Oddy has continued her post-doctoral fellowship at Curtin University. This involves continued collaboration with the Institute in the analysis of data from various studies. The work at Curtin has included being course coordinator in the Degree of Masters of International Health, Centre for International Health; responsibility for the 'Evaluation and Research in Health' unit, and the 'Maternal and Child Nutrition' modules in the 'Maternal and Child Health in Developing Countries' unit; development of the International Nutrition 600 postgraduate distance education unit; Development and delivery of lectures in the National Epidemiology 382 Unit to third year Nutrition students; supervision of two third year nutrition student research projects. The fellowship also involves collaboration with the Institute of Respiratory Medicine in Sydney.

Genetic Epidemiology

Genetic statistics and causal pathway modelling

University of Leicester Annexe - N Sheehan (Senior Research Fellow in Genetic Statistics), P Burton (Professor of Genetic Epidemiology and Head of GENESIS), K Scurrah (PhD student), in collaboration with L Palmer (Channing Laboratory, Harvard University) and J Hansen (Co-ordinator WATCH study).

This section considers that component of the research program of GENESIS (Genetic Epidemiology and Social Science, Department of Epidemiology and Public Health, University of

Leicester) that links directly to the research program of the Institute. This part of our research is subsumed within the NH&MRC program grant Epidemiological Studies in Maternal and Child Health. It has three underlying and interconnected themes.

Generalised linear mixed models (GLMMs)

Our first theme entails the development of complex mixed mathematical models to undertake genetic linkage or association analyses in pedigree data. This includes sibships, nuclear family data, twin family data, extended pedigrees and complex extended pedigrees with loops. Paul Burton has historically focussed on problems that are complex because they entail a mixture of unobserved polygenes (\pm a major gene), unobserved environment, a complex array of observed determinants (often genotyped loci) and may involve a non-normal phenotype (particularly right censored survival time data). Nuala Sheehan has mainly dealt with problems involving very complex pedigrees. Recently we have focussed on trying to incorporate ascertainment adjustment into our models.

Graphical modelling

Our second theme addresses one of the ways in which such models may best be fitted. Most of our pre-existing work has focussed on likelihood-based approaches or Markov chain Monte Carlo methods (principally Gibbs sampling). Many of these models can be expressed within the very general framework of Graphical Modelling. Indeed, much of our work has been based in WinBUGS, which explicitly permits Gibbs sampling models to be expressed via a graphical interface. Furthermore, Nuala Sheehan is co-principal investigator on a Biomedical Research Collaboration grant from the Wellcome Trust which funds joint work with Daniel Sorensen (Foulum, Denmark) on a graphical models approach to the problem of detecting quantitative trait loci by association with discrete marker data on general pedigrees. This work is based in the package HUGIN. The aim of developing a powerful graphical modelling framework within which many of our models can be fitted is an important component of both our current and proposed research plans.

Causal pathway modelling

Bayesian networks can be expressed as graphical models and a special case is the causal model, which shares much in common with structural equa-

tion models. Our third inter-related theme extends our work with graphical models to causal pathway modelling. This is our newest theme. We have recently been awarded a Leverhulme Research Interchange Grant as part of a consortium headed by Phil Dawid (University College, London). Our involvement within the consortium includes an investigation of graphical models to fit causal pathway models of the sort required to disentangle the causal pathways leading to newborn encephalopathy, cerebral palsy and asthma.

Suicide prevention

SR Silburn, SR Zubrick, A Brok, SJ Clark, JAM Cugley, SD Hillman, S Jackiewicz, N Kerr, K Northey, K Miller, D Robertson, A Robson, B Williams.

While still retaining a major focus on youth, the scope of the committee's activities this year have been extended to encompass suicide prevention across the lifespan. This was formalised by the Minister for Health (The Hon Bob Kucera, MLA) reconstituting the Youth Suicide Advisory Committee as the Ministerial Suicide Prevention Council (MCSP). This Council reports through the Minister for Health to all other Ministers on the Cabinet Subcommittee for Social Policy. The MCSP is responsible for advising government; coordinating and supporting effort across government and non-government agencies to reduce the morbidity and mortality associated with suicide and self harm; and advancing scientific and community understanding of suicide and its prevention.

The MCSP maintains the WA Coroner's Database on Suicide. This on-going 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. This was described in a report prepared for the Western Australian Drug Abuse Strategy Office. The Minister for Health launched the report Youth Suicide in Western Australia Involving Cannabis and Other Drugs in December 2001.

Hospital based studies of the clinical phenomenology and treatment of deliberate self harm have been

used to guide the targeting of clinical and population interventions. The findings from the four year follow up of an NH&MRC funded study evaluating enhancements to the hospital and community care for youth deliberate self-harm admissions in three Perth teaching hospitals has had an uptake in policy and practice at a state and National level.

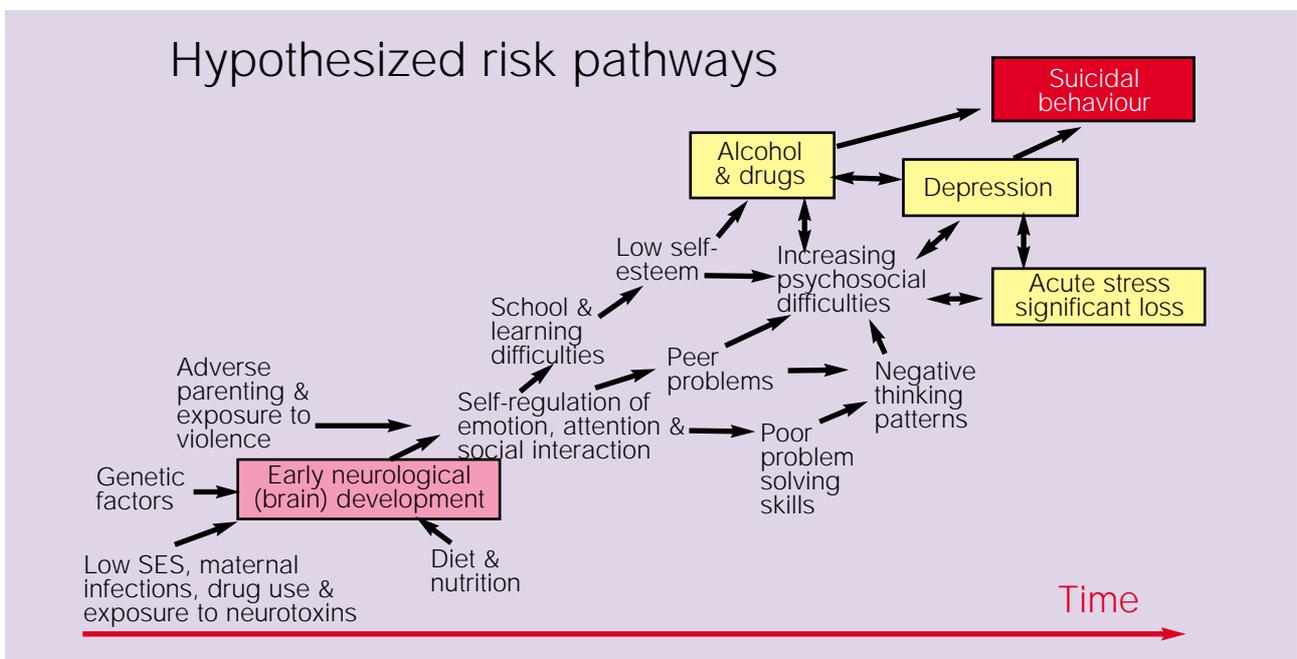
Prevalence data on suicidal behaviour and their risk associations are currently being explored through the 2000/2001 WA Aboriginal Child Health Survey. The early antecedents of depression and suicidal behaviour are also being investigated in the RASCALS study survey of a 10% random sample of recently 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. Figure 1 below delineates some of the hypothesised risk pathways that will be examined.

A policy and funding proposal for a long-term, across-government and inter-sectoral strategy to facilitate the community development of universally targeted, primary prevention strategies which address regional priorities was developed by the WA Aboriginal Suicide Prevention Steering Committee in 2001. This involved extensive consultation with Aboriginal communities and service providers and a review of the national and international literature. This proposal has been endorsed by the Health Minister and is to be considered by the Indigenous Affairs Advisory Committee in May 2002.

During 2001 National Suicide Prevention Strategy (NSPS) funding enabled an audit of information available to the general public and professionals regarding suicide prevention. This has been used to guide the development of the MCSP web site (www.ichr.uwa.edu.au/sp/). The Public Education strategy has also secured corporate funding over three years from January 2002 through a joint initiative between Woodside Energy and the Institute for Child Health Research, this will involve the systematic development of a national, resource and information system for suicide prevention.

NSPS funding has enabled the Council to produce an "Information and Support Pack for those bereaved by suicide or other sudden death". This was informed by research on the information and support needs of families bereaved through suicide. These are distributed through the Coronial counselling service and are also available on-line at the Council's website (www.ichr.uwa.edu.au/sp/).

A project to commence in 2002 is a record linkage study of four years of data from the Perth hospitals deliberate self-harm data system with the WA Coroner's database and the Department of Health's morbidity and mortality registers. Another project is a case-control study linking the Coroner's database and the Maternal and Child Health Research Database. This will examine the relationships between foetal growth restrictions, obstetric care and subsequent proneness to suicide.



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 MCHRDB:

- provides a sampling frame for epidemiological studies
- allows comparisons between study samples and the general population to enable appropriate generalisation of results
- 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 Child Health (WATCH) Study

J Hansen, H Ewart, P Alessandri, NH de Klerk, M Croft, A James (Sir Charles Gairdner Hospital), K Taylor (Curtin University of Technology), PR Burton (Leicester University), J Sleith

The WA Twin Register

The WA Twin Register was established by the Western Australian Twin Child Health (WATCH) study in 1997 with funding from Healthway, and comprised data on all WA multiple births between 1980 and 1992 inclusive. The aim of this study was to investigate the roles that genes and the environment play in the development of asthma and allergies. Families were asked to complete three lengthy questionnaires, one for the multiples, one for their parents, and one for any siblings they had. They covered a wide range of demographic, behavioural, health and medical questions, with the main concentration being on asthma and allergies. There were over 100 questions to answer for every member of the family, and estimates of time taken to complete ranged from 30 minutes to over 2 hours. After the study had been running for 8 months, we developed a much shorter questionnaire, as many families stated that they did not have the time to complete the longer versions. About 14% of families opted to complete this revised questionnaire. The percentage of families who returned completed questionnaires was about 60%, irrespective of whether the 'long' or 'short' questionnaires were used. Eighty eight percent of families indicated that they were happy for us to contact them in the future to be invited to take part in further studies. This percentage did not differ between 'long' or 'short' questionnaires, although families of multiples aged 10 years or younger were more likely to have responded positively to this request (92% vs 84%, $p < 0.001$).

There was no difference in response, to either the initial letter or to the questionnaires, between Metropolitan and non-Metropolitan residents. Mothers who were under 20 years old when their multiples were born, were less likely than older mothers to respond to the invitation to participate (46% vs 80%, $p < 0.001$). But once they had agreed to participate there was no difference in the proportion who returned completed questionnaires (82% vs 90%, $p = 0.174$).

Analysis of the questionnaire data shows that:

- 68% of families live in the Perth metropolitan area
- 54% of twins have older siblings
- 22% of fathers and 18% of mothers have tertiary education
- 86% of mothers and 85% of fathers are in their first marriage
- 98% of fathers and 66% of mothers work outside the home
- 64 fathers (2.3%) and 60 mothers (2.1%) are twins themselves
- 61% of fathers and 71% of mothers reported that they have not smoked regularly since the birth of their multiples

A number of outcomes in the twins show evidence of a genetic component as assessed by greater concordance between MZ twins compared with DZ twins. These include doctor-diagnosed asthma (DDA), had ever wheezed, had wheezed in the last 12 months, hay fever, eczema, and ADHD, both hyperactive and inattentive types.

Factors that appear to increase the risk of DDA are:

- having an asthmatic mother and asthmatic father
- being diagnosed with hay fever or eczema
- being born under 33 weeks' gestation.

Having older siblings seems to decrease the risk of DDA.

Factors that appear to increase the risk of ADHD (hyperactive type) include:

- mother reports to have smoked during pregnancy
- having ever wheezed
- male sex
- father being unemployed.

A grant from the NHMRC has allowed us to extend the Register to include WA multiple births in 1993 and 1994. A further 698 multiple birth families have been identified. Eighty five percent (561) of eligible families have been contacted and completed questionnaires have already been received from 337 (60%) of them. Follow-up of non-responders is continuing and the 1995 data will be included when they become available. The questionnaire developed for these families is much shorter than previous versions and only contains questions on family structure, pre- and post-natal factors such as periconceptual multivitamin use, smoking and breastfeeding, and several questions about asthma, including diagnosis by a doctor (DDA). The main

function of this questionnaire is to enrol the family on the WA Twin Register, and to ascertain concordance of DDA in twins.

Exploring the complexity of the asthma phenotype (WATCH for Asthma Study)

The next phase of the WATCH study, that is, phenotyping for asthma and allergies is now underway. Families consisting of the twins, their biological parents and any of their siblings aged 7 and over, are invited to attend our clinic at PMH to undergo a series of standard breathing, allergy and blood tests. Our plan is to recruit 60 multiple birth families from each of 6 birth years (1990 to 1995 inclusive), a total of 360 families. We are also offering a free zygosity test to families who are unsure of the zygosity of their twins. Since the clinic commenced in November, 28 families have attended and completed the testing.

WATCH Language Development Study

Participants

The sampling frame for this study consisted of all multiples born in Western Australia (WA) between September 1997 and August 1998 and 424 singletons in the same birth cohort as the multiples who were selected at random from the Midwives' Notification of Birth Records. The aim of the prospective part of WATCH was to investigate early language development and temperament in young twins and singletons and to recruit multiple birth families to the WA Twin Register.

Multiples

Mothers of multiples were identified using the Midwives' Notification System. There were 362 twin pairs and 12 sets of higher order multiples (i.e., triplets, quadruplets or quintuplets) born during this period (n = 374). The names of all multiples were checked against WA mortality data provided to the Institute by the Registrar General's office on a monthly basis. Nineteen families experienced the death of one or more of their multiples and were not contacted as part of this study. The names of mothers were sought on the WA electoral roll held at the Department of Public Health at the University of Western Australia to ascertain a postal address. Addresses that were not traced via the electoral roll were then sought in the Telecom White Pages. Of the 355 eligible families, 352 (99%) were traced to a postal address and were sent an information sheet

about the study and an Expression of Interest approximately one month prior to the multiples' first birthday.

Two hundred and sixty one (74%) families returned Expressions of Interest and agreed to participate; 25 (7%) families indicated that they did not want to participate; 55 (16%) families did not respond; and 11 (3%) of the letters were returned to sender. Questionnaires were sent to 261 families who agreed to participate just prior to the multiples' first birthday. The parents were asked to return the questionnaires within a month. Two hundred and twelve (81%) families returned year 1 questionnaires; 47 (18%) families did not respond and one family withdrew from the study. The 212 families who returned year 1 questionnaires, plus one family who did not complete the Year 1 questionnaire were sent Year 2 questionnaires just prior to their multiples' second birthday. The parents were again asked to return the questionnaires within a month. At the two-year-old follow-up, 145 (68%) questionnaires were returned; 62 (29%) families did not respond; 5 (2%) questionnaires were returned to sender; and one family withdrew from the study. Of the 261 participants, 144 (56%) families completed One-Year-Old and Two-Year-Old questionnaires.

Singletons

Four hundred and twenty four families with singletons in the same birth cohort as the multiples were selected at random from the Midwives' Notification of Birth Records. Four hundred and five mothers were traced and contacted following the procedures described earlier. Two hundred and forty two families (60%) agreed to participate; 57 (14%) families indicated they did not want to participate and 105 (26%) families did not respond to the Expression of Interest. The same procedures for sending questionnaires described earlier were followed. Of the 242 families who agreed to participate, 204 (84%) returned year 1 questionnaires, 35 (14%) families did not return questionnaires and 3 (1%) families withdrew from the study. At the two-year-old follow-up, 153 (75%) questionnaires were returned; 47 (23%) families did not respond; 3 (1%) questionnaires were returned to sender; and one family withdrew from the study. Of the 242 participants, 153 (63%) families completed One-Year-Old and Two-Year-Old questionnaires.

Results

These results are for 110/152 singletons and 212/278 twins for whom we have complete data at one- and two-years of age.

Language Development

Significantly more two-year-old twins (18.4%) than singletons (9.1%), $\chi^2 = (1, N = 322) = 4.86, p < .05$ presented with expressive vocabulary scores at or below the 5th percentile on the Macarthur Communicative Development Inventory: Words and Sentences instrument. This equates to a productive vocabulary of less than 48 words in boys and 70 words in girls. There were no sex differences in late talker status at two years of age for twins or singletons. The 5th percentile criterion for language delay is similar to the 50 words or less expressive vocabulary criteria adopted by other researchers. The finding that 9% of singletons scored below this cut-off is strikingly similar to the prevalence estimates of late talking in other research involving singletons. This finding that twice as many twins compared to singletons were late talkers at two concurs with previous research involving twins.

Temperament

When the temperament characteristics of twins and singletons were compared, the only difference was that twins were more rhythmical than singletons (ie their routines were more regular). Twins and singletons did not differ in approach/withdrawal; cooperation/manageability; distractibility; persistence, or reactivity. Subsequent analyses with the complete data-set will (1) investigate characteristics of language development at 12 months that predict language development (ie normal or delayed) at 24 months for twins and singletons and (2) compare temperament characteristics in twins and singletons with normal and delayed language development.

Benefits

Language impairment in children is a classic example of a complex, multifactorial disorder of human communication, with poorly understood interrelationships among factors that influence variability and susceptibility to disease and response to treatment. While the developmental outcomes for late talkers in the prospective part of WATCH are currently unknown, through WATCH we have identified a unique population-based birth cohort of late talking multiples and singletons who can potentially be recruited to further studies in language development and disorders in WA children.

RASCALS study

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

SR Zubrick, SR Silburn, JJ Kurinczuk (University of Leicester, UK), DE Parsons, MD Biggs, K Moore, K Dixon, 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 follow-up 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 and primary caregiver's mental well-being, will be used to identify possible causal factors and protective factors of mental health.

Recently, the RASCALS data have been used extensively to inform the national longitudinal study of Australian children and 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 those data suggest 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. This paper is in the process of being published.

Internationally, the RASCALS study is attracting great interest. One of the researchers, Associate Professor Leon Straker from Curtin University of Technology Western Australia, was recently invited to the John Hopkins University USA to report on the preliminary findings of children's computer usage.

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 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.

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.

To date, two thirds of the cohort has been contacted. Almost 1,200 children/families have attended the Institute and over 1,400 family questionnaires have been returned. The response from schools has been gratifying with data now available for over 1,100 children. Data collection is on track and it is expected that approx 1,800 children/families will attend and questionnaire data will be available for approx 2,200 children/families by the close of 2002. We are aware of the need to capture the enthusiasm of the children themselves as they move into adolescence, to ensure the long-term viability of the cohort. In this regard the position of Family Liaison Officer is about to be filled by a temporary recruit from the Avon Longitudinal Study of Parents and Children in the UK. This Institute employee will be available to the study to assist in the development of resources and strategies to engage the children and their families to enhance retention and data ascertainment. The study continues with an increasingly busy program of analyses to investigate the fetal and early life origins of childhood diseases.

WA Mortality Study

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

This study forms the major component of a doctoral thesis, being completed by Jane Freemantle. The research is based on data derived from the Maternal and Child Health Research Data Base. The research continues and enhances the work initiated by Dr Louisa Alessandri.

Every live birth recorded in Western Australia between 1980-1997 and every infant and childhood death (to age 19) occurring in Western Australia between 1980-1998 was included in the cohort. Data included demographic details of the parents, pregnancy details of the mother, race of the mother, pregnancy outcome (including weight, gestational age, sex and percentage of expected birth weight) and mortality details. The cause of death was determined using a 3 digit code devised by Dr Eve Blair. The classification of death code, which was developed for research purposes, was extended by Jane Freemantle to provide extra mortality information for childhood deaths. The place of death was also identified, including the location of death (in or out of

hospital) and the residence at the time of death. Data were validated through examining autopsy records and information contained on the Registrar-General's Data Base.

The specific focus has been to determine a comprehensive and complete mortality profile for Indigenous Western Australian infants and children, which includes geographical information associated with the mortality profile. The data have also provided cause specific and all cause mortality rates for Western Australian infants and children between the years 1980-1998.

These data will provide new and valuable information to advise health promotion and disease prevention strategies and policy in the following areas:

- regional targeting - the ability to determine the location, and health region associated with mortality statistics
- the antecedents to poor health outcome which may predispose to infant mortality and childhood deaths, such as low birth weight and prematurity which may be associated with an increased propensity to infection and SIDS
- improved services, particularly in the postnatal period, for Aboriginal children born with birth defects
- targeted programs for the prevention of death due to SIDS in the infant years, and infection and accidents in infant and childhood years that are informed by the prevalence of these causes of deaths and the description of the populations most at risk
- different causes of death in the different age groups
- review of specific causes of death eg pedestrian deaths, death due to drowning, location of suicides and motor vehicle accidents.

The prevalence of inflammatory bowel disease in the juvenile population of Western Australia

T Walters, R Hill (Department of Gastroenterology, Princess Margaret Hospital), C Bower.

Inflammatory Bowel Disease (IBD) is a problem of increasing significance in western society. Approximately 25% of cases occur in the paediatric age group. There is minimal published epidemiological data for IBD in Australia and no published data in reference to the juvenile population. The

Australian Paediatric and Adolescent IBD Data Base (APA IBD Database) is a national database organised on a state by state basis. National data collection commenced in February 1996. This study examined how well the database is identifying cases of diagnosed IBD in the juvenile population of WA.

Multiple search strategies were used. Identifiable data were obtained from the WA Hospital Morbidity Database, the PMH PIMS database, and the PMH Department of Histopathology database for all eligible cases with any diagnostic code that might indicate IBD. All registered gastroenterologists in the state were requested to identify eligible patients. A presentation was made at the Australian Crohns and Colitis Association's (ACCA) annual education meeting and database enrolment was encouraged. A letter was mailed to all ACCA members informing them of the study and encouraging them to confirm their enrolment. Unique identifiers linked the collated data.

Cases identified were confirmed manually by reference to the case-record or by direct communication with the patient's attending physician. The data were compared with the current APA IBD enrolment.

No single search strategy identified all confirmed cases. The APAIBD Database recorded approximately half of the cases identified by this study. The minimum estimated prevalence for juvenile IBD in WA is 16.7 cases per 100,000 under 18y/o (13.6 per 100,000 under 16 y/o) - comparable with international data.

Staff and Students

Head of Division
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John Stewart MBBS DCH FRACP MMedSci
Catherine (Kate) Taylor, BAppSc, PGradDipHlthSc, PhD FSPA (Human Communication, Curtin University of Technology)

John Vail BEc
Tom Walters MBBS DCH FRACP (Advanced Trainee)
Martha Werler ScD (Fogarty Senior International Fellow)

Theses Passed

KJ Brameld. Methods for the use of linked administrative health data in disease surveillance and measurements of patterns of care. PhD, Department of Public Health, University of Western Australia.
L Colvin. Childhood hospital morbidity profiles for children born with birth defects in Western Australia, 1980 - 1995. MPH, Department of Public Health, University of Western Australia.
L Milne. The effect of a school-based sun protection intervention on sun-related behaviour and the development of melanocytic naevi in children. PhD, Department of Public Health, University of Western Australia.
J Payne. Hospital admissions for Pneumonia before and after the introduction of Hib vaccines in children under five years of age in Western Australia. Master of Science (Public Health), Curtin University of Technology.

External Committees

H Bailey. Member, The National Newborn Hearing Screening Committee.
E Blair. Chair of Western Australian Branch, Perinatal Society of Australia and New Zealand (PSANZ).
E Blair. Member, Shaken Baby Syndrome Steering Committee initiated by the WA Child Protection Council
E Blair. Member, Scientific organising committee of the inaugural conference of the Australian Academy of Cerebral Palsy and Developmental Medicine to be held in Sydney, September 2002
C Bower. Member, National Perinatal Statistics Unit Management Advisory Committee.
C Bower. Member, Scientific Sub-Committee of the Human Research Ethics Committee, Curtin University of Technology.
C Bower. Member, Scientific Review Panel, Australian Paediatric Surveillance Unit, Sydney
C Bower. Member, National Child Health Information Advisory Committee, Australian Institute of Health and Welfare, Canberra.
C Bower. Member, Australian Birth Defects Society.
C Bower. Member, Confidentiality of Health Information Committee, for Health Department of

Western Australia.

C Bower. Member, Perinatal and Infant Mortality Committee.

C Bower. Member, Midwives' Notification System Advisory Committee.

C Bower. Member, Department of Public Health Research Committee (The University of Western Australia).

C Bower. Member, Western Australian Genetics Council, Department of Health WA

C Bower. Member, Prenatal Diagnosis Committee, Department of Health WA.

C Bower. Deputy Chair 2001, National Health and Medical Research Council Grant Review Panel.

H D'Antoine. Board Member, Disability Services Commission WA.

NH de Klerk. NHMRC New Program Grants Committee.

NH de Klerk. Advisor, Effects of routine immunization on child survival, Global Advisory Committee on Vaccine Safety, Geneva.

NH de Klerk. Busselton Population Research Foundation Board of Directors.

NH de Klerk. Perth Respiratory Epidemiology Group (P.R.E.G.) Executive Committee.

NH de Klerk. Scientific Advisory Committee of the Busselton Research Foundation Board.

NH de Klerk. Australian Radiation Health and Safety Advisory Council.

NH de Klerk. Clinical Drug Trial Committee, Sir Charles Gairdner Hospital.

NH de Klerk. Mesothelioma Committee of Western Australia.

NH de Klerk. Western Australian Air Quality Coordinating Committee Health Issues Group.

NH de Klerk. Management Committee, Data Linkage Project, Health Department of WA

NH de Klerk. Executive Committee, Australian Twin Register.

NH de Klerk. Medical and Scientific Advisory Panel, Cancer Foundation of Western Australia.

S Eades. Member, National Health and Medical Research Council Aboriginal Health Research Agenda Working Group.

S Eades. Trustee, Rio Tinto Aboriginal Foundation.

S Eades. Trustee, Robert Riley Law Scholarships, Australian Youth Foundation.

S Eades. Member, Aboriginal and Torres Strait Islander Health Council for the Federal Minister of Health.

J Freemantle. National Secretary, Public Health Association of Australia.

J Freemantle. Chair, Lady Lawley Cottage Management Committee, Australian Red Cross.

J Freemantle. Past President Australian Federation of University Women.

J Freemantle. Executive Committee, Public Health Association of Australia (WA Branch).

J Freemantle. Trustee for Public Health Education and Research Trust.

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, of Vaccine Impact Surveillance Network committee, WA

D Lehmann. Member, of The Meningitis Centre committee, WA

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

D Lehmann. Temporary Advisor, Vaccine Monitoring and Assessment, World Health Organization, Geneva

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.

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

L Milne. Convenor, Organising Committee for AEA Conference to be held in Perth in 2003.

L Milne. Member, Research Committee, Department of Public Health, UWA.

L Milne. Member, Teaching and Learning Committee, Department of Public Health, UWA.

L Milne. Member, Public Health Association of Australia

L Milne. Member, Australasian Epidemiological Association

L Milne. Member, Society for Epidemiological Research

L Milne. Member, Perth Epidemiology Group

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.

W Oddy. Member, National Food and Nutrition

Monitoring Project, Breastfeeding Reference Group (Australia).

W Oddy. Member, Breastfeeding Public Health Action Group, Health Department of Western Australia.

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.

A Read. Member, Editorial Board, Paediatric and Perinatal Epidemiology.

A Read. Member, Data Linkage Project Management Committee, Department of Public Health, The University of Western Australia and Health Information Centre, Health Department of Western Australia.

SR Silburn. Chair, Working Group on Youth and Drugs. WA Community Drug Summit

SR Silburn. Member, Ministry of Justice Suicide Prevention Taskforce Steering Committee.

SR Silburn. Member, Evaluation Committee MindMatters: National Mental Health in Schools Project.

SR Silburn. Chairperson, Ministerial Council for Suicide Prevention (formerly Youth Suicide Advisory Committee)

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

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 (formerly Youth Suicide Advisory Committee)

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. Epidemiology of cerebral palsy. Opening plenary session at 5th International congress on cerebral palsy - with Prof. Eva Alberman. Bled, Slovenia.

E Blair. Survival and Neurological childhood outcome in twins: relation to antenatal growth. International Congress on Twin Studies. London, UK - with Dr. Beverley Petterson.

E Blair. Dealing with measurement, the epidemiologist's view. Measurement tools: meaningful or mythical? An update. Melbourne.

E T d'Espaignet. First results of the first CATI population health and well being survey for the Northern Territory" Epidemiological Branch Seminar Series, Darwin.

E T d'Espaignet. Monitoring birthweight of Aboriginal communities taking part in the Strong Women, Strong Babies, Strong Culture project. The Infant and Child Mortality Conference, Canberra.

E T d'Espaignet. Monitoring SIDS in the Northern Territory. The Infant and Child Mortality Conference, Canberra.

NH de Klerk. Cancer incidence and RF radiation emitting from TV towers. 8th Biennial CDC and ATSDR Symposium on Statistical Methods, Atlanta.

NH de Klerk. Comparison of environmental and occupational exposure to crocidolite and the incidence of mesothelioma. USEPA 2001 Asbestos Health Effects Conference, Oakland.

NH de Klerk. Crystalline silica exposure and major health effects in Western Australian gold miners. Inhaled Particles IX, Cambridge.

NH de Klerk. Mortality in miners and millers of crocidolite in Western Australia: follow-up to 1999. Inhaled Particles IX, Cambridge.

J Freemantle. Trends in the mortality of Western Australian Infants, 1980-1998, University of Western Australia, Department of Public Health, seminar Series.

J Freemantle. Indicators of Child Health in Western Australia. New Zealand Public Health National Conference. Wellington.

J Freemantle. From two lands, a little story, a short journey, the voice of the children of the land. New

Zealand Public Health Association National Conference. Wellington.

J Freemantle. Where do Infants and Children Die? Public Health Association of Australia National Conference, Sydney.

J Hansen. Hospital admission for asthma for twins in Western Australia. International Society for Twin Studies, 10th Scientific Meeting, London.

J Hansen. How representative is the Western Australian Twin Register? International Society for Twin Studies, 10th Scientific Meeting, London.

J Hansen. The WA Twin Child Health (WATCH) study: preliminary results from a population-based Twin Register. "It Runs in the Family": Twins and Families in Biomedical and Behavioural Research Conference, Australian Twin Register, Melbourne.

H Leonard. Rett syndrome in Australia from the nineties to the new millenium. Grand Round, Women and Children's Hospital, Adelaide.

H Leonard. Symposiums - 2001 Annual Scientific Meeting of the Australian Association of Neurologists, Movement Disorder Workshop. Adelaide.

Silburn SR, Hillman SD, Zubrick SR and Green A. (2001) Drugs, alcohol and suicide: Options for reducing the incidence of suicide attributable to substance misuse. Paper presented at the Suicide Prevention Australia Conference. Sydney.

Silburn SR, Zubrick SR and Williams A. (2001) Promoting Developmental Health in Western Australia: How can research inform across-government policy and practice? Invited Keynote address at A Research Forum 'Well-Being: Through a Child's Developmental Stages' NSW Commission for Children and Young People. Parliament House, Sydney 7 March 2001.

Special Acknowledgements

The Health Department of Western Australia, 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 Centre for Health Services Research Linked Database Project for assisting with the provision of data and continued support.

Division of Virology

Overview

The division was recently set up to provide a nucleus for the development of research into infectious diseases of significance in childhood.

The diseases being studied are Murray Valley encephalitis, Japanese encephalitis and enterovirus encephalitis.

Our current research focuses on understanding how viruses cause disease within the central nervous system (CNS). This research covers a wide range of activity, including molecular studies of viral replication, studies of the pathogenesis of viral encephalitis using animal models, the development of community surveillance for viruses causing CNS infections and the development of improved diagnostic methods.

Molecular pathogenesis of flavivirus encephalitis

This is a long-term NHMRC-funded project on the pathogenesis of flavivirus encephalitis using the Murray Valley encephalitis virus (MVEV) – mouse model. Our work in this field is divided into three areas. Firstly, molecular genetic studies of MVEV virulence determinants using an infectious cDNA clone of MVEV developed in our laboratory. Secondly, examinations of virus-host interactions leading to the development of encephalitis and host determinants of susceptibility to infection using the mouse model. Thirdly, we are undertaking studies directed toward vaccine development against flavivirus encephalitis using a combination of molecular genetic and animal model approaches.

Studies on the epidemiology and pathogenesis of enterovirus 71 in Australia and Southeast Asia

We commenced this research in response to an epidemic of invasive neurological disease due to enterovirus 71 (EV71) in Perth during 1999. We have developed collaborations with research groups in Taiwan and Malaysia to study the molecular epidemiology of EV71 in the Asia-Pacific region, and this work is already far advanced.

In addition, we are studying the molecular genetics of virulence of EV71. This has involved the development of an infectious cDNA clone of EV71 and we

are also working towards the development of small animal model of EV71 encephalitis. Our approach here will be to develop a transgenic mouse expressing the cellular receptor for EV71, an approach used successfully to develop a mouse model of poliomyelitis. We are currently collaborating with research groups in Taiwan and Newcastle, NSW on the identification of the EV71 receptor molecule. The ultimate aim of this work is to develop a genetically defined, live-attenuated vaccine against EV71.

A study of intrauterine viral infection in Western Australian women

We are collaborating with Professor John Newnham and Dr Jan Dickinson (Women and Infants Research Foundation, KEMH) on a study of intrauterine viral infection in pregnancy. Specifically, we are comparing the prevalence of intrauterine viral infection in a group of women in whom fetal abnormalities have been identified by ultrasound with a group of women who have amniocentesis undertaken for other reasons. This will be a long-term cohort study to determine the prevalence of intrauterine viral infection in WA and to determine the overall impact of these infections on the outcome of pregnancy and on child development. In addition, a major technical aim of this study is to develop a rapid, sensitive and specific PCR test to detect intrauterine viral infection from prenatal amniotic fluid and fetal tissue samples. The work on development of a multiplex PCR assay is well advanced.

Staff and Students

Head of Division
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Students
Beng Hooi Chua - PhD Preliminary (UWA)
Lara Herrero - Honours (UWA)
Sharon Szefczyk - Honours (Curtin)

Theses Passed

R Hurrelbrink - Doctor of Philosophy (Distinction) - UWA Microbiology. An Infectious cDNA Clone for the Analysis of Virulence Determinants in Murray Valley Encephalitis Virus. Supervisor: Dr Peter McMinn

L Herrero - Bachelor of Science (First Class Honours) - UWA Microbiology. A Study of the Molecular Epidemiology of Enterovirus 71 in the Asia-Pacific Region. Supervisors: Dr Peter McMinn and Dr Rob Hurrelbrink

S Szefczyk - Bachelor of Science (First Class Honours) - Curtin University. Molecular Genetics Surveillance of Neurotropic Enteroviruses in Child Care Centres in Perth, Western Australia by Rapid Molecular Typing. Supervisors: Dr Peter McMinn and Dr David Townsend

External Committees

Peter McMinn. Member, State Arbovirus Control Committee, 1996-present

R Hurrelbrink. Scientific Committee Secretary, Australian Society for Microbiology (Annual Scientific Meeting and Exhibition, Perth)

Invited Presentations

Peter McMinn. Invited speaker. Communicable Disease Control Conference, Canberra, Australia. Title: "Community surveillance for enterovirus 71 and other neurotropic enteroviruses by rapid molecular typing."

Peter McMinn. Invited speaker. Infection Control Association of WA Annual Conference, Perth, WA, Australia. Title: "Community surveillance for enterovirus 71 and other neurotropic enteroviruses by rapid molecular typing."

Peter McMinn. Invited speaker. ASM Annual Scientific Conference, Perth, WA. Title: "The molecular epidemiology of enterovirus 71 in the Asia Pacific region, 1997-2001."

Peter McMinn. Invited Keynote Speaker. Australian Virology Group Conference, Fraser Island, Qld. Title: "The molecular basis of viral encephalitis: a study of two models."

R Hurrelbrink. "Reverse Genetic Studies on Murray Valley Encephalitis Virus", Australian Virology Group, 1st Annual Meeting, Fraser Island QLD, and Combined Biological Sciences Meeting, 12th Annual Meeting, Perth WA

R Hurrelbrink. "An Infectious cDNA Clone for the Analysis of Virulence Determinants in Murray Valley Encephalitis Virus", Science at the Shine Dome, Canberra ACT

Awards (2001)

R Hurrelbrink. New Investigator Award, National Health and Medical Research Infrastructure Council of WA (Combined Biological Sciences Meeting), Perth WA

R Hurrelbrink. Early Career Development Award, Australian Academy of Science, Canberra ACT

L Herrero. Swan Brewery Prize in Microbiology
S Szefczyk. Student Travel Award, Australian Virology Group Meeting

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