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**WELLINGTON MEDICAL  
RESEARCH FOUNDATION**

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## Editorial

This edition of the Wellington Medical Research Foundation Research Review details the outcomes of research funded the Foundation over the past twelve months. In accordance with the objectives of the Foundation, this funding is made available for medical research activities at the request of individual scientists or research groups; however, this is not provided on an *ad-hoc* basis, but is made available following a complex vetting process that ensures the scientific validity of any proposed project. This process is integral to the business of any granting organisation as it ensures that scarce resources are invested wisely and that the outcomes of research make a valid contribution to the existing body of scientific knowledge.

One of the most striking features of the research portfolio supported by the Foundation is that it encompasses a wide and varied range of projects drawn from the fields of social and clinical medicine, public health and basic biomedical research. It is beyond the expertise of any individual to adequately assess the validity of research proposals that may focus on any small part of this broad base of medical science. For this reason the Foundation has appointed a Research Advisory Committee to assess the various requests for funding and make recommendations to the Board of Management.

The members of the Research Advisory Committee are drawn from the various health-related research providers active within the Wellington Region and every nominee for membership of the Committee must have an established research record. Appointments to vacancies on the Committee are made in those specialist area that complement the research experience and expertise of existing members.

Two grant rounds are undertaken annually and the applications received for each round are forwarded to each member of the Research Advisory Committee for evaluation. In addition to this general review process, each application is assigned to an individual Committee member who has relevant research expertise. It is the responsibility of this member to provide a detailed objective assessment of the scientific merit of the application to the other members of the Committee. The Committee also has the benefit of expert reports from external reviewers forwarded to the Secretary of the Foundation prior to the meeting which provide an independent group of opinions regarding the validity of each research proposal. If the application is considered worthy of funding, the proposed budget is reviewed and a determination is made regarding the appropriateness of the amount requested. In some instances the total is revised downwards in order to maximise the funds available to other worthy projects. On occasion the total value of approved applications exceeds that available for the grant round and in those instances projects are marked on the basis of merit and recommendations for funding are made accordingly. The recommendations of the Research Advisory Committee are presented to the Board of Management of the Foundation by the Research Committee Chair and

following discussion and deliberation, a final list of successful applicants is approved.

The review process of the Foundation is, of necessity, complex, however it does provide both transparency and validity, and further ensures that the Foundation receives a maximum return on its investment in medical research.

Professor Brett Delahunt  
Editor

### **Research Advisory Committee Membership**

Professor Brett Delahunt (Chair)  
Professor Peter R Crampton  
Dr Sunny Collings  
Professor Carl D Burgess  
Associate Professor John H Miller  
Professor Graham Le Gros  
Dr T William Jordan  
Associate Professor Duncan C Galletly  
Professor Thomas Bäckström

## **Reports of research work funded by grants prior to the current year**

### **Malaghan Institute of Medical Research**

#### **Mechanism of action for suppression of eosinophilic lung inflammation by phosphatidyl inositol mannan (PIM)**

J Harper

Phosphatidyl inositol mannan (PIM) is one of the cell wall components of mycobacteria. Previous studies showed that administration of PIM intranasally resulted in suppression of cell infiltration into the lung in an OVA-induced model of lung inflammation. The aim of this project was to investigate the effect of PIM on immune cells and how this might contribute to the anti-inflammatory effect observed *in vivo*.

Earlier work showed expansion of T cell subsets in response to PIM treatment *in vivo*. Our *in vitro* studies have now shown that PIM alone does not directly affect proliferation of splenic T cells. This result indicated that PIM activity arises from interaction with an alternative cell type, most likely either macrophages and/or dendritic cells (DC).

Bone marrow-derived macrophages and dendritic cells were both found to produce IL-10 in a dose-dependent manner following exposure to PIM *in vitro*. Interestingly, this was associated with slight upregulation of MHCII and CD80, albeit significantly lower than MHCII expression in the presence of LPS, and was not significantly affected by the absence of the lipoglycan receptor CD1d. Nor was this effect greatly altered by competition for the mannose receptor through the addition of mannan. It therefore appears that neither CD1d nor the mannose receptor play a leading role in the recognition of PIM by macrophages or DC.

It is not clear what role MHCII upregulation might play in the inhibitory effect of PIM *in vivo*, however, we found that macrophages treated with LPS, expressed lower levels of MHCII when pre-treated with PIM. PIM may therefore modulate MHCII expression by alternative stimuli such as LPS, thereby lowering the ability to augment an inflammatory response *in vivo*.

Further work is planned to investigate this effect on DC and macrophages *in vivo*, and to continue searching for the recognition pathway for PIM.

#### **Suppression of experimental multiple sclerosis by a modified superantigen complex**

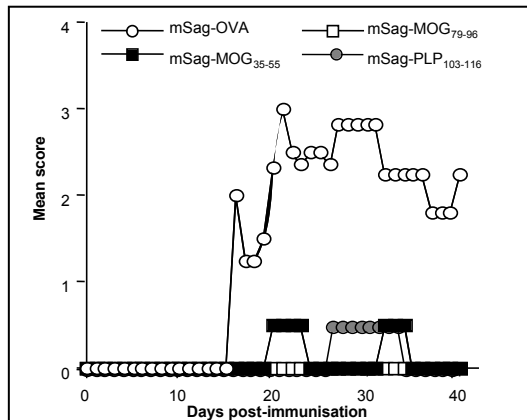
A McNeill, BT Bäckström

Multiple sclerosis is a chronic disease of the central nervous system (CNS) that affects approximately 1:1400 New Zealanders. The molecular pathogenesis of MS involves the activation of myelin-reactive T cells in the

periphery. Following activation, these cells migrate into the CNS and cause myelin destruction.

EAE is the most widely used animal model of demyelination and MS. EAE can be induced by immunisation of susceptible animals with CNS homogenate, or proteins and peptides derived from the CNS, such as myelin oligodendrocyte glycoprotein (MOG).

An ideal treatment for MS would be non-toxic and specific to the disease causing auto-antigen, but able to induce “bystander suppression” to other disease causing auto-antigens that may be exposed as a result of epitope spreading. We have recently shown that treatment of C57BL/6 mice with a novel modified superantigen (mSag) linked to a central nervous system (CNS)-specific peptide MOG35-55 (mSag-MOG35-55) prevents the development of EAE when administered in low doses at early stages of disease initiation. The inhibition of disease involves the activation of antigen-



**Figure 1: Bystander suppression of EAE**

Mice were immunized as described in Figure 1 and then treated 5 days later with mSag-MOG<sub>35-55</sub>, MOG<sub>79-96</sub> or mSag-OVA<sub>323-339</sub> or PLP<sub>103-116</sub>. Mice were scored daily for clinical signs of EAE.

(C57BL/6 x B10.Q) mice were immunized with MOG35-55 to induce EAE and then on day 5 mice were treated with mSag-MOG35-55, mSag-MOG79-96, or mSag-PLP103-116 (Figure 1). This indicated that our treatment can induce bystander suppression to different epitopes of the same myelin-derived protein and also to an epitope of a different myelin-derived protein.

We hypothesised that treatment with mSag-MOG35-55 renders antigen-presenting cells (APC) particularly efficient at activating regulatory T cells in preference to effector T cells. Bystander suppression is proposed to occur in this situation because in addition to presenting the antigen coupled to the mSag, these APC will present other myelin antigens.

specific regulatory T cells. These features potentially make for a very favourable treatment for MS and other autoimmune diseases.

This project aimed to determine whether the treatment induces bystander suppression. One likely mechanism of bystander suppression is through the production of immunomodulatory cytokines such as IL-10, hence the second aim of this project is to determine the role of IL-10 in the suppression of EAE.

In order to determine whether bystander suppression occurs after treatment with mSag-MOG35-55 F1

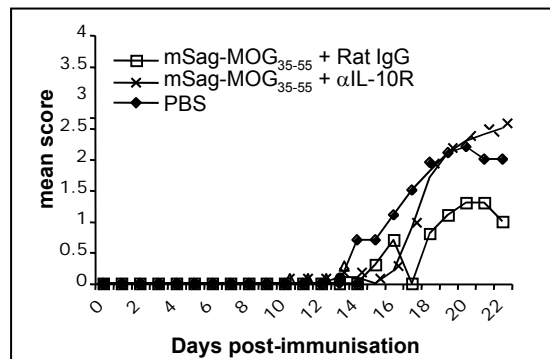
## The role of IL-10 in the suppression of EAE

The second aim of this work was to determine the role of the immunomodulatory cytokine IL-10 in the suppression of EAE particularly in regards to the role of IL-10 in bystander suppression. To date we have used an anti-mouse IL-10 receptor (IL-10R) monoclonal antibody to determine the role for IL-10 in suppression of EAE in C57BL/6 mice after treatment with mSag-MOG<sub>35-55</sub> (Figure 2). It is clear from this initial study that IL-10 plays an important role in the suppression of EAE, as when mice are treated with the  $\square$ IL-10R the mSag-MOG<sub>35-55</sub> treatment does not suppress EAE. We initially

chose to determine the role of IL-10 in the suppression of EAE using the C57BL/6 model rather than the model of bystander suppression because it is a more robust model with both suppression and the severity of disease being more complete in this model. After this initial study in the C57BL/6 mice to determine the concentration of antibody to give and the time at which to administer the antibody we will next determine the role of this immunomodulatory cytokine in the bystander suppression model.

To date we have generated an F1 (C57BL/6 x B10.Q) cross between two strains of mice that are susceptible to EAE induced with different myelin peptides. We have demonstrated that these mice are susceptible to disease induced with two different MOG peptides and as such this model was used to study bystander suppression of EAE. Using this F1 (C57BL/6 x B10.Q) model we have also shown that following immunisation with MOG<sub>35-55</sub>, treatment with mSag-coupled to various myelin peptides induces bystander suppression.

IL-10 is an immunomodulatory cytokine that has been implicated in protection against autoimmunity and bystander suppression. Hence, the second aim of this project is to determine the role of IL-10 in the suppression of EAE. The initial experiments undertaken in the C57BL/6 model has shown that IL-10 is required for suppression mediated by mSag-MOG<sub>35-55</sub>. In the next part of this work we aim to determine the importance of IL-10 in the induction of bystander suppression in the F1 (C57BL/6 x B10.Q) model. These results will provide information regarding this novel superantigen complex, which may lead to a potential treatment for MS.



**Figure 2: The role of IL-10 in suppression**

Mice were immunized as described previously and then treated 5 days later with mSag-MOG<sub>35-55</sub> or PBS. Mice treated with mSag-MOG<sub>35-55</sub> were given 100  $\mu$ g Rat IgG or  $\alpha$ IL-10R on day 5 and day 7 post-immunisation. Mice were scored daily for clinical signs of EAE.

## Victoria University of Wellington

### Imagination in children with autism spectrum disorder

J Low  
School of Psychology

From early in life, children actively use their imagination to reflect on the world around them. And yet, there is one important group of children for whom acts of imagination appear to be formidable: children with autism (CWA). The crucial features of autism are deficits in social interaction, communication *and* imagination.

One established way of studying imaginative thinking is through the seminal 'Draw an impossible person' task. The results from the few studies investigating the production of drawings of imaginary entities in CWA have been mixed.

The primary aims of the proposed research, were as follows. First, to assess the nature of imagination in children with autism. The aim was achieved through studying whether children with autism (in comparison to typically developing controls) were able to generate novel ideas when completing an imaginative drawing task. The second aim was to test three candidate theories that attempt to explain underpinnings of imagination deficits in autism. Specifically, we examined whether performance in central cognitive constructs such as mental state understanding (theory of mind), planning and inhibition (executive functioning), and/or holistic stimuli organisation (central coherence) predict(s) imaginative drawing ideas in children with autism.

Two groups of children ( $n = 22$  per group) participated in the study. Children with autism spectrum disorders (ASD) were recruited through various ASD associations in Wellington providing support and intervention services. Among the children with ASD, 15 had received diagnoses of autism and 7 had received diagnoses of Asperger Syndrome (AS). Typically developing (TD) control children ( $n = 22$ ) were recruited from various mainstream primary schools in Wellington, and were individually matched to children with ASD on gender, handedness and verbal ability.

The research was multi-session, with three data collection time points. Following the first time point, the second time point took place 1 week later; the third time point took place 1 month after the second point. Measures of verbal ability, theory of mind, executive functioning, central coherence, and imaginative thinking were administered at the first time point. For the remaining two time points only the imaginative thinking measure was administered.

Verbal ability was measured using the Test of Reception of Grammar (TROG-2). The test assesses receptive understanding of grammatical contrasts.

The theory of mind task battery was comprised of an Unexpected Contents Task, two versions of the Unexpected Transfer Task, and two versions of a Second Order False Belief Task.

Two components of executive functioning were measured: planning and inhibition. Following extant research, planning ability was measured through the Mazes Subtest and the Opposite Worlds Subtest was used to measure inhibitory control.

The Children's Embedded Figures Test was used to measure central coherence while imagination was measured using the Impossible Person Drawing Task. Results showed, with respect to within-group correlations for the ASD and TD groups respectively (chronological age and verbal mental age were partialled out of the analysis), that there were no significant correlations found between theory of mind, planning and inhibition and central coherence while for theory of mind, an ANOVA showed that the ASD group obtained significantly lower overall total scores than the TD group.

In terms of the total number of drawings generated (pooled across the three time points), a univariate ANOVA showed no significant difference between the ASD group and the TD group.

Analysis of associations between theory of mind, executive functioning and central coherence with proportion of type of drawing features generated over time showed that verbal ability or chronological age could discriminate between the ASD and TD groups independently of theory of mind, executive functioning or central coherence.

Analysis of group associations between theory of mind, executive functioning and central coherence with the proportion of type of drawing features over time showed that for the TD group, none of the partial correlation coefficients of critical theoretical interest reached statistical significance. For the ASD group, theory of mind performance was significantly and negatively correlated with proportion of typical drawing features generated at Time 1 and theory of mind performance was significantly and positively correlated with proportion of unusual drawing features generated at Time 3.

Overall, our findings confirm that children with ASD are impaired in their imaginative thinking, and this impairment holds over time. Only very few children with ASD were able to sustain long term imaginative drawing solutions and these children were the ones who possessed some understanding of theory of mind. Since children with ASD, were found to possess intact executive functioning and also showed a processing style that favoured attention to detail, the next step in research is to establish how these assets can be put to good use in order to trigger long term imaginative thinking.

## Long-term cellular effects of ecstasy - adaptation or degeneration?

J.H. Miller, D.J. Day, B.M. Kivell, S. Schenk, B. Lake, J. Colussi-Mas  
Schools of Biological Sciences and Psychology

Ecstasy (MDMA) is a popular recreational drug in New Zealand. MDMA leads to short and long-term changes in serotonin and dopamine neurotransmitter levels and decreases the density of the serotonin transporter (SERT) in the brain, specifically in regions such as the dorsal raphe, nucleus accumbens, striatum, and frontal cortex. These changes have major effects on behaviour, an endpoint that can be easily measured as an increase in movement (hyperactivity). We are using an animal model of drug abuse to investigate the changes in brain neurochemistry that occur after experimenter-administered or self-administered (rat lever press) MDMA. Understanding the neurochemical changes will help us determine whether brain function can recover following long-term use of MDMA. In one aspect of this research, we are attempting to correlate short and long-term behavioural responses to MDMA during behavioural sensitization and tolerance with changes in specific brain regions by linking locomotion intensity to the expression of cFos, a transcription factor that marks neuronal activation of particular regions of the brain.

Because of the role of serotonin in the behavioural responses to MDMA, we are examining the effect of the drug on serotonin levels in specific brain regions by HPLC and on SERT expression and distribution. Our *in vivo* studies so far have shown that the observed decrease in SERT density in some brain regions is not a result of a decrease in SERT mRNA, nor is it due to a decrease in the amount of SERT protein. This has been shown by others as well and suggests that either SERT is functionally modified, post-transcriptionally or post-translationally, or that its localisation in the neuronal cell membrane is altered. The problem in this latter case could be trafficking, in which SERT protein is present in endocytosed vesicles in the cytoplasm and therefore not available to transport extracellular serotonin out of the synapse. We can determine the percentage of SERT on the membrane of synaptosomes (cell synapse fragments isolated from brain tissue) by biotinylating the exposed proteins to mark them for Western blotting. Internalised SERT would not be biotinylated.

As it is difficult to study the kinetics of this process in the intact brain, we are also using an *in vitro* approach by transfecting hSERT into cells with a neuronal phenotype (N2A cells) to determine the effect of direct MDMA exposure in culture on the turnover of SERT in the membrane. We have access to GFP-hSERT for transfecting cells, and this green fluorescent fusion protein will improve the sensitivity and definition of our measurements. We will also address the changes in SERT function using a new method developed at the National Institute for Drug Abuse in the USA which uses live cell confocal microscopy to measure uptake of a fluorescent substrate (ASP+) for the serotonin transporter. Changes in the kinetics of ASP+ uptake will give an indication of whether MDMA is activating transporter trafficking. We hope that the answers to these questions on what neurochemical changes are

induced by MDMA will suggest an approach for treating the accumulated behavioural problems encountered following chronic MDMA use.

### **Prenatal morphine exposure and development of the neocortex in the embryonic mouse**

DJ Day, JH Miller, TJ Sargeant, D Foo  
School of Biological Sciences

Opioid analgesia is now widely used in neonates for postoperative pain management. The lack of supporting research in paediatric opioid pharmacokinetics and hence appropriate therapeutic dosages has led to concerns regarding use of opioids in the developing neonate. Such concerns are heightened as opioid receptor expression undergoes significant large changes near parturition and in the early neonatal period. Signalling through mu-opioid receptors (MOR) is generally inhibitory in nature, with evidence to suggest that MOR signalling inhibits neuronal proliferation. This project aims to examine the effect prenatal exposure to morphine has on the developmental of the neocortex and cerebellum in embryonic mice. In particular, the effect of morphine activation of MOR in the germinal zone of the lateral ventricle, where neurons divide and migrate to occupy the locations in which they will reside in the mature cortex, is being examined. Opioid receptor signalling has been implicated in the integrated control of neuronal division, differentiation, migration and death, but the role of morphine in modulating these processes is, at best unclear, and often controversial. Using an immunohistochemical (IHC) approach we have shown that MOR protein is expressed in the germinal zones by neuronal progenitor cells in the neocortex but not in the cerebellum of the embryonic mouse; we have previously shown that MOR mRNA and protein is expressed in the adult cerebellum. We postulate that the lack of MOR expression in the embryonic cerebellum is a consequence of the immaturity of this structure at parturition, but that later postnatal developmental stages will show a burst of MOR expression as the structure develops, akin to that seen in the neocortex. Within the neocortex, our quantitative RT-PCR studies have shown that prenatal morphine exposure reduces both mRNA and protein expression of the neuronal migration-specific marker doublecortin (DCX). Sufficient and correct temporal expression of DCX is required to ensure newborn neurons migrate to and take residence in their correct laminar locations within the mature cortex. RNAi knockdown of DCX expression and human deficiencies in DCX expression lead to lissencephaly that is characterised by mental retardation, and a smooth double-cortex lacking gyra. Our initial findings indicate that the decreased DCX expression caused by prenatal morphine treatment may induce aberrations in cortical lamination in the mouse brain. The mechanism whereby MOR signalling reduces DCX expression remains elusive, as our IHC study has shown that migrating neurons do not express MOR. Our hypothesis that neuronal migration is inhibited by interactions between the migrating neuron and the radial glial scaffold network that they translocate upon is supported by IHC staining that localises MOR to radial glia. Current studies using BrdU labelling and fate mapping aim to examine

the effect of prenatal morphine exposure on proliferation, migration and survival of newly divided cells.

### **Proliferation and involution of haemangioma involves vascular endothelial growth factor receptor, STAT-3 and cyclophilin-A**

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Haemangioma (strawberry birthmark) affects 1 in 10 Caucasian babies. They are disfiguring but can also be life-threatening or cause functional problems such as blindness, airway obstruction and ulceration. Haemangioma is a tumour of the endothelial cells that make up capillary blood vessels. The endothelial cells divide and grow rapidly for about a year after birth but for some unknown reasons regress spontaneously over several years. Approximately 1 in 10 affected children need active treatment during infancy and 1 in 4 will need surgery and/or laser treatment during childhood. The molecular mechanisms underlying haemangioma progression remain poorly understood. We hypothesize that signal transduction through the vascular endothelial growth factor receptor (VEGFR) and signal transducers and activators of transcription (STAT) proteins, in endothelial cells modulate haemangioma progression and involution. Signalling through STAT-3 has been implicated in activation of cell proliferation while STAT-1 induces apoptosis. Using quantitative real-time PCR (qRT-PCR) and immunohistochemical (IHC) labelling, differences in expression of candidate genes were examined in haemangioma in varying stages of progression.

qRT-PCR showed high relative expression of STAT-3 and VEGFR in haemangiomas that are rapidly growing followed by a concomitant decrease in expression when they regress. There was a strong correlation between STAT-3 and VEGFR expression, such that those biopsies expressing high levels of VEGFR also expressed high levels of STAT-3. IHC staining showed a similar pattern of protein expression with the plump endothelial cells that make up haemangioma expressing high levels of STAT-3 and VEGFR.

An unexpected finding, which we are further investigating, is that during the involution phase of haemangioma, concomitant with a large influx of mast cells, there is a marked increase in the expression of the enzyme cyclophilin A (CYCL-A). CYCL-A catalyses the isomerisation of proline bonds, is induced by oxidative stress, and can have a proliferative or pro-apoptotic effect on endothelial cells. Our current model has a key role for CYCL-A activity in haemangiomas progression.

Signal transduction through STAT-3 and VEGFR is elevated in the endothelial cells of proliferating haemangioma, and both mRNA and protein expression decrease with ongoing involution. Our data suggests that signal transduction through STAT-3 and VEGFR may enhance haemangioma endothelial proliferation. The large increase in CYCL-A expression seen during involution

may be triggered by stress, or may be a consequence of secretion by infiltrating mast cells. The role of CYCL-A in haemangioma is unknown but its pro-apoptotic role, and timing of the increased expression, suggest a functional role.

## **Regulation of the cell cycle of *Mycobacterium* species**

Ronan O'Toole  
School of Biological Sciences

Tuberculosis, a disease which has afflicted mankind since ancient times, is the leading cause of death worldwide due to a bacterial infection today. An estimated 1.75 million people died from tuberculosis in 2003 and currently, 45 million people suffer from the disease

The ability of microbes to adapt and mould their behaviour to suit their surroundings is crucial if they are to exist in diverse and dynamic habitats. Microbes sense their environment in a number of ways. One process involves the production of molecules whose sensory targets are intracellular. Here the information is generated in the form of cyclic nucleotide monophosphates (cNMPs). How cNMPs are integrated into the cell signalling of mycobacteria and what their role is during infection are unknown.

In the 1980s, the cyclic nucleotide cyclic di-guanosine-monophosphate (c-diGMP) was recognised as a signalling compound involved in the control of glucose condensation into cellulose polymers in *Acetobacter xylinum*. Recent studies have shown that cyclic di-GMP controls many other important cellular properties in bacteria such as cell morphology, exopolysaccharide production and cell-cell communication. Unlike the other cNMPs, cAMP and cGMP, c-diGMP has not been detected in eukaryotic cells. Therefore, c-diGMP production may provide a selective target for antibacterial drug development.

The aim of this study is to investigate the role of c-diGMP in controlling the growth and development of *Mycobacteria*.

We have identified an enzyme which produces c-diGMP, in the genome of *Mycobacterium smegmatis*. A mutation in the coding gene of this enzyme, *cdgM*, has been constructed in the non-pathogenic strain, *Mycobacterium smegmatis*, using the overlap extension PCR method followed by allelic exchange. *M. smegmatis* has a number of advantages over pathogenic species of mycobacteria as it permits a greater scope of *in vitro* experiments to be performed. To determine the role of c-diGMP in the physiology of mycobacteria, the *cdgM* mutant will be tested in a range of physiological assays to examine growth and survival under nutrient-rich and nutrient-depleted conditions, biofilm formation, sliding motility, cell morphology and resistance to environmental stressors. Possible outcomes of this work are an understanding of the importance of c-diGMP production to mycobacterial physiology and the determination of its potential as a drug target in the treatment of tuberculosis.

## Role of the opioid system in respiratory disease

JH Miller, DJ Day, EM Mrkusic  
School of Biological Sciences

The brain's opioid system consists of protein receptors and small opioid peptides that activate those receptors. Signalling through the mu-opioid receptor (MOR), for example, is important in pain control and drug abuse. MOR also modulates many homeostatic physiological processes, and has major inhibitory effects on respiratory activity in the neonate and adult. There is some evidence that opioid modulation plays only a minor role in breathing control in healthy individuals but may become more important in respiratory disease. MOR is expressed throughout the brain in a regional specific manner, with expression being highest within the cortex, olfactory bulb and several hindbrain nuclei involved in breathing. One of the opioid peptides that activates MOR in the brain is enkephalin. The intention of this study is to make use of a genetically modified mouse, the preproenkephalin (PPENK) knockout mouse that lacks the ability to synthesize enkephalin. We will examine the role of opioid signalling in this mouse in relation to respiratory responses to low oxygen and high carbon dioxide tensions, conditions that simulate chronic obstructive pulmonary disease (COPD). High-resolution fluorescence *in situ* hybridization (FISH) will also be used to determine differences in mu-opioid receptor mRNA expression between normal and PPENK knockout mice.

We have completed some preliminary studies on MOR mRNA expression in normal and PPENK knockout mice and found significant differences in the level of MOR gene expression in specific brain regions of the PPENK knockout mice relative to the wildtype. The habenula and medial thalamic nucleus had the largest increases, 25% and 27%, respectively; whereas, the piriform cortex exhibited a significant decrease (16%) in the level of MOR staining. Most of the regions analysed showed no change in staining between control and knockout mouse. Within the thalamus, there were regional specific differences in the observed staining density. The anteriomedial thalamic nucleus showed no change; whereas, the medial dorsal thalamic nucleus showed a 27% increase. These results suggest that a previously reported up-regulation of MOR in the PPENK knockout mouse is due to a combination of mechanisms that are region specific. Dr Dona Boggs of the Department of Biology, Eastern Washington University, USA, who will be visiting our laboratories between July and September 2004, will carry out measurements of respiratory activity in these mice.

The overall aim of our project is to understand how opioid peptides function in the brain to control development and neuronal activity, in particular development and activity of the neural pathways involved in breathing. This information will provide insight into the role played by opioids in normal brain development and the changes that occur in respiratory disease.

## **Targeting multiple sclerosis with anti-mitotic drugs**

AC La Flamme, K Crume, JH Miller  
School of Biological Sciences

The goal of this research was to determine the effectiveness of anti-mitotic drugs at treating multiple sclerosis (MS). This goal had three main parts. First, we investigated the timing and location of T cell proliferation during the mouse model of MS, experimental autoimmune encephalomyelitis (EAE). Results from these experiments indicated that most T cell proliferation occurred in the periphery and not the central nervous system. This finding suggests that to target proliferating auto-reactive T cells, the anti-mitotic drug does not need to pass through the blood-brain barrier.

Second, the effects of the anti-mitotic, microtubule-stabilizing drugs, paclitaxel and peloruside, on immune cells *in vitro* were compared. Our results from these studies showed that paclitaxel induces the production of pro-inflammatory-mediators by macrophages through toll-like receptor 4. In contrast, peloruside does not induce cytokine production and, moreover, reduces LPS-stimulated TNF- $\alpha$  production. Together these findings suggest that for the treatment of pro-inflammatory-mediated diseases (e.g. MS), the use of a drug that reduces pro-inflammatory cytokine production like peloruside may be advantageous.

The third part of the study was to determine the effectiveness of paclitaxel at treating MS, using the EAE mouse model. These studies indicated that at high doses, paclitaxel significantly delays the onset of EAE. However, treatment did not have any observable effect on autoreactive T cell expansion or responses suggesting that the protection afforded by paclitaxel is not due to a specific deletion of auto-reactive T cells. Despite the effectiveness of paclitaxel at delaying disease, the treatment regime was not well tolerated, possibly due to the ability of paclitaxel to directly induce pro-inflammatory mediator production. Therefore, we are currently investigating the use of peloruside at inhibiting MS as an alternative to paclitaxel.

## **The effects of tobacco smoke components on nicotinic receptors and neurotransmitter systems**

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Research, Kenepuru

There is evidence that components of tobacco smoke other than nicotine can interact with nicotinic acetylcholine receptor (nAChR) expression or function, and influence neurogenic amine release or breakdown by monoamine oxidases (MAO). We initially examined the effect of total particulate matter (TPM) from cigarette smoke on nAChR's in a human neuroblastoma cell line (SH-SY5Y). TPM but not nicotine alone inhibited cell growth, and both nicotine and TPM caused an increased specific binding of the high affinity nAChR ligand 3H-epibatidine, suggesting up-regulation of nAChR. Most

significantly, up-regulation was consistently greater after pretreatment with TPM than in cells pretreated with nicotine alone (at the same nicotine concentration as in the TPM sample).

Monoamine oxidase enzymes are known to modulate monoamine neurotransmitter concentrations in the brain and have been linked to alcohol addiction. In a preliminary study, we evaluated relative gene-transcript abundance of MAO-A and MAO-B in SH-SY5Y cells in response to 100 mM ethanol and found that each isoform was significantly transcriptionally up-regulated in response to ethanol exposure, suggesting a role of transcriptional regulation for MAO in alcohol abuse. Similar addictive consequences may occur as a result of smoking effects on MAO activity, since both isoforms of this enzyme are significantly inhibited in tobacco smokers. Nicotine alone, however, does not inhibit MAO activity, and therefore other neurobiologically active compounds in tobacco smoke must function as MAO inhibitors, contributing to the development of tobacco dependence. To test this possibility, we are investigating MAO-A and MAO-B activity using the Amplex Red enzyme activity assay in SH-SY5Y cells and in a neuroglial cell line, U-118, and will examine the effect of TPM extracts on their enzyme activities. Preliminary tests have shown that both SH-SY5Y cells and U-118 cells express MAO-A and MAO-B, although expression in U-118 cells is low. We currently have TPM samples collected from three different types of cigarettes for this study: a normal New Zealand brand and two brands from overseas, one with low nicotine and the other nicotine-free. To further characterise the changes in MAO enzyme activity in these cell lines, we will use quantitative real-time PCR analysis and Western blotting to measure mRNA and protein changes, respectively, in the cells. We are currently developing these methodologies in our laboratories.

As there appears to be an indirect involvement of the opioidergic system in the maintenance of tobacco dependence, it has been hypothesised that exposure to nicotine or tobacco smoke may result in down-regulation of the mu-opioid receptor (MOR). Given this opioid link to tobacco addiction, we will also test whether there are changes in MOR gene expression and protein abundance in cultured cells following exposure to TPM.

From the results of these studies on the neurochemical effects of TPM extracts, an increased understanding of the complex biology of tobacco dependence may help provide effective strategies for assisting smoking cessation.

## Wakefield Hospital

### Genetic changes in chromosome 8p21-22 during the metastasis of colorectal cancer

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The Wakefield Gastroenterology Research Institute

Every year 2400 New Zealanders are diagnosed with colorectal cancer (CRC) and approximately 1100 die from it. Although colorectal cancer originates in the large bowel, in many patients cancer cells will spread (or metastasize) to other parts of the body, most commonly the liver. Since liver metastases are the major cause of death from CRC, prediction, prevention, and treatment of metastatic CRC disease may be the most practical approach to reducing the mortality rate from CRC.

Our research seeks to identify genes that prevent the metastatic spread of colon tumours to the liver (metastasis suppressors). Analysis of these genes may allow us to identify those tumours that are likely to spread so that patient management can be altered to either prevent metastasis developing or detect it earlier so appropriate treatment can be initiated sooner.

Previous research undertaken at the Wakefield Gastroenterology Research Institute by Dr Serena Rooker found that DNA loss at chromosome 8p21-22 occurred in a significant number of CRC liver metastases compared with their matched primary colon tumour. The financial support provided by the Wellington Medical Research Foundation has facilitated the continuation of this research, allowing us to complete this analysis and to undertake quantitative expression analysis of selected genes located in that specific region of DNA loss to determine whether the genetic loss results in altered gene expression.

#### Metastasis-Specific DNA Changes on Chromosome 8p21-22

Research on this aspect of the project is now completed. DNA from over 50 liver metastasis samples were analysed for loss at 8p21-22 by measuring the loss of heterozygosity (LOH) of microsatellite markers in this region. To complement the LOH analyses, a mutation screen of four candidate metastasis-suppressor genes located in the region of DNA loss was also undertaken. These analyses suggest that DNA mutation may not be the major "second hit" of 8p21 gene inactivation in CRC metastasis. DNA analysis was also expanded to include polymorphism analysis of candidate genes. A known polymorphism in one of the candidates demonstrated a significant association with colorectal tumour APCS staging ( $p < 0.01$ ), while a known polymorphism in another candidate demonstrate a significant association with primary tumour site ( $p < 0.01$ ).

The expression of fourteen genes in the region of loss, was quantified using real-time PCR in 34 liver metastases, and their matching colon tumour (14/34 patients) and normal colon (6/14) where available. Although none of the candidate genes demonstrated a consistently significant metastasis-specific

decrease in gene expression, a degree of co-ordinate regulation at the transcriptional level in liver metastases was observed.

In addition to RNA expression, protein levels of the candidate metastasis-suppressor genes were also examined in colorectal tumour tissues by western blotting and immunohistochemistry. This analysis has revealed a striking pattern of protein expression for one of the candidates.

Additional investigations including polymorphism and protein expression analyses were also incorporated into the project. The study has provided a number of avenues for future research to determine whether candidates in this region of DNA may provide the basis for prognostic markers for identifying CRC patients at risk of metastatic spread. We gratefully acknowledge the financial contribution provided by the Wellington Medical Research Foundation.

### **Is the improvement in insulin resistance after gastric bypass due to reduced energy intake?**

RS Stubbs, J Krebs  
Wakefield Gastroenterology Centre and Research Institute

We have previously demonstrated that insulin resistance (IR) is substantially lost within six days of gastric bypass surgery – a finding which explains the resolution of type 2 diabetes seen within the same time frame after gastric bypass surgery. The work was conducted with financial support from Wellington Diabetes Inc, through the Wellington Medical Research Foundation and it may lead to an understanding of the fundamental cause of insulin resistance. Its importance lies in the fact that effective treatment for IR has the potential to lead to resolution of such conditions as type 2 diabetes, hypertension, high cholesterol, and perhaps obesity itself. Currently no such treatment exists.

Our work has led us to believe that IR occurs as a result of the presence of an as yet unidentified substance carried in the blood, released from that part of the stomach or duodenum which is bypassed by the gastric bypass operation.

In an extension to this earlier work we proposed a series of studies to compare the benefits to IR achieved by a very low calorie diet (600 kcal/day) for 6 days and that achieved by the gastric bypass over the same time period. The study was intended to shed light on whether the benefits of the surgery were the result of substantial reduction in energy intake or as a result of a fundamental physiological change brought about by the gastric bypass.

IR is a phenomenon which may occur in one or more of the following tissues - liver, muscle and fat. Levels of IR in one tissue do not necessarily reflect levels in another. In our previous studies we have measured IR using the HOMA index, which is a mathematical relationship between fasting glucose and fasting insulin levels. We have also used intravenous glucose tolerance tests. Both are a measure of IR in the liver. In our proposed study looking at

IR before and after VLCD and gastric bypass we chose to use an insulin tolerance test (ITT), which is a measure of whole body IR (ie the sum of IR in liver, muscle and fat). To our surprise we have found that IR measured by ITT is worse following both gastric bypass and VLCD. This has been confirmed in 8 patients undergoing gastric bypass and 6 patients taking VLCD. Thus the original aim of the study could not be achieved, and substantial changes to the study as proposed have been undertaken.

The implications of the findings of deterioration in whole body IR, yet resolution of central (liver) IR after gastric bypass, coupled with the knowledge that type 2 diabetes is almost always resolved by gastric bypass, is that type 2 diabetes is primarily a disorder of liver (and not whole body) insulin resistance. This is a new and important finding which will assist us and others in the pursuit of the cause of type 2 diabetes and an effective treatment for that condition.

## Wellington School of Medicine and Health Sciences

### A fluorescence and image analysis microscopy system for the Dental Research Group

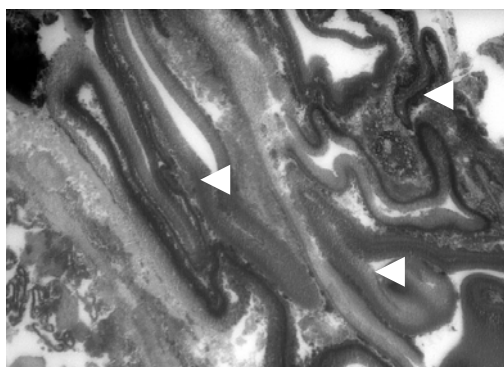
S Filoche

Dental Research Group, Department of Pathology and Molecular Medicine

The Research Grant that was awarded by the Wellington Medical Research Fund was used to purchase the essential accessories to the Leica DM RE microscope recently purchased by the Dental Research Group (DRG). These additional components consisted of a beam splitter to accommodate a specialised fluorescence camera, a red spectrum filter cube and a long distance working objective.

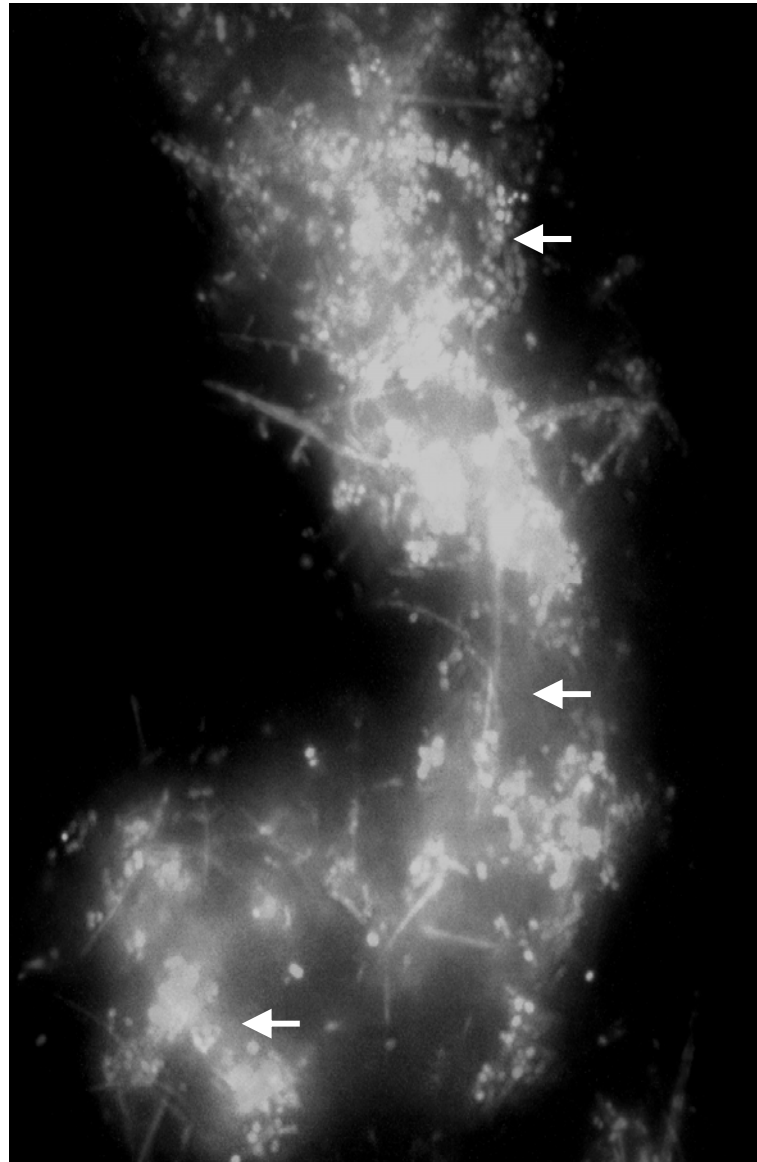
With the purchase of these items, the Leica DM RE microscope covers current and predicted microscopy studies for the DRG. Presently the microscope is used for the analysis of plaque structure, viability assessments, and cell culture analysis. Analysis of plaque structure is key to understanding how current clinical treatments are effective and for the development of more efficacious regimes (Figure 1 and 2). Future investigations also aim to use image analysis techniques, including using fluorescent in situ hybridisation (FISH) and green fluorescent protein (GFP) techniques, which without these purchases would not have been possible.

The grant has enabled five main goals to be achieved: (i) to support the research at DRG, particularly regarding investigations into plaque structure and morphology (Figure 1), and analysis of cell cultures; (ii) to establish an imaging database for the analysis of plaque structure; (iii) to develop a bacterial viability assay, a crucial determinant for assessing the effect of oral antiseptics (Figure 2); (iv) to enable preliminary investigations using FISH technology, a novel approach for studying bacterial associations in plaque; (v) to support research outside of the DRG, including work carried out at the Bioactivity Investigation Group (BIG) at the Wellington School of Medicine.



**Figure 1** Digital photomicrograph of an *in vitro* grown plaque at 40x magnification, which had been treated with the oral antiseptic chlorhexidine digluconate (0.2 %). Plaque morphology was shown to be affected by treatment with chlorhexidine, and other oral antiseptics, forming dense ileum-

*like structures comprising of distinct cell types, indicated by the arrows. Such structures, may contribute to plaque biofilm recalcitrance to clinical treatment by limiting penetration of antiseptics to the bacteria within.*



**Figure 2** *Digital photomicrograph of in vitro grown plaque bacteria which had been made into suspension for viability analysis, at 100x magnification. Even after homogenising in suspension the plaque bacteria maintained structural integrity and cell-to-cell associations within an exopolymer-like matrix, as indicated by the arrows. The formation of these aggregates may also contribute to the survivability of plaque bacteria in saliva and in the plaque biofilm to treatment with oral antiseptics.*

### **A pilot study into the permeability of the gastrointestinal tract in preschool children with eczema compared to age match controls**

TV Stanley  
Department of Paediatrics

The study continues and a further two children have been enrolled in the study today. This takes the total number of eczema children between 1-4 years of age to 49. Unfortunately, controls have not been so easy to acquire. We have at present only got 6 patients and the plan is to try and get 25.

All that remains after that is to collect the samples and send them to Australia.

I am pleased to also report that the information gathered so far has been helpful with regard to another study I am involved with (in cooperation with the Wellington Asthma Research Group). In this we plan to arrange patch testing of children with atopic eczema. I was able to advise that on the basis of my study I can confirm there is no direct relationship between patch testing and severity of eczema. This has not been previously reported.

### **Air endotoxin levels in homes of smokers and non-smokers**

R Siebers  
Wellington Asthma Research Group

Endotoxin, a component of the outer membrane of Gram-negative bacteria, is ubiquitous in the indoor environment. It is one of the most potent inflammatory mediators known and may explain the high prevalence of respiratory disorders among smokers. Inhaled endotoxin causes inflammation in the airways and tobacco smoke contains large quantities of endotoxin. A recent study in the laboratory setting showed that endotoxin levels were 120 times greater in a smoke-fined room, compared to a smoke free room.

Our pilot study aimed to quantify levels of biologically-active endotoxin in the every day domestic setting of homes with smokers, compared to non-smokers. Information from this study could potentially be of use in alerting the public of the potential adverse effects of cigarette smoke exposure on smokers, and non-smokers exposed to second hand smoke.

It was intended to obtain air samples from 10 houses where inhabitants smoked inside, and from 10 control smoke-free houses. To date we have obtained air samples from some houses of smokers. As there is wide public concern about indoor smoking we have had some trouble obtaining enough homes with smokers. However, we are continuing to find such homes in order to finish the study.

## **Cat allergen in beds of infants and atopic status**

R. Siebers  
Wellington Asthma Research Group

Development of atopy to indoor allergens, such as house dust mite and cat, is a major risk factor for asthma in children. However, recently intriguing overseas studies have suggested that high exposure to cat allergens is associated with decreased sensitisation to cat. We have collected dust samples from bedding and living rooms of infants at three months from a prospective infant cohort study set up to determine factors associated with development of atopy and asthma later in childhood.

Most of the dust samples have now been analysed for the cat allergen. At present we are cleaning the data collected on asthma and allergy symptoms when the infants were 15 months, two, three and four years of age. At present the children are being assessed for atopic status at age six together with objective markers of airway inflammation. Thus, a very large amount of data has been collected that will be analysed in respect to the association with the early life exposure to cat allergens. This analysis is complex and will take some time. However, ultimately we will be able to answer the question of whether early life exposure to cats is protective for development of allergic diseases and asthma in childhood, or not.

## **Effects of "back migration" on atopic markers and asthma symptoms in Tokelauans: a pilot study**

R. Siebers  
Wellington Asthma Research Group

Previous New Zealand research has demonstrated that native Tokelauans in Tokelau very rarely have atopic diseases but after migration to New Zealand have the same prevalence as New Zealanders. The reasons are likely to be multifactorial, but the high indoor allergen levels in New Zealand may be a major factor. Indoor allergens (dust mite, cat, dog and cockroach) are 100 to 1000 fold lower in Tokelau, compared to New Zealand (*Clin Exp Allergy* 2005; 35: 479-82).

This study was set out to determine whether asthma and atopic symptoms and severity, and lung inflammation (assessed by non-invasive techniques) improve when New Zealand Tokelauans return to Tokelau for a medium time period. If so, this would argue in favour of major allergen avoidance in atopic diseases.

Ten asthmatic Tokelauans, resident in Wellington and intending to visit Tokelau for sporting, cultural, church group, or personal reasons for at least six weeks, and intending to return to Wellington after their visit were to be recruited in the study. It has taken quite a while to obtain ethical approval and also we were going to assess bronchial hyperresponsiveness using a new agent, namely mannitol (Aridol). We have been in negotiation with the

company to use this and have recently obtained the material transfer agreement from them. Participants are being recruited at present.

### **Falls assessment clinical trial (F.A.C.T.)**

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Department of Primary Healthcare and General Practice

Falls are a major cause of morbidity for older adults, with between 30% and 60% of those living in the community falling each year. There is evidence that several medical interventions, removal of home hazards and exercise programmes can substantially reduce the risk of falling.

This study was undertaken to assess whether a multi-component intervention based in primary care can reduce falls in older adults.

A randomised controlled trial has commenced within primary health care practices in the Hutt Valley. People over the age of 75 years (over 55 for Maori or Pacific peoples) who have had a fall in the previous 12 months are eligible to participate. Intervention participants are assessed by a community falls and fracture nurse coordinator who performs a home-hazards assessment and falls-related medical assessment at home and oversees referral to appropriate services to address identified risk factors. Referral is also made to a physiotherapist or trained nurse for the home-based Otago Exercise Programme. Control participants receive usual care from their primary care practices and two 'social visits'. Outcome measures include the number of falls over 12 months (recorded prospectively with monthly calendars), injuries, physical activity, quality of life, physical functioning (strength and balance) and fear of falling (confidence).

To date three hundred and twelve participants have been enrolled in the trial. Enrolment proceeded ahead of schedule, with all participants enrolled, randomised and those in the intervention group having started their interventions by January 2006.

Initial analysis has been completed using data collected at baseline and using data collected by a Masters student on the cost of falls.

While there were nine participants between the ages of 60 and 75 (they qualified because they were either Maori or Pacific), all other participants (n=303) ranged from 75 to 98 years of age.

Increasing number of falls was associated with higher BMI and poorer SF36 physical component summary score. The average provider and personal cost of a fall in the first 6 months was NZ\$78/fall, with 60% of falls causing injury and 18% requiring medical help.

Ninety-three participants have finished the study and have had their follow-up assessments completed. In addition to the home based follow-up assessment, participants have also been contacted by phone following

completion of the study and have been asked questions relating to how they found participating in the study. The overall response has been good with participants appreciative of the study related visits they have received. A recurring theme is that participation has made them more vigilant about falls risks and taking care when in high-risk situations such as climbing stairs etc.

### **In-flight medical events in international air transportation: the development of an evidence-based categorisation system**

PH Mahony

An in-flight medical event is defined as an acute event that has affected the physical or mental health of a passenger between aircraft door closure and arrival at the destination. A range of in-flight medical events in international air transportation have been reported and include traumatic injuries, gastrointestinal disturbances, syncope, asthma attacks, psychiatric emergencies, acute myocardial infarction, convulsions and cardiac arrest. Previous research to determine the types and relative frequencies of these events has been limited because of difficulty in obtaining data from airlines and because there has been no standard reporting process for in-flight medical events in international air transportation.

The majority of in-flight medical event data available to date have been derived from telemedicine providers, airport medical clinics and ambulance emergency services, and airline generated in-flight medical reports. All of these sources are limited: telemedicine patches are not used for all medical events, and relatively small numbers of passengers seek attention in airport medical clinics or are attended to and transported by emergency services to hospital. Health care professional attend only a small proportion of passengers exhibiting ill health in flight, and one third of passengers experiencing medical problems in flight decline treatment, so that these events have not previously been identified as medical events. The aim of this research project was to derive a symptom-based classification tool from passenger health data that would more clearly quantify and characterise in-flight medical events, and enable the comparison of data from different sources.

The first part of the research was the determination of the types and relative frequencies of in-flight medical events. The research data set was all in-flight medical reports from 1996 to 2004 and was collected retrospectively from an international airline that carried in excess of 10 million passengers per year, on a combination of long-haul and short-haul sectors. The data included detailed information on presenting problems, vital signs, medical history, treatment and outcomes. Cabin crew routinely logged all events in the cabin, including service issues, complaints and use of equipment, and as such can be considered a representative description of anything that caused crew to deviate from normal service duties. The data contained epidemiological information on age and gender of the passenger, flight number, date, sector, time of day, aircraft type, symptoms and signs, vital signs, treatment rendered, whether a physician or nurse was involved, diversions and outcome. Cabin

crew generated reports of in-flight medical events and any health care professional rendering assistance was asked to complete a medical report. Some events were reported twice and as part of data collection, these records were merged to avoid duplications. Events where primary symptoms could not be identified or which were ambiguous were classified as 'unwell'. Cases where passengers only sought advice, or which were non-specific in nature, were also assigned to this category.

A total of 11,244 cases were identified over a period of 9 years, (mean 1249 per year; range 1060 to 1417,); mean 167 per million passengers carried, providing higher rates than have been cited in previous studies. Ages ranged from newborn to 88 years (mean age 44 years); 39% were male and 68% were female.

The data revealed the following relative frequencies of medical events in order of decreasing frequency. The gastrointestinal category included symptoms of nausea, vomiting and diarrhoea; fainting and near fainting; the cardiovascular category included all cases of chest pain, cases of suspected heart attack, and disturbances of pulse rate. The respiratory category included acute shortness of breath resolving with oxygen therapy, and suspected asthma attacks. Neurological cases included principally headache, suspected migraine and convulsions. The psychological/behavioural category included mostly cases of hyperventilation associated with anxiety, and confusion. Cases in the trauma category included soft tissue injuries such as abrasions and minor lacerations to fingers as well as superficial burns from hot liquid spills. Minor categories included obstetric and gynaecological symptoms, musculoskeletal symptoms including joint pain and muscle pain; dermatological symptoms including rashes and minor allergic reactions. The ENT category included epistaxis as the most common event, followed by endocrine which mainly included cases of hypoglycaemia. The remaining categories were where passengers or crew sought health related advice from telemedicine providers or physicians or nurses on board, but received no treatment. The final category was where symptoms were ambiguous or not clearly reported.

The clinical outcomes of each case were grouped into one of four categories: improved; unchanged; deteriorated; and deceased. In cases where a health care professional was involved, events considered to have improved were those where the presenting problem had resolved (87.5%) i.e. the presenting symptoms had disappeared, events unchanged were those where the problem persisted but no improvement or deterioration was reported (9.96%), and deterioration referred to cases where the problem escalated (2.54%). There were 7 reported deaths in-flight including 4 attempted resuscitations.

Based on the data collected, a symptom based categorisation system was derived and consists of 14 categories. The categorisation system allows for additional event descriptions to be incorporated within each category. The categories and relative frequencies of events bear some similarities to those described in previous studies where similar patterns of syncope, gastrointestinal symptoms, and respiratory symptoms were described,

however, this study found much higher relative frequencies of each type of in-flight medical event than previous studies.

This study is the first of its kind to produce a classification scheme based on empirical evidence. The methodology employed produced consistent results for each year of the airline's data and there was good agreement with data published by a telemedicine provider.

The use of the classification system derived from this research provides a set of standard definitions based on presenting symptomatology which provides a suggested platform for further research in this area.

### **Methods of mandibular advancement splint (MAS) titration**

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Department of Medicine and Dental Department, Capital and Coast District Health Board

MAS devices have a therapeutic role in Obstructive Sleep Apnoea (OSA) but research is needed to define optimum methods of titration.

This study was undertaken to compare two methods of MAS titration (self adjustment with and without polysomnography feedback).

Twenty nine patients (25M, 4 F, mean age 49 years, mean BMI 27.6kg/m<sup>2</sup>) with OSA (mean Apnoea Hypopnoea Index (AHI) 25.7/hr range 10-46/hr) and symptoms (Epworth Sleepiness Score > 8/24, snoring, choking, poor sleep quality) were treated by MAS set at 70% of maximal protrusion.

A subjective group (n=17) self-adjusted according to symptoms and comfort, while an objective group (n=12), fixed setting then given self-adjustment advice based on polysomnography result at 3 weeks. Outcome variables (AHI, symptoms) at 6 weeks were compared by t-tests, and chi squared tests.

The study showed that the two groups did not differ in mean baseline AHI (p=0.73), BMI (p=0.54) or age (p=0.80). MAS was used by the majority of patients every night (83% objective, 59% subjective) and all night (82% objective, 71% subjective).

Objective feedback was associated with a progressive reduction in AHI (baseline 26.5 ±12.0/hr, 3 weeks 15.3±13.5/hr, 6 weeks 11.7±10.0/hr, p=0.01) and increasing proportion of patients whose OSA resolved (AHI < 5/hr) or improved (decrease by > 50% but AHI > 5/hr), 7/12 at 3 weeks and 9/12 at 6 weeks p=0.33).

In the subjective group AHI was reduced by a lesser extent (baseline 25.1±7.4/hr, 6 weeks 15.5±13.7/hr, p=0.053) but a similar proportion resolved or improved their OSA at 6 weeks (10/17, versus objective p=0.43). Symptomatic benefit was reported by both groups (daytime alertness: objective p=0.0007, subjective p=0.0004, refreshed on waking: objective

p=0.0003, subjective p=0.002 and sleep quality: objective p<0.0001 subjective p=0.004).

It was concluded that a titratable MAS improved or resolved OSA in the majority of patients. Objective feedback was associated with a greater reduction in AHI but a similar proportion of patients gained symptomatic benefit. Objective follow up is recommended.

### **Randomised adolescent pedometer trial (RAPT): Pedometers as motivational tools for increasing physical activity in adolescents with type 1 diabetes**

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Departments of Paediatrics and General Practice

This study is investigating whether pedometers and text messaging over a period of 12 weeks can increase physical activity, and improve diabetes management and quality of life in adolescents with type 1 diabetes.

The study commenced recruiting in September 2005. So far, 44 adolescents between 11 and 18 years of age have been recruited into the study. Twenty seven participants have completed their 12-week involvement in the study. Most of the data has been successfully collected with some gaps where adolescents have lost their log sheets. There have been some lost pedometers due to them falling off their waistbands and safety pins have been an economic securing device. So far, three adolescents have opted out of the study.

There has been no analysis of results as yet but data is being entered weekly into Excel databases. Data collected is baseline measurements (HbA1c, height, weight and blood pressure; number of steps after 4 days taped shut pedometer wearing; physical activity and quality of life questionnaires and log sheets filled in for the 4 days of pedometer wearing); logs of all texts and phone calls made for the 4 days of pedometer wearing and for those randomised to the intervention-group who are texted weekly; end of study measurements (same as baseline measurements).

Recruitment is slower than expected across the three DHBs and an extension is being sought through the Ethics Committee to include Christchurch. Professor Darlow is currently working on site approval and Maori consultation prior to the application being sent to Ethics. However, with a lower than expected drop-out rate and extension to Christchurch, we expect to complete the study and data analysis in the expected timeframe.

## **To establish a fetal lamb model for prenatal study of spina bifida**

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Neural tube defect (NTD) is one of the commonest birth defects in humans. Spina bifida (failure of closure of the caudal end of the neural tube) is associated with significant lifelong disability such as paralysis of the lower limbs, collection of fluid in the brain, sexual dysfunction, skeletal deformations, incontinence and mental impairment. Although antenatal detection with ultrasound examination is possible, allowing termination of affected pregnancies and fortification of food with folic acid reduces the occurrence of NTD, the live birth rate for spina bifida remains 0.39/1000 (22 live births) in the year 2000 in New Zealand.

It is thought that the exposure of spinal cord to the toxicity of amniotic fluid in the uterus and mechanical trauma during delivery (2-hit theory) lead to the neurological dysfunction of the baby after birth. There are reports on healing power of fetal tissue in-utero, out of these are reports that surgical closure of spina bifida before birth reverses some of the associated neurological changes in animals and humans and the reduction in need for shunting of hydrocephalus after birth in humans. However, the methodology of prenatal repair is yet to be examined systematically in either animals or humans. This pilot study was set up aiming at establishing a fetal lamb model for future study of prenatal repair of spina bifida on animal and human fetuses.

The initial study examined the effect of gestation on creation of spina bifida lesion in fetal lambs. Hysterotomy was performed on the ewes at the assigned gestation (50, 60 and 70 days). Spina bifida lesion at L1-4 level was created surgically in fetal lambs by removal of the spinal processes and laminae. The spinal cord was exposed to amniotic fluid. At delivery at term by Caesarean Section, the neurological outcome of the fetal lambs was examined. The findings were compared with a normal control group of comparable gestation. The ewes received field block as post-operative analgesic and were returned to the farm once recovered. The lambs were euthanased within 24 hours of birth.

Spina bifida was created surgically in 7, 7 and 8 lambs at 70, 60 and 50 days gestation. In the group with spina bifida created at 70 days gestation (n=7), 4 were sacrificed at term and 3 at 90 days.

In the group with spina bifida created at 60 days gestation (n=7), 2 were sacrificed at term and 3 at 80 days, 1 was stillborn prematurely, and 1 aborted spontaneously. The 2 sacrificed at term succumbed soon after birth.

In the group with spina bifida created at 50 days gestation (n=8), 2 were sacrificed at term, 3 at 70 days and 3 aborted spontaneously.

The spontaneous fetal loss rate was 0% when spina bifida was created at 70 days, 29% at 60 days and 38% at 50 days.

All surviving newborn showed defective motor function at hind limbs. The sensation of the hind limbs was preserved in all. Anal spincter response was present in all surviving newborns except one (5/6) which also had urinary incontinence. The lesion could be recognised by scar over the skin but the skin tended to heal over the underlying defect.

For models of prenatal study of spina bifida, the lesion should be created as early as possible to mimic the naturally occurring lesion which is formed at embryonal stage in human. Technically it is possible to create spina spina lesion surgically in fetal lambs at 50, 60 and 70 days gestation. However the spontaneous loss rate is high (38%) at 50 days. When spina bifida is created at 60 days, there is perinatal loss (forbidding postnatal neurological examination) in addition to the spontaneous fetal loss rate of 29%. The fetal loss rate is acceptable at 70 days, being 0% in this study and the condition of the newborn is satisfactory for postnatal neurological examination.

Creation of spina bifida at 70 days gestation is optimal for future prenatal study of surgically created spina bifida at a spontaneous fetal loss rate of 0% after the procedure in this study. However, with further experience, it will be possible to create the spina bifida lesion earlier in gestation with an acceptable loss rate, and a better success rate.

### **'What's going on?' Transsexual people and their relationships with clinicians**

J Humpfer  
Department of Psychological Medicine

Transsexuality has previously been researched from a clinical perspective with regard to aetiology, diagnosis, sex reassignment surgery, client profiles and pathology. Occasionally the literature has acknowledged that the therapeutic relationship between transsexuals and clinicians is not always ideal: transsexuals have been described as being dishonest, manipulative or antagonistic towards health care providers. However the issues around the relationship between clinicians and transsexuals have been largely disregarded by scientific research.

This study aimed to address this oversight by exploring the nature and consequences of the therapeutic relationship between transsexuals and clinicians in New Zealand. The main research question was: "What is happening between transsexuals and their clinicians in New Zealand?" By applying a grounded theory framework, I aimed to discover and explore matters within the client-professional interaction that might work for or against a positive therapeutic relationship in this very specific clinical situation. Data collection and analysis used the Grounded Theory method that emphasises theory generating rather than theory testing. Therefore, no specific hypotheses were formulated. Altogether I interviewed 37 participants, 26 transsexuals and 11 clinicians. Six transsexuals and one clinician were interviewed twice. Two types of interviews were conducted: focus group

interviews and individual interviews. Transsexual participants were recruited for focus group discussions and individual interviews. Clinicians were recruited for individual (one-to-one) interviews only. The types of clinical specialists most relevant to transsexual participants were identified through the first focus group and the first round of individual interviews with transsexual participants.

Research questions were of an exploratory nature and therefore intentionally broad. The main research question was: What is going on between transsexual people and their clinicians in New Zealand?

While data collection and analysis progressed the following research questions emerged:

- What are the underlying reasons for transsexual people to contact clinicians?
- What factors and preconceptions influence transsexual people in their contact with clinicians?
- What quality of service do transsexual people receive when they contact clinicians?
- What kinds of interactions occur within the relationship?
- What behaviours do transsexual people show when in contact with clinicians?
- What factors influences transsexual people on showing these behaviours?

Striving for access was identified as the overarching theme and basic social process, in all transsexuals' narratives in regard to their relationship with clinicians. Striving for access occurred in a repeated manner and was the main challenge for developing a positive relationship with clinicians as it influenced transsexuals' behaviour in their interactions with clinicians. It was found that transsexuals' striving for access were concerned with accessing information, support in decision-making, hormone prescription and gender reassignment. All transsexuals encountered difficulties in regard to clinicians' availability, waiting time and funding for services. By the time of initial clinical contact many transsexuals harboured negative expectations. These were a consequence of pre-disposing factors such as relationship motive, relationship dilemma and relationship dynamic. Striving for access occurred as a consequence of transsexuals' expectations and negative experiences that caused them to fear that their access to treatment would be endangered. As a result the data show that striving for access is a condition that influenced transsexuals who fear access-denial to present themselves in selective or fictional ways during clinical contact. Transsexuals who harbour fears that access to certain treatment may be denied will present themselves in the most favourable way possible to increase their chances of successfully accessing desired treatments. However such presentation will create a vicious cycle for transsexuals, as it will reinforce their non-trust of clinicians. By responding in certain ways clinicians can reinforce or reduce transsexuals striving for access strategies. Clinicians can either foster fear in transsexuals that their access may be endangered or alternatively they can foster trust building, by adopting a number of approaches. In order to achieve a positive relationship with transsexuals, clinicians need to engage in extensive trust

building behaviours. The findings of this research can inform clinicians and health service managers on important aspects of current service delivery and how these impact on the therapeutic relationship. They can also be used to better educate clinicians on transsexuals' needs in relations to the therapeutic relationship.

### **Leura Trask Trust Renal Research Grant**

Lidijja Jovanovic and Brett Delahunt  
Department of Pathology and Molecular Medicine

Two projects were completed during the tenure of the grant:

#### **1. Genetic assessment of primary renal carcinomas showing dual clear cell and papillary morphology**

Renal cell carcinoma is a group of tumours each of which have unique histological, genetic and clinical features. Of these tumours clear cell renal cell carcinoma and papillary renal cell carcinoma are most commonly encountered. Clear cell carcinomas characteristically show loss of heterozygosity of the Von Hippel-Lindau gene (3p 25-26) while loss of heterozygosity in papillary renal cell carcinomas involves a variety of genetic loci including chromosome 7, 16 and 17. In particular allelic imbalances at 3p are not a feature of papillary renal cell carcinoma. Despite the apparent genetic diversity of these two tumour types, renal tumours are encountered which show a dual clear cell/papillary morphology. The genetic nature of these dual morphology tumours is unknown and in particular it is uncertain if they represent collision of two primary tumours or an evolution of morphology from one tumour type to another.

In this study we have examined thirteen dual morphology clear cell/papillary renal cell carcinomas utilising a total of 21 genetic markers in order to determine the presence or absence of allelic losses at chromosomes 3p 25-26, 3p14.2, 7q 31 and 10q 23.

Patterns of LOH in a series of tumours examined in our study indicated that complex genetic mechanisms are involved in the development of these tumours. In 71.4% of cases, allelic losses were detected in at least one tumour. Sixty percent of tumours with LOH displayed concordant genetic changes in both papillary and clear cell tumour compartments. This finding supports the hypothesis that a certain proportion of renal cell carcinomas with dual morphology are multifocal tumours and that histologically different tumours develop from a single malignant clone that proliferates and differentiates to give rise to tumours with heterogeneous phenotypic characteristics. In 66.7% of cases, discordant allelic losses were present alongside concordant allelic changes, indicating that cells of the original clones continued to accumulate genetic alterations and evolve into morphologically distinct clones. In 40% of cases with LOH, allelic losses were discordant between clear and papillary tumour compartments; in all of these cases LOH was present only in the clear cell renal cell carcinoma

compartment. This finding, although indicating oligoclonal development, does not necessarily exclude the possibility of a monoclonal origin. The presence of discordant LOH patterns can be attributed to on-going genetic alterations, possibly through clonal selection. In 28.6% of cases we failed to detect any genetic losses at markers used in the study.

In conclusion, the LOH analysis data suggest that both clear cell and papillary components of renal cell carcinomas with dual morphology, in the majority of cases, are derived from the same progenitor cell. The finding of unique genetic alterations in some of tumour compartments indicate that clonal divergence occurs during tumour progression.

## **2. Assessment of tumour suppressor gene loss of heterozygosity at *VHL* and *FHIT* loci for multilocular cystic renal cell carcinoma**

In 1928, Perlmann described a multilocular cystic renal tumour containing a small population of clear cells as a lymphangioma. In 1957, Robinson reported a second case and recognised the clear cells as epithelial. These appear to be the first descriptions of what today is called *multilocular cystic renal cell carcinoma*. Since that time, the clinical and histological features of more than 40 of these renal tumours have been published.

In practice this lesion is much more common than the reported figures would indicate as they constitute a significant proportion of referral practice of specialised urological pathologists.

The most frequently encountered form of malignant renal epithelial neoplasm is conventional (clear cell) renal cell carcinoma and it is a popular belief among pathologists that multilocular cystic renal cell carcinoma is a variety of this common form of malignancy. To date, however, the evidence for this is at best circumstantial. The most compelling observation to connect conventional (clear cell) renal cell carcinoma and multilocular cystic renal cell carcinoma is the existence of cells containing abundant amounts of glycogen in the cytoplasm of both tumours. Very recently the expression of the renal cell carcinoma associated protein, 250 (RCC, 250) was demonstrated in a significant proportion of tumours in a small series of multilocular cystic renal cell carcinoma suggesting a neoplastic origin for these lesions. It is, however, of interest that not one single case of multilocular cystic cell renal cell carcinoma has metastasised despite a follow-up period that in some cases extends beyond two decades.

Conventional (clear cell) renal cell carcinoma is characterised by genetic losses at 3p. Hereditary conventional (clear cell) renal cell carcinoma is associated with von Hippel-Lindau disease where the genetic defect has been shown to occur at 3p25-26. In addition to a tumour suppressor gene at 3p25, we also have shown that loss of heterozygosity at the *FHIT* gene locus (3p14.2) is an early event in conventional (clear cell) renal cell carcinogenesis.

In this study we investigated a suite of well characterised microsatellite markers which map to the *VHL* or *FHIT* gene region in a multilocular cystic renal cell carcinoma.

Utilising computer-assisted microdissection of unstained paraffin-embedded sections of multilocular cystic renal cell carcinoma, samples were obtained from 14 tumours for detailed genetic analysis.

Surprisingly, the analysis of 3p region in our panel of multilocular cystic renal cell carcinomas failed to detect any genomic deletions and microsatellite instabilities. This finding indicates that genetic alterations involved in pathogenesis of clear cell renal cell carcinoma are not typical features of multilocular cystic renal tumours and that other chromosomal regions and/or genetic mechanisms must be involved in pathogenesis of these tumours. This finding has great implications for the current classification of clear cell renal tumours.

## Project Grants 2006

The following projects were approved for funding in May 2006 and will be reported on in subsequent Annual Reports of the Foundation.

### David Ackerley

Victoria University of Wellington

#### **Development of high-throughput screening methods to detect enhanced activation of chemotherapeutic prodrugs by evolved bacterial nitroreductase enzymes**

The overriding aim of the proposed research is to develop an effective screen for detection of enhanced prodrug-reducing activity of bacterial oxidoreductase enzymes, which will enable the subsequent generation and identification of enhanced enzymes by directed evolution. To achieve this, four high-throughput screening methods will be developed and tested using *Escherichia coli* K12 strains that express different levels of prodrug activating enzymes, to identify the most appropriate screen. Reductive prodrugs such as CB 1954 kill cells through generation of extensive DNA damage, and one of the screening methods will examine autoinduced bacterial cell death by comparing the turbidity of replicate CB 1954 challenged and unchallenged cultures, grown in 96-well plates. The remaining three screens will employ novel *sfiA* promoter fusion strategies to compare levels of DNA damage between the different control strains. *sfiA* is an E.coli SOS-regulated gene whose expression is up-regulated by DNA damage, and it has previously been demonstrated that activation of CB 1954 by E. coli induces the SOS response in these cells. Development of an effective screen for detecting enhanced enzymatic activation of CB 1954 offers great promise for developing novel gene therapy approaches to treat a wide range of human cancers.

### Thomas Bäckström

Malaghan Institute of Medical Research

#### **Investigating the interaction between regulatory T cells and antigen presenting cells: lessons to learn for potential therapeutics of multiple sclerosis**

MS is a chronic inflammatory disease, in which the autoimmune response is directed against the myelin sheath that surrounds axons of the central nervous system. The disease usually starts between 20 to 40 years of age for humans and leads to substantial disability through deficits of motor and sensory nerve function. The socio-economic importance of MS is second only to trauma in young adults. EAE is the murine model for human MS, which is widely used to study the cause and potential treatments of MS. Though much effort has been made, the mechanism for the cause of MS is still poorly understood.

The study aims to investigate the cellular and molecular mechanisms of Tregs suppression, with the focus at the molecular interactions between Tregs and APCs during immune suppression. The findings of the study may provide

insights into the cause of EAE and MS and shed light on potential therapies that involve immune modulation of Tregs and MS and other autoimmune diseases.

### **Kylie Hood**

Wakefield Gastroenterology Unit

#### **Investigation of candidate metastasis suppressor proteins in colorectal cancer**

Colorectal cancer is the second most common cancer in New Zealand and around 2400 new cases are diagnosed in New Zealand each year. Detection or prevention of early liver metastasis is the most likely factor to influence CRC patient outcome since the spread of CRC to the liver is the major prognostic indicator for survival. TRAIL therapy is in preclinical trials for the treatment of liver metastases, despite the lack of knowledge regarding the TRAIL-sensitivity of non-experimentally-induced tumours.

Previous Wellington Medical Research Foundation research undertaken by the Wakefield Gastroenterology Research Institute has highlighted the TRAIL receptor gene products as being likely candidates to play a role in metastasis prevention. Recent preliminary data supports this hypothesis. The Current proposed research is required to validate the function of the TRAIL receptors and TRAIL and its clinico-pathological relevance to CRC metastasis.

### **Joanna Kirman**

Malaghan Institute of Medical Research

#### **Signalling Lipid Compounds as a Novel Therapy for Tuberculosis**

In New Zealand and abroad, the prevalence of drug resistant strains is increasing, compounding the Tb emergency. To this end, in New Zealand 18.1% of culture-positive Tb cases in 2004 were resistant to at least one antimicrobial agent. Strains resistant to both isoniazid and rifampicin are complicated, expensive and sometimes impossible to treat. Thus, there is a strong sense of urgency to develop novel approaches to treat Tb. If drugs developed can be used as adjunctive therapy to shorten treatment regimens, it would reduce treatment costs, medication and medical care. Shortened treatment courses would also lead to increased compliance that will help curb the development of drug-resistance. The proposed pilot study aims to evaluate a potential immunotherapeutic agent for pulmonary Tb.

### **Anne La Flamme**

Victoria University of Wellington

#### **Regulation of susceptibility to autoimmunity by interleukin-4 receptor $\alpha$ (IL-4R $\alpha$ )**

The work outlined in this project builds upon the research expertise of its senior investigators in Th2 response regulation, M $\Phi$  activation and function, generation and analysis of immune response induction, and the pathological consequence of inflammation and aims to reveal new layers of complexity in the induction and regulation of autoimmune inflammation. The proposed

research will investigate the pathway by which autoimmune regulation occurs and will concentrate on identifying the specific factors involved in this process. By identifying factors that can suppress disease progression, new therapeutic targets or strategies may be developed to inhibit the induction or reduce the severity of immune-mediated diseases like MS.

### **Ronan O'Toole**

Victoria University of Wellington

#### **Cell division as a source of new anti-tubercular drug targets**

Tuberculosis is the most serious bacterial disease affecting mankind causing 1.75 million deaths per year. The method of prevention, diagnosis and treatment are grossly outdated and this has hindered its control and eradication. The project proposes to address one of the most pressing needs in tuberculosis treatment, the identification of new classes of antibiotics which are effective against *M tuberculosis*.

### **Thorsten Stanley**

Wellington School of Medicine and Health Sciences

#### **Probiotics for the prevention of allergy – what is the mechanism of action?**

The Wellington Asthma Research Group is the lead centre in collaboration with the Dept of Paediatrics, University of Auckland in conducting a trial on the effect of probiotics on the development of atopy and eczema in children with a family history of allergic disease. This study, funded by the Health Research Council and Fonterra New Zealand is a randomized double-blind controlled trial.

The WMRF grant is for the additional measurement of atopy patch tests, which have recently been used to study the mechanisms underlying the development of eczema.

Atopy patch tests differ from skin prick tests in that they provide a way of assessing the cell-mediated inflammation that occurs in the skin in response to allergen. With a positive test the inflammatory infiltrate in the skin is similar to that seen in atopic eczema.

An understanding of how probiotics act to influence the development of allergic disease would strengthen the case for using probiotics in clinical practice. If we can influence the development of allergic disease by intervening with probiotics in early childhood and can prevent the development of asthma it will reduce the substantial burden of illness associated with asthma in childhood and in later life.

**P Stoitzner**

Malaghan Institute of Medical Research

**Epicutaneous immunisation as a new cancer immunotherapy?**

Dendritic cells are specialised antigen presenting cells that play a central role in induction of immunity and tolerance. They have the special ability to take up, process and present foreign antigens and thus are potent stimulators for naïve T cells. Stimulated T cells start to proliferate and acquire the capacity to produce cytokines that recruit other immune cell populations. In the presence of inflammation in the skin, skin dendritic cells migrate from the skin to the draining lymph nodes where they activate T cells. The resulting effector T cells migrate back to the inflamed site and help to eliminate infected cells.

There are two major populations of dendritic cells in skin, Langerhans cells, which are located in the outermost layer of skin, or epidermis, and dermal dendritic cells which are located in the dermis below the basement membrane. These two populations are distinguished by their location, and by the expression of a specific marker, Langerin, which is expressed in Langerhans cells but not in dermal dendritic cells.

Immunotherapy with tumour antigen-loaded dendritic cells is being evaluated in clinical trials worldwide. So far the results are promising but there still many unknown factors and difficulties that we have to investigate and overcome to improve the outcome of the immune responses induced. The Immunisation strategy through the skin is a very attractive approach since it is easy to perform and not as cost intensive as other immunotherapies. Thus, this strategy might be an interesting alternative to other immunotherapies, but we will have to further improve it in a more “patient-like” situation. Hopefully the knowledge we will acquire in this study will help us improve existing and new study designs and, in the end, lead to an improved efficacy and applicability of dendritic cell-based cancer vaccines.

**L Wong**

Wellington School of Medicine and Health Sciences

**Analysis of *in vitro* and *in vivo* dental plaque microbial populations by Checkerboard DNA:DNA hybridisation and the intraplaque physicochemical environment**

This grant will enable the purchase of a spectrophotometer to measure DNA, RNA, protein and mineral ions in microlitre volumes.

Dental caries is plaque-induced, and is still probably the most widespread human disease. It is a major but under-recognised NZ health problem. In children, the improvement seen for many years has stopped, and caries rates are increasing, eg up by 30% in Christchurch since 1998. Caries, and also periodontal disease, is concentrated in sub-populations groups. Poor oral health is prevalent especially for Maori, Pacific Island and low socioeconomic/socially deprived populations and in areas of no water fluoridation. Dental plaque is the key aetiological factor in dental caries and periodontitis.

It causes infection, pain and misery and can lead to a lifetime of health disadvantage, including enhanced risk of cardiovascular disease, diabetes and premature birth. To halt this decline in overall health it is necessary to prevent caries to improve lifetime oral health. This study aims to further our understanding of how plaque causes caries.

## **Travel Grants**

### **35th Australasian Society for Immunology**

J Qin

The 35th Australasian Society for Immunology conference was held in Melbourne, Australia on the 4-8th of December 2005. I am grateful for the financial support provided by the Wellington Medical Research Foundation to allow me to attend this conference. At the conference I was able to present a poster on the interactions between dendritic cells and T cells and how it influences dendritic cell function and survival.

The meeting of over 800 delegates from Australasia and around the world was the largest in the ASI history. The theme of the meeting was genetics and the immune system, which was incorporated into its comprehensive program of presentations featuring researchers of all facets of immunology. It was great to be able to meet and listen to many notable and experienced researchers. I was able to gain detailed understanding of the current research taking place in immunology, which was very helpful as a new researcher.

As part of the conference I was also able to attend the tumour immunology workshop that featured numerous researchers presenting their latest results. This workshop was very useful personally as this is my area of research. Of particular interest was a talk by Delia Nelson and her lab's work on  $\alpha$ CD40 in the treatment of tumours, which is related to my current research.

The conference was my first chance to present my work outside my institute to international researchers. My poster detailed the work in our lab investigating how to develop the potential of dendritic cells into better vaccines against tumours by examining various treatments in vitro such as activating the CD40 receptor and how that affects the dendritic cell's ability to protect against tumours in vivo. It was positive to be able to present and discuss the work with many different international researchers.

As a new researcher in the field of immunology the conference has to a great extent expanded my understanding of the many areas of current immunological research. It has also provided me with a clear view of the future of the field, and where I should focus my future research. I again thank the foundation for providing me with the opportunity to attend this conference.

### **Allergy, Allergic Inflammation and Asthma**

N van Panhuys

With the help of the WMRF, earlier this year I attended the Keystone symposia on Allergy, Allergic Inflammation and Asthma in Breckenridge, Colorado. This conference provided a great opportunity to broaden my knowledge on aspects of asthma which, I had not had the chance to hear talks on first hand in New Zealand. Several topics that, I feel are becoming extremely important emerging areas were discussed as part of the meeting; such as the role of functional genomics, the genetic determinants of

pharmacological responsiveness and talks on novel drugs and emerging therapies. It was also an excellent opportunity to meet some of the more distinguished scientists in the field of asthma/allergy research. Like Bob Coffman who along with Tim Mossman initially described the TH1/TH2 paradigm, Bill Paul a collaborator of ours, who opened the meeting with a brilliant address on cytokine control networks and the organisation of allergic inflammation and Anjana Rao who has characterised major epigenetic regulators involved in TH2 differentiation, as well as many others whose studies covered areas, which I have been closely following during my studies. By presenting my own work on the role of Interleukin 4 and STAT6 in TH2 immune responses and protective immunity at the conference and at several seminars I gave after, I got excellent feedback from members of the international community as to future directions, novel techniques and ideas, which will help me to further my research.

### **European Networks of Immunology Institutes (ENII) Summer School 2006**

L Goldsack

This year I attended the ENII summer school held on the 13<sup>th</sup>-20<sup>th</sup> of May in Sardinia, Italy. This was an extremely valuable experience as I had the rare opportunity to meet and interact with leading international immunologists. In addition I have made valuable connections with fellow students and post docs who will be important contacts in the future. As an outcome of the summer school I have gained a greater depth of knowledge in the field of immunology, which I believe will provide me with an excellent grounding to begin my PhD study.

The summer school was based on advanced immunology covering a broad range of topics. The lectures included the different cells of the immune system, the receptors and signalling events that are required for immunity, innate and adaptive responses and how the immune system interacts with invading pathogens. Lastly models of autoimmunity, inflammation and cancer were discussed. Klaus Rajewsky was the Keynote speaker; his talk covered the latest research in B cell development and the signals required in their differentiation, which was really interesting, as I have not had much opportunity to learn about B cells. It was very exciting to listen to talks given by prestigious scientists from the field of research that I am interested in, as they presented new developments and techniques in the area of Th1 differentiation and memory, and host interactions with *Mycobacterium tuberculosis*. This opportunity has allowed me to develop new research ideas, which I can apply to my PhD project.

The layout of the summer school was very efficient. Lectures were given in the morning and evenings, leaving the afternoon for interacting with other students and scientists. Each evening, tutorials were held by the speakers who had given lectures earlier in the day. This provided the perfect opportunity to talk to the scientist, ask questions and create discussion in a friendly environment. One of the most enjoyable and informative sessions of the course was the poster sessions. I was able to present a poster on my

PhD project "Which memory T cells protect against Tuberculosis: isolating memory T cell subsets". My work was well received and I was given valuable feedback which will be useful in future research. The poster sessions allowed me to develop skills and confidence required to discuss and debate research. However the whole week was not always about science. During a cultural afternoon we were fortunate enough to visit the Grotto of Neptune, a beautiful limestone marine cave that is carved into the cliff face off the coast of Capo Caccia. We were looked after extremely well with three course meals, three times a day from a delicious Sardinian cuisine.

The summer school was extremely beneficial. I encourage other PhD students from New Zealand to attend the European summer schools, as they provide a great opportunity to form networks with international scientists, meet the leading immunologists of their field and gain an understanding of how research is carried out overseas. I greatly appreciate having had the opportunity to attend this course and my research will benefit significantly from this experience.

### **International Congress of Parasitologists XI, Glasgow 2006**

M Harvie

I was fortunate this year to attend the International Congress of Parasitologists (ICOPA XI) from the 6<sup>th</sup> – 12<sup>th</sup> of August in Glasgow Scotland. It was an intense time of sharing and developing ideas in this field, and I found it to be a stimulating and interesting meeting. During the course of the meeting I had the opportunity to meet with some of the worlds leading parasitologists, making valuable contacts for the future.

The congress was a large event attracting over 2000 participants from around the world. Many diverse topics were covered with each day organised into themes including parasite-host interactions, genetics, proteomics and development of parasite vaccines. There was a lot of information on offer with a total of thirteen concurrent sessions available. Of particular interest for my research was the helminth immunomodulation series that covered the immune responses generated by helminth worms and how they are able to manipulate our immune system. These talks covered interactions of parasites and their secreted antigens with various facets of the immune system including dendritic cells and T cells which are of particular interest to my research. I found the talks on secreted antigenic products to be particularly helpful and have returned with many new ideas and renewed enthusiasm for this area of study.

The schedule of the ICOPA meeting was comprehensive with talks throughout the day and opportunities in the evening to meet with and interact with other scientists. Poster sessions were held on both the Tuesday and Thursday night, these sessions were a good opportunity to see what other researchers are focusing on and also to discuss thoughts and ideas with them in a less formal environment. I was able to present a poster of my own research "Comparing gut vs. lung priming for Th2 protective immunity in *Nippostrongylus brasiliensis*" at the Thursday poster session. My poster

generated significant interest and it was a valuable opportunity for me to showcase my work and also to receive feedback and establish connections with other scientists with similar research interests. I really enjoyed this opportunity to meet and interact with highly regarded scientists in my area of research. I feel that this conference has allowed me to make valuable contacts and also given me insight into new ideas and methods which may be beneficial to my research.

Overall I have found the ICOPA XI meeting to be an enriching experience which has enabled me not only to meet and learn from experts in my field but also to put my own work forward on the international stage, gathering valuable input and developing new research ideas. I greatly appreciate having had the opportunity to attend the congress and feel this valuable experience has contributed to my development as an informed and involved research scientist.

### **Respiratory Syncytial Virus Symposium in Oxford, United Kingdom**

F Rich

In September 2005 I travelled to Oxford, UK to attend the Respiratory Syncytial Virus (RSV) Symposium with the aid of a travel grant from the Wellington Medical Research Foundation. As the only research group studying RSV in New Zealand attending this meeting was very beneficial for the alignment of our work with the international field of RSV research.

RSV is the single most important cause of lower respiratory tract infections in infants worldwide with nearly all infants experiencing an RSV infection by the age of two. Significant disease is also increasingly recognised in the elderly. RSV infection accounts for up to 90% of bronchiolitis and up to 70% of viral pneumonia cases.

In New Zealand the bronchiolitis hospitalisation rate of 6% is double that of other industrialised countries. The greatest health burden in New Zealand is found amongst infants of Pacific Island and Maori ethnicity with rates of 103/1000 and 83/1000, respectively.

The RSV Symposium is a multidisciplinary conference and brings together investigators from all aspects of RSV research. Session topics included basic viral biology, immunology, epidemiology and impact, RSV in older persons, clinical aspects, host genetics and animal models as well as a session attempting to address the current grand challenges and reviewing the latest therapies. Also included were three debates on topical issues of RSV research that encouraged lively discussion. Interestingly, clinical therapy research is quite advanced with some well-established products available and several others in advanced stages of development, while research at the basic viral biological and immunological level is still in an early stage of discovery. The down stream benefit of advancing basic research is the fuelling of the development of prophylactic, antiviral and vaccine therapies and this was particularly exciting to see.

My abstract, titled '*Distinct patterns in the molecular evolution of RSV isolates from New Zealand over a 37 year period*', was selected for oral presentation and this gave me the opportunity to discuss our work with international researchers. In this first study of the RSV strains and subgroups found in New Zealand, we have identified viruses here to be part of the globally circulating pool and thereby eliminated viral factors as a likely cause of the high bronchiolitis hospitalisation rates. In addition we identified for the first time a significant difference in the evolutionary rates of the two RSV subgroups, RSV A and B, where RSV B is evolving at a faster rate. This finding held true for international strains of RSV not just those found within New Zealand. Many investigators were interested in the very high bronchiolitis rates seen in Pacific Island and Maori infants while others were interested in the carefully chosen phylogenetic methods we employed on our unique data set.

Many of the leading researchers gave oral presentations. Research highlights included the developments in attenuated mutants, which help explain viral genetic regulation and immune modulatory activities of RSV complementary to their use as vaccine candidates. The most promising immunology research used an antagonist to the late costimulatory molecule OX40 ligand and had success in a murine model at reducing pathology and viral loads even when given after the development of clinical symptoms. This was significant as effective treatment strategies after the development of symptoms are infrequently found for RSV.

I am very grateful to the Wellington Medical Research Foundation for their support to attend this conference. It has been an invaluable experience and I am looking forward to the using contributing the information gained in the completion of our current projects investigating RSV in New Zealand.

## Summer Student Research Reports



### **An assessment of the effects of adult literacy on health care outcomes**

Sasmira Bhatt

Low levels of adult literacy affects awareness and comprehension of personal medical issues. This study was carried out in an attempt to assess the relationship between health literacy and health outcomes including length of stay in hospital and self-reported understanding of medical illness and medication. We built upon the established association between poor reading and poor literacy. Undetected problems with patient literacy may interfere with all aspects of health care, including preventative care, screening programs, compliance with medical regimens, and participation in clinical research.

The total number of inpatients included in the study was 77 and the ages ranged from 19 to 94 with a mean age of 64. Forty-seven percent were women; 58% NZ European/Pakeha, 21% European (Other/Not Defined), 9% NZ Maori and the remainder composed of Cook Islands/Maori, Samoan, Indian, Chinese, South East Asian and Latin American/Hispanic.

A 6 week evaluation of the literacy levels of patients involved the use of Rapid Estimate of Adult Literacy in Medicine (REALM), an internationally accepted screening instrument to detect patients with low reading skills. A questionnaire was also supplied to the patients. It was composed of questions that probed into their understanding of their medical problems, medication as well as their compliance with medication. Depending on what raw REALM reading test score the patients achieved, they were placed in one of four grades [Grade I to Grade IV]. Each of the four grades was then examined for their average length of stay in the hospital as inpatients as well as the most commonly reported destination of discharge and ethnicity. All four grades were also compared with each other with the focus on the patients' answers to the questionnaire.

We established that poorer reading scores reflecting lower literacy levels were associated with longer hospital stays. Almost all patients in the study cohort demonstrated a disciplined medication routine. Regardless of the literacy level, the patients reported to adhere religiously to medication routines. Low scoring patients also reported a poorer understanding of their medical problems and medication than the high scoring patients.

Our literacy study has made way for potential of an amplified, more intense study looking into the link between poor health outcomes and low literacy. The implications of establishing one are widespread. Further studies aimed at

the research questions and hypotheses proposed in this paper are likely to produce significant policy implications at a local level. Vigorous interventions to improve patient health literacy and following them up over a period of time in order to detect an effect, if any, upon their health outcomes may prove beneficial for future intervention planning and application. Although it may appear to be a realistically unreachable standard, a scenario of uniform literacy amongst patients about their health problems could hold promises of astronomical heights. It would not only simplify matters for healthcare providers and the patients but may also aid the minimisation of health provision costs and enhancement of health care access.



### **Learning clinical skills - the medical student as the principal stakeholder**

Alistair Escott

International trends in curriculum change at medical schools around the world have involved the development of clinical skills teaching due to the changing nature of medical practice. The lack of information about medical students' perception towards clinical skills teaching before any curriculum change, triggered the Faculty of Medicine at the University of Otago to commission this project to inform any potential developments in the teaching of clinical skills at the Faculty. The project aimed to seek the medical student opinion towards current clinical skills teaching in terms of opportunities, teaching methods, patients and assessment. The project involved sending a questionnaire to all 2005 University of Otago medical students. There were 256 responses from 1028 students and four follow up focus groups were conducted. Information gathered from the focus informed the conclusions from the questionnaire results. There was a significant difference between preclinical and clinical students in both the opportunities to watch and perform clinical skills. Preclinical and clinical students felt that they are not exposed to enough clinical skills in second and third year. Clinical students were concerned at the variability that exists in the attachment to clinical teams, as well as the objectives, assessment and feedback of clinical skills learning. The project recommended that the Faculty Curriculum Committee continue to promote the development of clinical skills teaching across the Faculty of Medicine and heed student concern about preclinical exposure, run attachment assessment and feedback.



### **Clinical indicators of community-acquired lobar pneumonia in 309 hospitalised children and infants**

Chloe Heyhoe

Lobar pneumonia is a life-threatening infection of the lung, most commonly caused by *Streptococcus pneumoniae*. Approximately 1 million children less than 5 years of age die from pneumococcal pneumonia each year in developing countries. A definitive diagnosis of pneumococcal pneumonia can only be made if *Streptococcus pneumoniae* is found in the blood, however, studies have shown that the bacteria is identified in only 1-3% of cases. As a result, we must rely on the early identification of the clinical signs and symptoms for the appropriate management of children. The World Health Organization has suggested rapid breathing is the most reliable sign of pneumonia in children but this work has been done in developing countries where children may be much sicker before they come to medical attention.

The medical records of 309 children hospitalised with community acquired lobar pneumonia confirmed by x-ray were reviewed for presenting symptoms and signs.

This study showed that pneumococcal pneumonia was characterised by high fever ( $>38.5^{\circ}\text{C}$  in 64%), cough (in 84%) and lethargy (in 91%). Fast breathing and chest indrawing (inward movement of the lower chest wall - a sign of increased work of breathing), the most common clinical signs of pneumonia in developing countries, were only found in 53% and 25% of patients respectively. Of the 309 patients, 105 children or 34% would have been missed if fast breathing had been the only criteria for diagnosis. The most common clinical signs detected were fast heart rate and crackles over the area of pneumonia.

It was concluded that children with pneumococcal pneumonia present quite differently in developed countries like New Zealand, making diagnosis more difficult. A chest x-ray should be considered in any sick febrile child, even where no clinical symptoms or signs of pneumonia are present.



### **Review of endovascular stent metals, design and manufacturing methods**

Hamish S McLaren

Endovascular stents are mesh tubes inserted inside blood vessels which have collapsed or have become blocked by atherosclerosis. They are expanded and hold the vessel open. Stainless steel was the original metal used to construct stents, chosen for its 'good' biocompatibility and strength, it is still used for manufacturing stents today. However, surgeons are attempting to open smaller and more tortuous vessels, with this requiring smaller, more flexible stents, without compromising strength. Nitinol and cobalt-chromium alloys have filled this desire. Nitinol is superelastic and can weave through curvy vessels and cobalt-chromium is extremely strong, allowing for smaller stents. Also added into the mix is that of the stent geometry. The shape and thickness of the stent and the mesh is an important factor in determining the success of a stent. Thin and fine mesh helps improve performance. Whether the stent is balloon expanded or self expanding is important. Balloon inflatable are usually stiffer, whereas self expanding stents are more flexible and conform to the vessel architecture. The manufacturing of stents is becoming more intricate. Methods such as electro discharge machining using high voltage carrying electrical wire to cut the stent design and photochemical etching which uses photosensitive chemicals to cut the stent metal were used in the past. Now the more recent water-jet guided laser is employed. The laser can cut finer and cleaner than the other two methods, reducing cutting time and production costs as well as making the stent more biocompatible.



### **Clinical features of Wellington subjects enrolled in the New Zealand multicentre genetics of rheumatoid arthritis study**

Janine Pilcher

A number of projects are underway to untangle the web of genetic and environmental interactions that cause rheumatoid arthritis. The University of Otago's "Multicentre Genetics of Rheumatoid Arthritis Study" aims to examine the relationship of three genes (HLA-DRG1, PTPN22 and RANK) with the various clinical characteristics that can present with this disease. So far, patients from Dunedin and Wellington have been recruited for the project.

This study collected and analysed the clinical characteristics of patients from the Wellington cohort. Surprisingly, much of the clinical data gained was significantly different to that from the Dunedin cohort. Possible explanations include differences in data recording, population genetics and environmental exposures at the two centres. A potentially important link between prescribing pattern and disease outcome was noted through the observation that the Dunedin cohort had greater disease severity (indicated by increased rheumatoid nodules and bone erosions), and less disease modifying anti-rheumatic drug prescriptions.

Despite the fact that a number of factors are thought to work together to influence rheumatoid arthritis, many studies single out an individual factor and analyse it independently of the others. In response, a literature review was performed which found that acknowledgement of interactions between phenotype, genotype and environmental exposure, has great potential to influence a study's results and conclusions.



### **Atopic dermatitis - what happens after the allergy clinic?**

Linda Zhou

The aim of this study was to assess outcomes of children with eczema after undergoing allergy testing. We wanted to investigate the amount of information that parents recalled and whether this information has changed the child's management and improved their life.

100 patients who had attended the Paediatric Allergy Clinic were selected on the basis of the tests they had undergone. 81 interviews were completed to evaluate patient outcomes.

We found that all parents remembered having their children tested and whether any allergies were identified, recalling an average of 3 allergens. 88% of the parents reported allergies to house dustmite, 48% to eggs and 46% to peanuts. 88% of the parents changed the way they managed their child's eczema as a result and 64% felt these changes were beneficial. We also found improvement in the quality of life for both the children (including severity, sleep and behaviour) and their families (including fatigue and emotional distress). Over half of our patients have also used alternative medicines.

Our study found that although allergy testing and avoidance is not the perfect solution to all childhood eczema, it was beneficial for the majority of patients and has improved their and their families' lives.

