



*Wellington  
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Foundation*

Research Report 2009

**WELLINGTON MEDICAL  
RESEARCH FOUNDATION**

**RESEARCH REVIEW**

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# WELLINGTON MEDICAL RESEARCH FOUNDATION RESEARCH REVIEW 2009

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## Editorial

The past twelve months has seen a marked increase in demand for the research funds made available by the Foundation.

During the last calendar year the Research Advisory Committee has held two grant rounds. For the round in October 2008 thirteen applications for research funding were received and the number of applications grew to eighteen for the April 2009 round. Such has been the volume of applications received by the Foundation that the Research Advisory Committee experienced difficulty in assessing these in the time available. For this reason the Committee has established a special subcommittee to triage applications, with the aim of selecting the leading fifteen in each round that will go forward to the full committee for assessment.

This dramatic increase in demand for funding from the Foundation is in line with the experience of previous years and is in part due to the a further erosion of funds for basic research that have been made available through the Health Research Council.

In past years the Health Research Council implemented a scoring process that gave precedence to studies that fulfilled the Council's "investment strategy". In this nine strategic funding areas were defined, which had the effect of focusing applications away from the basic sciences. The Health Research Council has continued this policy for the 2009-2010 funding round and for the coming year \$68.5M will be made available through the contestable fund, with \$33M of this being allocated for project contracts. This total amount, however, will not be made available for funding of projects assessed purely on the basis of research excellence, as \$25M will be deducted from this over the next three years as funding for specific research activities.

This year the Council has developed what they have termed, a targeted health funding strategy. Central to this, the Council will identify an annual targeted stream or research focus that they determine will produce "significant health as well as productivity and economic gains for New Zealand".

For the 2009-2010 research round it has been decided that the targeted funding stream will focus upon research related to New Zealand health delivery. In the investment signal that detailed this funding strategy, the Council noted that the targeted stream will allow it to be responsive to health sector challenges. The Council further anticipates that the research will "strengthen the evidence base for decision making by health practitioners, managers, policy-makers and consumers to improve health sector experiences and ultimately health outcomes for all New Zealanders".

Specifically excluded from this funding is research that focuses upon basic science or discovery, and infrastructural support for research units or centres.

The overall effect of this is that there is further erosion of funds available for investigative research that falls outside the priority determinants specified by the Council.

For the 2007 funding round 284 applications were received by the Health Research Council and this increased to 302 for last year's round. Final figures are not available, but it is anticipated that this will be substantially increased for the 2009 round. The Council has stated that "at least" 25 new projects will be supported for the coming year; however, there is a limit of \$1.2M per project, with the permissible annual budget being capped at only \$0.4M. When overhead costs are deducted from this, this figure represents a significant decrease in funding available nationally.

It is of concern that the Health Research Council has chosen to implement this policy at a time when sponsorship money is particularly scarce. This is the more so, when it is considered that much of the work relating to improving health service delivery would be more appropriately funded by the Health Ministry.

The Wellington Medical Research Foundation remains committed to supporting medical research with funding priorities determined solely by the excellence of the proposed work.

Professor Brett Delahunt  
Editor

## **Research Advisory Committee Membership**

Professor Brett Delahunt (Chair)  
Dr David Ackerley  
Professor Carl D Burgess  
Associate Professor Duncan C Galletly  
Dr T William Jordan  
Dr Jeremy D Krebs  
Professor Graham Le Gros  
Professor John H Miller  
Dr David Slaney

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

### Capital and Coast District Health Board

#### **Assessing insulin sensitivity and glucose excursions in patients with type 2 diabetes in response to altered macronutrient composition in the diet**

D Bell and J Krebs  
Endocrinology

We are undertaking this research as a sub study of a multicentre HRC funded trial, the DEWL study. Wellington is the major centre coordinating this trial, there were 188 participants recruited in Wellington, and 419 recruited in total.

#### **Specific objectives:**

- To assess whether a high-protein low-carbohydrate diet is more effective than a low-fat high-carbohydrate diet in improving insulin sensitivity in patients with type 2 diabetes over 1 year.
- To compare the glucose excursions in patients with type 2 diabetes on the different diets via the 7hr continuous glucose monitor.

We have currently performed continuous glucose monitoring (CGMS) and oral glucose tolerance testing on;

- Baseline 30 people
- Six months 18 people
  - 11 people withdrew from the study
  - One remained in the study, but did not have the six month tests
- 12 months 12 people
- 11 people had all both measurements, at all three time points.

We had aimed to recruit 50 participants. From our power calculations to detect a 10% difference in insulin sensitivity would require 25 people in each arm of the study to meet the 80% power and  $P < 0.05$ . Primarily because of the additional time commitment for participants we were unable to recruit the 50 people for the study from the 188 people already enrolled in the dietary intervention. In addition there has been a large rate of withdrawal from the study.

With the number of people we were able to perform CGMS and glucose tolerance testing on, it may be that our study will be under powered to identify a possible difference between the arms of the dietary intervention. The available results will still provide information on insulin sensitivity and glycaemic excursions within these groups. The dietary intervention the people were following is unknown at present as we cannot unblind the DEWL

study at this point. The DEWL study is due to be completed in January 2010 and analysis of this sub study will then be possible.

The American Diabetes Association is currently using an equation to convert HbA1c to provide an estimated Average Blood Glucose (eAG). This was defined in a study using both CGMS and finger prick glucose measurements in 600 people. Our study will be well placed to comment on their version of the average blood glucose estimation in a group of people undergoing a dietary intervention, to see the effect of diet on HbA1c derived eAG.

The availability of CGMS and the cost reductions of the sensors are making CGMS more accessible for the routine management of patients, but this is generally still reserved for those people treated with insulin. We will be able to provide insight into its use with non-insulin treated type 2 diabetes patients.

The study involves both an oral glucose tolerance test and the CGMS, thus the combination of these may still provide a statistically significant measure of difference in glucose handling of the two dietary interventions.

### **Does the time of day affect the outcome of the Dix-Hallpike manoeuvre when testing for Benign Paroxysmal Positional Vertigo (BPPV)?**

A Burston

Benign Paroxysmal Positional Vertigo (BPPV) is the most common cause of vertigo with incidence increasing with age. Spontaneous remission may occur over weeks to months, with a group whose symptoms last for longer than one year if left untreated. Symptoms can be very debilitating and have serious social consequences. There is very strong evidence supporting the use of repositioning manoeuvres as the most effective treatment. The gold standard diagnostic test is the Dix-Hallpike manoeuvre. However false negatives have been reported which may lead to misdiagnosis.

**Purpose:** The aim of this study was to establish if there is a variation in the results of the Dix-Hallpike manoeuvre if it is performed at different times of the day.

**Methods:** A randomised cross over trial was undertaken with 50 participants referred to the Kapiti Health Centre for Physiotherapy for BPPV or vertigo. Data collection began in July 2007 and was completed at the end of August 2008. Participants were randomised into two groups. Both groups had the Dix-Hallpike manoeuvre performed on two consecutive days. Group One had the tests performed in the afternoon on the first day, repeated the following morning. Group Two had the tests performed in the morning followed by the afternoon the following day. The manoeuvres were videoed using infrared goggles, with a second camera to record the manoeuvre. Both views were recorded digitally onto a laptop computer using a picture within a picture programme. To reduce the chance of bias from misreporting by a single researcher, a second assessor, who was blinded to the participant's details, the time of day, and the findings of the researcher performing the tests, also

viewed the digital recordings and rated each Dix-Hallpike manoeuvre as positive or negative. To reduce the chance of bias from the possibility of the afternoon tests being performed more slowly than the morning tests, the time taken to perform each test was analysed and compared.

**Results:** Fifty participants were recruited with all of them completing the two days of testing and nobody pulling out.

There were 27/50 participants with positive Dix-Hallpike manoeuvres on at least one of the days. A positive Dix-Hallpike manoeuvre was one that produced both vertigo and appropriate nystagmus. There were 6/27 with discordant results (participants with a positive test on one day and negative the other day). On analysis of the discordant pairs, the difference in marginal proportions was 0% (95% CI -9.6 to 9.6) and McNemar's chi-squared test had a test statistic value of zero,  $P=1.0$ . There was no difference between the numbers testing negative in the morning and positive in the afternoon, to those testing negative in the afternoon and positive in the morning. Therefore time of day does not appear to be a factor in false negatives with Dix-Hallpike manoeuvres. However 22% of those with a positive Dix-Hallpike test, tested negative on one of the days, with half of these (11%) testing negative on the first day of testing and half (11%) on the second. These figures are very similar to results reported in previous research.

A Kappa statistic for agreement between the Dix-Hallpike manoeuvres on the two occasions had a value of 0.76 (95% CI 0.58 to 0.94). This result shows that the Dix-Hallpike manoeuvre is a very good diagnostic test for BPPV with high levels of agreement between tests performed at different times.

A Kappa statistic for agreement between the researcher and blinded assessor had a value of 0.92 (95% CI 0.87 to 0.98) indicating an excellent level of agreement.

**Conclusions and Recommendations:** The Dix-Hallpike manoeuvre remains the most reliable test for diagnosing BPPV with high levels of agreement between tests performed at different times. However approximately 11% of patients may test negative initially. Because the treatments for BPPV are so effective and some patients who do not go into spontaneous remission within the first 1-2 weeks can become quite debilitated with serious social consequences, it is important to have a correct diagnosis and opportunity for treatment. These results support the clinical practice of retesting patients on more than one day if they have a history suggestive of BPPV, are still symptomatic, but a negative Dix-Hallpike test initially.

## Institute of Environmental Science and Research (ESR)

### Mucosal Immunity: Implications for Meningococcal Carriage and Spread

JK MacKichan  
Communicable Disease Group

**Project Overview:** *Neisseria meningitidis* (meningococcus) is one of the leading causes of infectious disease worldwide, resulting in meningitis or severe sepsis (meningococemia). Even with prompt antibiotic therapy, the fatality rate is 10-20%, with a further 12-19% suffering long term effects, including neuronal deficits, loss of hearing, or limb amputation. The natural habitat of meningococcus is the human throat, where it rarely causes disease or symptoms. About 8-25% of the general population carry meningococcus asymptotically. Occasionally, highly virulent (eg disease-associated) strains can emerge and cause widespread outbreaks or epidemics, as occurred in New Zealand beginning in 1991, in contrast with strains primarily associated with carriage or sporadic disease (eg carriage-associated). Genetic differences between strains likely underpin observed differences in behaviour, but have yet to be identified and remain a significant unknown. Invasive disease occurs when meningococcus in the throat gains access to deeper host tissues, including the bloodstream. The means by which this process occurs are unclear. Factors that damage the throat epithelium, including smoking, inhaled dust, and respiratory illnesses, increase the risk of acquiring invasive meningococcal disease. This suggests that wounded tissue may be one possible portal of entry for meningococci to gain access to the bloodstream.

The lack of animal models for carriage and invasive disease has hampered study in this area, and *in vitro* assays have to date provided limited insights. Meningococcus lacks many of the canonical virulence factors (eg secretion systems or toxins), and nearly all of the “virulence factors” identified to date (eg type IV pili) enable meningococcus to colonise its host, but typically are equally present in disease- and carriage-associated strains. Using the extensive collection of meningococcal strains at ESR, the genetic and phenotypic differences between carriage- and disease-associated strains were examined.

**Summary of Experimental Findings:** To further explore the interaction between meningococci and wounded tissue, an *in vitro* experimental model was developed, measuring the rate of wound repair in tissue culture cells in the presence of meningococci. It was noted that disease-associated meningococci inhibited the wound repair process, while carriage-associated strains did not. This suggests that virulent meningococci have the capacity to manipulate host cell processes. Occasionally, a strain classified as disease-associated (such as the New Zealand epidemic strain isolate) is found in the throat of an asymptomatic carrier. One isolate indistinguishable from the NZ epidemic strain type was recovered from the throat of a carrier, also a household contact of a disease case. This isolate inhibited wound repair, similar to the disease-associated isolates recovered from clinical cases,

suggesting that the ability to inhibit wound repair is not dependent on the site the isolate was collected from (eg throat, cerebrospinal fluid).

Meningococci are known to have many genes that undergo phase variation, a process by which genes can be reversibly switched on or off in expression. Many of these genes are involved in virulence. These genes can be turned “on” or “off” by point mutations or the acquisition or loss of base pairs, frequently through slipped strand mispairing in repeat regions. Many phase variable virulence genes would be expected to have reduced expression during laboratory passage, as they do not confer any benefit outside the host. One representative disease-associated isolate was extensively subcultured on laboratory media over a period of months, with variants frozen down at one and three months. It was found that the high-passage variant lost the ability to inhibit wound repair, compared to the low passage clinical isolate it was derived from, after only one month of continuous passage. These two variants were used to identify candidate genes responsible for wound repair inhibition. Global gene expression was compared between the two variants, using a serogroup B *N. meningitidis* microarray. Only a handful of genes differed between the two variants, and candidate genes are currently being tested. These genes are being deleted by allelic replacement, with the resulting mutants being tested in the wound repair assay.

**Conclusions:** In New Zealand, meningococcal disease remains a significant health burden, indicating a need for improved treatment strategies and a universal vaccine. By using cell-based assays for evaluating the virulence properties of meningococci, novel virulence factors may be identified and characterised. This approach will provide an opportunity to shed light on how some meningococci cause such devastating disease.

## Malaghan Institute of Medical Research

### Adoptive T cell transfer therapy of cancer

R Perret and F Ronchese

Adoptive cell transfer (ACT) is a method of cancer immunotherapy that involves isolating tumour-specific CD8<sup>+</sup> T cells from a patient's blood or tumour tissue, activating and expanding these cells *in vitro*, and infusing them back into the patient. In some clinical trials, ACT in combination with lymphodepletion and the administration of exogenous IL-2 has led to the development of objective responses in up to 50 % of the patients with advanced metastatic melanoma, while other similar trials have produced little or no protective effect. The T cell characteristics that are at the basis of these differences are only partly understood.

In this study we have used a mouse model of anti-tumour immune response to examine the specific elements required for the generation of tumour-specific T cells that are capable of rejecting tumour challenge *in vivo*. The conditions for the generation of tumour-specific CD8<sup>+</sup> T cells involved culturing naïve CD8<sup>+</sup> T cells with dendritic cells (DC) loaded with antigen for 4 days, and then further expanding these T cells by culture in IL-2-containing medium for a further 3 days. The CD8<sup>+</sup> T cells thus generated were then transferred into mice and their ability to survive and reject tumours, either immediately or after 4-weeks, were examined.

In initial experiments we examined the importance of the activation status of the DC used to stimulate the CD8<sup>+</sup> T cell cultures. We observed that DC that had been activated by exposure to products of infectious agents were able to stimulate CD8<sup>+</sup> T cells that were very effective at attacking tumours, both immediately and after 30 days "rest", while CD8<sup>+</sup> T cells cultured with untreated DC were significantly less effective (Figure 1). Surprisingly, this substantial difference in anti-tumour activity between the two CD8<sup>+</sup> T cell populations could not be correlated to differences in surface phenotype or cytokine secretion *in vitro*, nor to differential survival or cytotoxic activity after transfer *in vivo*. Further studies, carried out in collaboration with Drs Bill Jordan, Pisana Rawson and Lifeng Peng at the Centre for Biodiscovery at Victoria University in Wellington, are now attempting to identify relevant differences between the two CD8<sup>+</sup> T cell populations by using two-dimensional fluorescence difference gel electrophoresis. Initial experiments have identified a small number of differences between the two CD8<sup>+</sup> T cell populations, these will be confirmed and characterized with the support of further funding from Cancer Society and Health Research Council of NZ.

Additional studies have examined the susceptibility of *in vitro* activated CD8<sup>+</sup> T cells to Treg mediated suppression *in vivo*. Our previous studies had shown that anti-tumour immunity elicited by DC vaccination can in some cases be substantially enhanced by Treg depletion *in vivo* (Prasad et al, J Immunol 2005). In contrast to those findings, anti-tumour immunity induced by ACT was not affected by Treg depletion, suggesting that Treg mediated

suppression of anti-tumour immunity likely acts by preventing the initial activation of tumour-specific CD8 T cells, rather than by inhibiting the effector function of already activated CD8+ T cells.

By determining the importance of the relationship between the antigen presenting cells and T cells during activation, and investigating the in vivo requirements for survival and anti-tumour function, this research might be able to further improve the success rate of ACT and guide the development of effective immunotherapies for cancer.

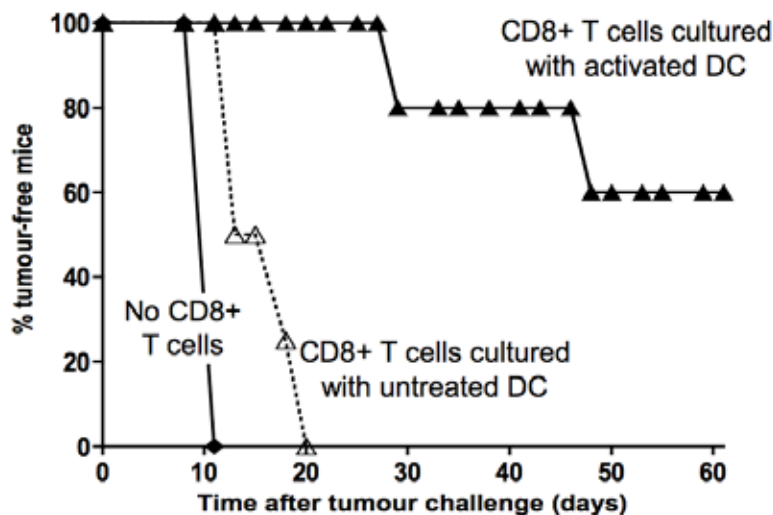


Figure 1: Tumour appearance in mice treated with different populations of CD8+ T cells. Mice were left untreated or injected with  $1 \times 10^6$  in vitro activated CD8+ T cells generated using different conditions as described in the text, and challenged with tumours. Tumour appearance was recorded over 60 days.

### Completion of a 3-year multi-centre rotavirus strain surveillance study in NZ

JR Kirman

We would like to thank the Foundation for this grant-in-aid, which enabled us to complete our strain typing for the final collection year of a 3-year study. From May 2007 to June 2008, we received 681 rotavirus positive samples that fulfilled our inclusion criteria, from 8 collection centres throughout New Zealand: Auckland Hospital, Waikato Hospital, Wellington Hospital, Aotea Pathology, Hutt Hospital, Medlab South, Christchurch Hospital and Dunedin Hospital. Stratified random selection of these samples generated a sub-group of 385 samples to undergo strain typing by semi-nested multiplex RT-PCR or sequencing.

Rotavirus has 11 individual RNA segments that can be easily exchanged during a co-infection, leading to the frequent emergence of novel strains. Of particular importance are the genes that encode Viral Protein 4 (VP4) and

Viral Protein 7 (VP7), the two viral surface proteins that are recognised by host antibodies. Genotyping the gene for the glycoprotein VP7 designates the viral G-type. Rotavirus surveillance studies predominantly evaluate G-types circulating in a particular region to provide the key information needed for the implementation of a vaccine program.

We found that most samples (nearly 75%) were G1, and the second most common strain was G2 (almost 20%), with G3, G4 and G9 making up the remaining samples. Two samples were identified as the uncommon G12 genotype by sequencing.

There was a geographical trend amongst the G2 samples, with over 80% of those identified as G2 coming from South Island samples. Amongst the Canterbury samples typed, G2 accounted for over half of rotavirus cases, contrasting with the other collection centres where G1 was the predominant genotype.

It is important to have knowledge of rotavirus strain prevalence, and any yearly changes in strain prevalence, in NZ before any vaccines are introduced into the nationwide vaccine schedule. This will ensure the successful implementation of a vaccine programme to reduce rotavirus-associated hospitalisations and illness in New Zealand.

### **Development and testing of novel DNA and protein "dormancy" vaccines against *Mycobacterium tuberculosis***

J Kirman

#### **Project Objectives:**

- To develop and test prototype vaccines against *Mycobacterium tuberculosis*, targeting mycobacterial antigens expressed by dormant tubercle bacilli.
- To initiate a collaborative link between the Infectious Diseases Group at the Malaghan Institute of Medical Research in Wellington and the Structural Biology Group at the School of Biological Sciences, University of Auckland.

**Research Summary:** Four plasmid DNA vaccines encoding each of four mycobacterial antigens expressed by latent tuberculo bacilli (Rv1738, Rv2624c, Rv2625 and Rv2626c) were developed using the plasmid pcDNA-DEST40 (Invitrogen, CA, USA). Large amounts of plasmid DNA were grown in *Escherichia coli* using endotoxin-free MegaPrep kits (Qiagen, CA, USA) for testing *in vivo*. Plasmids were tested by PCR to ensure they encoded the correct Tb gene. Recombinant proteins were made to use as antigenic stimuli in *in vitro* assays. Groups of male 6-8 week old C57B1/6 mice were immunised with 100µg of one plasmid DNA vaccine, as well as groups of mice receiving saline, empty plasmid vector or the current Tb vaccine, BCG, as a positive control.

Mice receiving DNA were injected 3 times subcutaneously, at 3-weekly intervals. Two weeks after the third immunisation, mice were culled and the

inguinal lymph nodes (draining the vaccination site) and spleens were removed, and lymphocytes from these tissues examined for immune responses induced by the DNA vaccination.

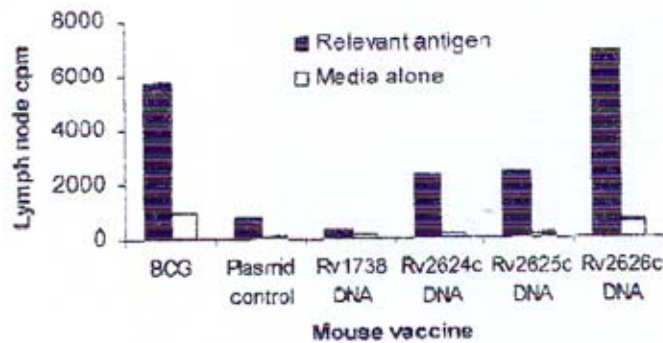


Figure 1: Lymphocyte proliferation in response to recombinant protein antigen stimulation was measured by [<sup>3</sup>H]-thymidine incorporation.

The DNA vaccine pcDNA-2626c was found to be the most immunogenic of those tested, although pcDNA-2624c and Rv2625c also induced proliferative responses and low levels of IFN-gamma production (measured by ELI Spot, data not shown).

Groups of vaccinated mice have also been challenged, by aerosol, with *Mycobacterium tuberculosis*, and three months after challenge will be culled to examine whether any of the DNA vaccines have afforded protection against the virulent challenge, by measuring bacterial burden in the lungs, and by examining immunopathology.

This study has formed a solid collaborative link between the Infectious Diseases Group at the Malaghan Institute of Medical Research in Wellington and the Structural Biology Group at the School of Biological Sciences, University of Auckland. We have since applied for joint funding to continue and expand this line of research.

### **Development of an immunophenotyping panel for multi-parametric flow cytometry for the characterization of CD4<sup>+</sup> T cell subsets that mediate protection against Tuberculosis**

JR Kirman

We would like to thank the Foundation for this grant-in-aid, which enabled us to initiate a study to identify a phenotype and the function of CD4<sup>+</sup> T cells primed by a BCG vaccination that mediate protection against *Mycobacterium tuberculosis*.

We have purchased all antibodies required for our panel, and undertaken the initial, critical steps to titrate the Ag85A class II tetramer, by generating an in vitro culture of antigen-specific CD4<sup>+</sup> T cells. We then determined the optimal time, temperature and concentration of antibody for staining the cells. Shortly we will be assessing the antibody panel together with the tetramer to

develop an immunophenotyping panel for multi-parametric flow cytometric analysis, and will submit a final report to the Foundation with these findings.

### **Imino sugars as inhibitors of *M. tuberculosis* – Evaluation of a new class of Mycobacterial Arabinan Biosynthesis Inhibitors**

BL Stocker

**Introduction:** Tuberculosis (TB) is on the increase worldwide and is no longer a disease of developing countries. With limited efficacy of current vaccine formulations and the emergence of drug-resistant strains, there is an increasing need for more effective anti-mycobacterial drugs. In view of this, we aim to develop a novel class of TB drugs that will disrupt the biosynthesis of the mycobacterial cell wall by acting on arabinotransferases, the enzymes responsible for the formation of the arabinan chains found in the cell wall. Arabinose is xenobiotic to mammals, and hence drugs designed to specifically inhibit these glycosidases are not expected to affect the human host.

**Project Progress:** Recently we developed new methodology for the synthesis of imino sugars, a class of compounds that have potential as novel drug therapeutics for the treatment of *M. tuberculosis*. Using our new methodology, we synthesised our first library of novel TB therapeutics and screened these for their ability to inhibit the growth of BCG. To our delight, one of our library members showed nanomolar growth inhibition. Though the mode of action of our successful drug candidate still needs to be ascertained, the identification of a compound with nanomolar growth inhibition is exceptionally encouraging. This drug candidate represents a novel class of TB therapeutics and therefore has much potential for further drug development and optimisation.

We are currently synthesising a second-generation TB-drug library based on modifications to our parent compound with the hope of discovering an even more potent drug. The synthesis of additional library members will also enable us to better understand the correlation between structure and the mycobacterial inhibitory properties of our drug-class. The synthesis of this second generation library will be completed and tested using our BCG-Alamar Blue assay within the forthcoming month. From these studies, we will be able to make further modifications to our basic drug class, if required. A more robust evaluation of the pharmacological profile of the lead candidates and their assessment in other TB-assays will be performed in due course.

### **NK-T Cell Activation And Dendritic Cell Survival *In Vivo***

HA Simkins and F Ronchese

NK-T cells are a population of T cells expressing an invariant T cell receptor and markers of NK cells such as CD56 in humans and NK1.1 in mice. Upon intravenous injection of the appropriate antigen ligands *in vivo*, NK-T cells become activated and rapidly produce cytokines that lead to the activation of

other immune cell populations, and support the establishment of immune responses.

Intravenous injection of the NK-T cell ligand  $\alpha$ -galactosyl ceramide ( $\alpha$ -GalCer) is known to induce activation of dendritic cells (DC), a population of powerful antigen presenting cells that are critical to the initiation of immune responses. Because of this ability to induce DC activation,  $\alpha$ -GalCer is used as an adjuvant to support the induction of T cell and B cell immune responses to soluble antigens, and anti-tumour immune responses.

We have observed that activation of DC after  $\alpha$ -GalCer injection was followed in about 18h by a substantial reduction in the subpopulation of DC that carry the surface marker CD8 (Figure 1). Other CD8<sup>-</sup> DC subpopulations, which are also activated after  $\alpha$ -GalCer injection, appeared not to be affected. Further characterization of the affected subpopulation of DC revealed that it can be defined by the co-expression of the markers CD8 and CD207, as CD8<sup>+</sup>CD207<sup>-</sup> DC survived after  $\alpha$ -GalCer treatment, while CD8<sup>+</sup>CD207<sup>+</sup> DC were depleted. CD8<sup>+</sup>CD207<sup>+</sup> DC are thought to carry out a specific function in the immune response, and selectively take up and cross-present soluble antigens injected intravenously. Future experiments will attempt to establish whether depletion of the CD8<sup>+</sup>CD207<sup>+</sup> DC prevents hosts from generating CD8<sup>+</sup> T cell responses to soluble antigens injected intravenously. We have attempted to define the mechanism that leads to the depletion of CD8<sup>+</sup> DC, as preventing their loss might improve the ability of  $\alpha$ -GalCer to support immune responses. Initial experiments examined the possibility that DC loss is due to direct contact between the CD8<sup>+</sup>CD207<sup>+</sup> DC and NK-T cells, however, this possibility was not supported by the experimental results. We then examined the role of cytokines produced by NK-T cells. Injection of  $\alpha$ -GalCer induces NK-T cells to rapidly secrete TNF- $\alpha$ , a cytokine known to induce activation and then death of DC in vitro. Injection of a neutralizing anti-TNF- $\alpha$  antibody was effective at reducing the serum levels of this cytokine in treated mice, and allowed larger numbers of CD8<sup>+</sup>CD207<sup>+</sup> DC to survive, suggesting that TNF- $\alpha$  is critically involved in the death of DC during  $\alpha$ -GalCer treatment. However, anti-TNF- $\alpha$  treatment also prevented the DC activation observed after  $\alpha$ -GalCer treatment, and did not lead to the generation of enhanced immune response.

We conclude that the depletion of CD8<sup>+</sup>CD207<sup>+</sup> DC observed after  $\alpha$ -GalCer treatment does not affect the ability of  $\alpha$ -GalCer to act as an adjuvant for the induction of immune responses. However, the ability to generate some immune responses may be transiently decreased after use of  $\alpha$ -GalCer.

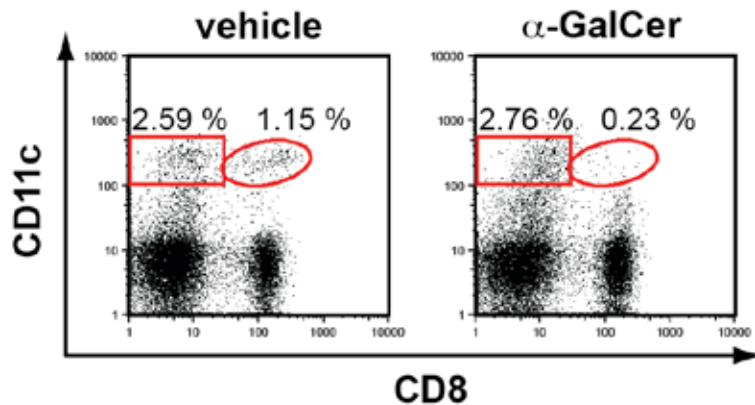


Figure 1: Depletion of CD8+ DC following treatment with  $\alpha$ -GalCer. Mice were injected iv with PBS (vehicle) or 200 ng  $\alpha$ -GalCer, and spleen DC were examined 24 hours later by flow cytometry. Each dot plot refers to spleen cells from one mouse; the DC populations are identified by expression of the CD11c marker. The CD8+ and CD8- DC populations are highlighted by an oval and a rectangle, respectively; the percentages of cells in each subpopulation are shown.

## Sirtuins, Stress and Survival: A Problem in Anti-Tumour Therapy

MJ McConnell

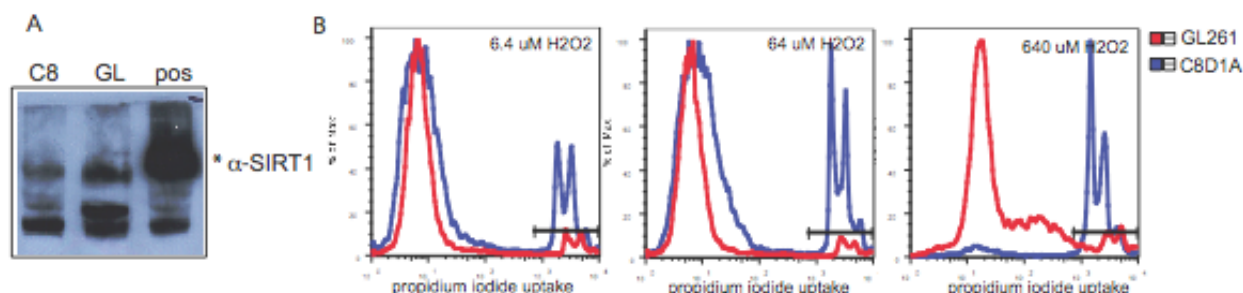
When proliferating cells are stressed by unfavourable conditions they express a deacetylase enzyme SIRT1 which regulates survival pathways. This study investigates SIRT1 as a potential target for inhibiting cancer cell growth and the relationship between stress, induction of SIRT1 and cell survival in both T-cell malignancy and in normal T-cells. It is hypothesised that malignant T-cells will be more vulnerable to blockade of SIRT1-mediated stress responses than normal T-cells, due to a higher reliance on survival pathways mediated by SIRT1. This study will investigate whether normal cells are more resistant to a range of clinically relevant stresses than malignant cells, whether the stress response is dependent on SIRT1, and whether SIRT1 loss preferentially affects malignant cells. If this occurs, then tumour specificity could be leveraged from SIRT1 inhibitors in a rational approach to combination drug therapy.

**Results:** Normal murine T-cells expressed very low, often undetectable levels of SIRT1 protein. This made it difficult to consistently determine SIRT1 protein levels, which in turn made any short hairpin knock-down of SIRT1 protein technically challenging, and potentially of limited experimental value. In light of this finding, a decision was made to change the to a model where SIRT1 expression could be routinely detected in the normal cell counterpart. A murine glial cell line C8D1A, was chosen as the normal counterpart to the malignant murine GL261 glioblastoma cell.

### i. Are normal cells more resistant to stress than malignant cells?

Several stresses have been explored, including chemotoxic stress upon doxorubicin treatment, nutrient stress after serum withdrawal, and oxidative

stress induced by hydrogen peroxide. The outcome of the cell has been measured by propidium iodide exclusion to directly measure changes in viability over time. The current focus is on oxidative stress, but the experiments will be performed with the range of stresses described in the grant proposal.



**Figure 1:** SIRT1 level corresponds with resistance to stress. A. Western blot analysis of SIRT1 expression in untreated cells with a polyclonal antibody for SIRT1. Asterisk indicates specific SIRT1 band. C8, C8D1A normal; GL, GL261 malignant; pos, SIRT1 positive control. B. Flow cytometry analysis of propidium iodide (PI) uptake. Non-viable cells take up more PI and have higher fluorescence, falling further along the x axis. Cells were incubated with increasing doses of H<sub>2</sub>O<sub>2</sub> for 41 hours before addition of PI, then PI uptake measured after 5 mins.

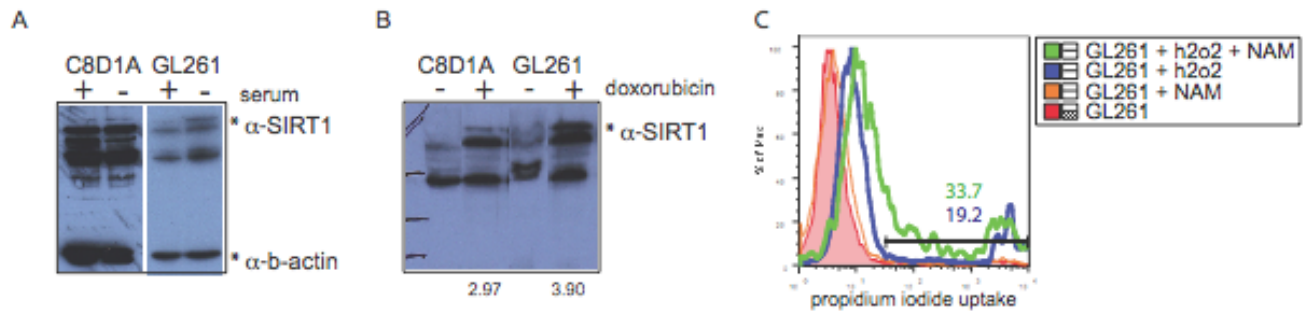
We have demonstrated that malignant GL261 cells are more resistant than normal C8D1A glial cells to hydrogen peroxide treatment, and able to withstand 20-100x higher concentration. This correlates with an increased basal level of SIRT1 expression in malignant cells (Fig 1). Interestingly, the differential SIRT1 expression between malignant and normal cells was observed in both T-cells and glial cells, suggesting conservation of stress responses across tissues.

A new monoclonal antibody has been purchased, which has greater specificity for murine SIRT1 than the polyclonal antibody used for the experiments presented here.

## ii. Is response to stress SIRT1-dependent?

Under both chemotoxic stress (doxorubicin treatment) and serum deprivation, both malignant GL261 glioblastoma cells and normal C8D1A glial cells up-regulated SIRT1 expression. This demonstrated that SIRT1 is a component of the stress response in both normal and malignant cells.

The non-specific SIRT inhibitor nicotinamide, while having no effect on cell viability on its own, increased death induced by hydrogen peroxide in malignant GL261 cells from 19% to 33%. This implied SIRT1 activity was necessary for the successful protective response in GL261. These experiments will be repeated with the SIRT-specific inhibitor sirtinol, and will be confirmed with a shRNA knock-down of SIRT1.



**Figure 2:** SIRT1 is up-regulated under stress. A. Cells were grown in 10% (+) or 0.1% serum (-) for 24 hours, and lysates collected for western blot of SIRT1. B-actin is shown as a loading control. B. Cells were exposed to 5 uM doxorubicin for 24 hours and lysates collected for western blot analysis. The SIRT1 band was quantified using ImageJ, and the fold increase in SIRT1 expression indicated underneath each line. C. GL261 cells were exposed to either 320 uM H<sub>2</sub>O<sub>2</sub>, 10 mM nicotinamide, or the combination, for 18 hours. PI was added and incubated a further 5 mins, and uptake analysed by flow cytometry.

### iii. Does SIRT1 loss preferentially affect malignant cells?

Experiments comparing inhibition of SIRT1 in malignant GL261 and normal C8D1A glial cells are in progress. Preliminary data suggests that loss of SIRT1 activity in normal cells might have less effect on viability than observed in malignant cells. This would confirm the hypothesis, that normal cells are less reliant on SIRT1 for survival under stress.

## Unplugging Food Allergy

EE Forbes

This research proposed to identify the key cellular and molecular mechanisms responsible for the elicitation of food-induced allergic skin sensitisation. This approach is based on the rationale that the onset of food allergy is often preceded by atopic dermatitis, commonly known as eczema, in which the normal skin barrier is defective. The majority of young children with food allergies and atopic dermatitis go on to develop respiratory allergies and asthma, often identified by allergists as the “allergic march”. In addition, children with food allergies and asthma are more likely to suffer from severe asthma, and are at greater risk for severe and possibly fatal anaphylactic reactions. The goal of this research is to develop an animal model that is relevant to human exposure with allergen sensitisation through the skin, and the outcome will be an enhanced understanding of the inter-relationship of food allergy and other allergic disease processes.

To date, we have established a model of food-induced skin sensitisation within our laboratory, and have examined the kinetics involved in dose and time intervals that generate optimal sensitisation. Utilising our elegant IL-4

reporter mouse, we have demonstrated the development of a T helper type 2 (Th<sub>2</sub>) response, and have obtained reproducible results. We are now moving forward to identify the specific contribution of distinct potential effector types, including the basophil in establishing food-allergen induced allergic sensitisation in the skin.

### **Worms and Germs: Do helminth infections impair the efficacy of the Tuberculosis vaccine, BCG?**

Kirman J, Connor L and Le Gros G.

In this study we have:

- Repeated our preliminary experiment, in which we found in a murine model that helminth infection partially abrogated the efficacy of the Tb vaccine, bacille Calmette Guérin (BCG) against aerosol challenge with virulent *Mycobacterium tuberculosis*. Tissues have been harvested for bacterial counts, which will take an additional three weeks to grow on agar.
- Investigated whether regulatory T cells induced by helminth infection suppress the development of Th1 memory by the BCG vaccine. We have shown that after *Nippostrongylus brasiliensis* infection, regulatory T cells increase in number in the lungs, but not in the spleen or lymph nodes. Of note, we have shown that regulatory T cells in the lung, lymph nodes and spleen become activated after *N. brasiliensis* infection, by measuring expression CD103 on regulatory T cells.
- Shown that the recently identified cytokine IL-17 is made by similar numbers of CD4+ and  $\gamma\delta$  T cells in the lungs of BCG-vaccinated mice after mycobacterial challenge, irrespective of whether they had been infected with *N. brasiliensis* ( $\pm$  Treg depletion) before vaccination.
- Determined that Th2 cytokine production is elevated in *N. brasiliensis* infected-BCG vaccinated mice compared to BCG vaccinated controls. However, the level of the Th1 cytokine interferon-gamma remains the same in BCG vaccinated mice irrespective of whether they were infected with *N. brasiliensis* or not.
- We were unable to obtain conclusive results from our studies in Stat6-deficient mice, as these animals failed clear the *N. brasiliensis* infection, even following anti-helminthic therapy.

**Conclusions:** We have found that regulatory T cells are activated and increase in number in the lungs of *N. brasiliensis* infected mice. We know that Th1 cytokine production is not decreased in the spleen or lymph nodes of BCG-vaccinated *N. brasiliensis* infected mice, and Th17 cytokine production is not impaired in the lungs of these mice after challenge, suggesting that the local Th2 response induced in these tissues does not inhibit the development of a Th1 or Th17 response.

## Massey University

### Akt as a therapeutic target for muscle wasting induced by acidosis

JA Edge and MJ Short  
Institute of Food, Nutrition and Human Health

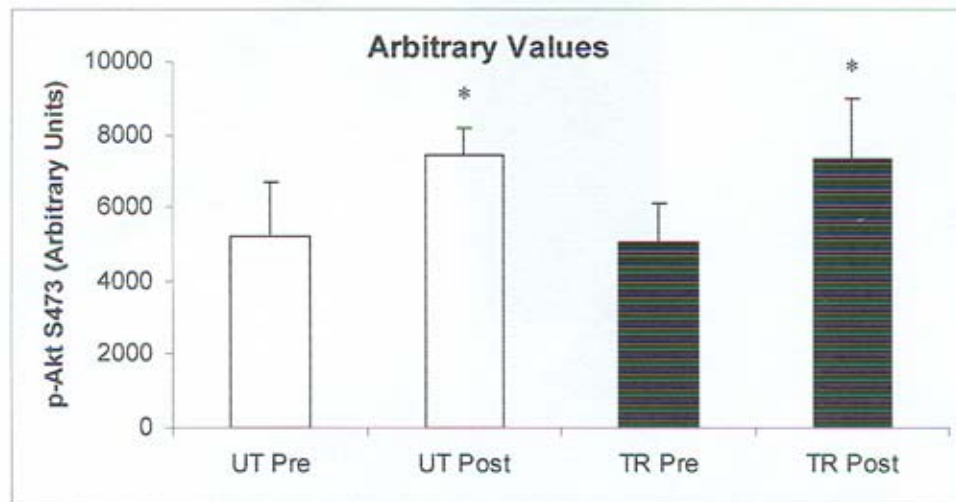
**Introduction:** The loss of skeletal muscle is a serious clinical problem due to its relationship with morbidity and mortality. Many conditions such as chronic kidney disease, cancer, sepsis and insulin deficiency result in increased breakdown and loss of skeletal muscle. Metabolic acidosis accompanies a number of disease conditions such as chronic kidney disease and some insulin disorders. Metabolic acidosis has been shown to have a negative effect on skeletal muscle development and accelerate muscle degradation in animal models. In contrast; regular physical activity promotes the maintenance and growth of skeletal muscle and may provide protection against many disease related muscle wasting disorders. To date few studies have examined the effects of metabolic acidosis on human muscle and whether physical activity has a protective effect against the negative consequences of metabolic acidosis. A key signalling protein involved in the regulation of skeletal muscle development is Akt. Therefore, identifying changes to activation of this protein following metabolic acidosis and physical activity may provide insight into how metabolic acidosis affects skeletal muscle development and determine how long term involvement in exercise may be of benefit to skeletal muscle.

**Aim:** The aim of this project was to determine the effects of (1) short-term metabolic acidosis on the molecular response (ie phosphorylation of Akt) of skeletal muscle in healthy humans and (2) the effects of the physically trained status of skeletal muscle on the molecular response during metabolic acidosis.

**Methods:** Sixteen healthy humans aged 38 - 60 y volunteered to participate in this study. Eight of the participants were healthy and active. Participants reported to the laboratory 4 h after consuming a standardised light meal. After a 30 min rest period, participants had a venous blood sample and they were each given food packages that contained all of their food to be eaten over the following 72 h. Participants were also given a set of tablets containing ammonium chloride to induce metabolic acidosis to be taken over the following 72 h. Approximately 72 h ( $\pm 1$  h) after their first blood sample and muscle biopsy, the participants returned to the laboratory for a second blood sample and muscle biopsy 4 h after their last provided meal. Food diaries were checked to determine that all the provided food had been consumed and pills ingested. None of the participants reported any gastrointestinal discomfort from consuming the tablets.

**Results:** The well trained runners had a significantly higher maximal oxygen uptake than the but not well trained participants ( $60.5 \pm 4.0$  vs  $47.1 \pm 4.3$  mL·kg<sup>-1</sup>·min<sup>-1</sup> respectively;  $P < 0.05$ ). There were no differences in lean thigh volume or leg strength between the two groups ( $P < 0.05$ ). Following the

three days of metabolic acidosis, there was a significant decrease in venous blood pH ( $7.48 \pm 0.03 - 7.34 \pm 0.08$  mean both groups;  $P < 0.05$ ) and bicarbonate  $28.4 \pm 1.5 - 17.8 \pm 2.4$  mean both groups;  $P < 0.05$ ), with no differences between groups. There was a significant decrease in skeletal muscle pH also ( $7.19 \pm 0.04 - 7.05 \pm 0.06$  mean both groups;  $P < 0.05$ ), with no differences between groups. There was a significant decrease in skeletal muscle pH also ( $7.19 \pm 0.04 - 7.05 \pm 0.06$  mean both groups;  $P < 0.05$ ), with no differences between groups. There was a significant increase in Akt phosphorylation following the three days of metabolic acidosis (Figure 1;  $P < 0.05$ ), with no differences between groups.



**Discussion:** The three day metabolic acidosis protocol resulted in a significant drop in pH in the blood and muscle of both groups of participants. This decrease in blood pH is similar to that reported by others following 2 - 5 days of induced metabolic acidosis and in untreated renal patients. We also demonstrate that this protocol can affect tissue pH, specifically muscle pH. Therefore, these results show that systemic metabolic acidosis can result in skeletal muscle metabolic acidosis. This could have long term effects on the development of muscle tissue and could partly explain some of the muscle loss reported in patients with systemic metabolic acidosis.

However, in contrast to our hypothesis and to previous reports using a rat model, short term metabolic acidosis increased the phosphorylation of Akt, a protein involved in muscle growth. There were no changes in phosphorylation of mammalian target of rapamycin (data not shown), which is a downstream target of Akt involved in muscle growth, suggesting that muscle growth may not have been stimulated by the increased Akt activation following metabolic acidosis.

### **SPIRIT study: Beneficial health effects for Polynesians with Type 2 diabetes?**

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**Introduction:** Progressive resistance training (PRT) is now well established as a safe and beneficial exercise modality for healthy adults, the elderly and certain chronically diseased cohorts. A few studies have also demonstrated that PRT may be of particular importance in type 2 diabetes by potentially improving glycaemic control and inducing additional adaptations beneficial for diabetes management. Such PRT-induced adaptations include: improved insulin sensitivity, reduced visceral adiposity, increased skeletal muscle glycogen content and reduced pro-inflammatory cytokines.

**Aim:** To investigate the effect of aerobic (AER) and PRT exercise on obesity and diabetic hormonal biomarkers and gene expression in the skeletal muscle of Polynesian individuals participating in this study.

The specific objectives of the WMRF funded research project was to determine if 16 weeks of PRT or AER exercise may;

- 1) Improve glycaemic control (HbA1c), insulin sensitivity and additional indices related to diabetic status in Polynesians diagnosed with type 2 diabetes mellitus
- 2) Alter specific hormonal indicators (such as leptin, cortisol binding globulin (CBG), sex hormone binding globulin (SHBG) and cortisol) of obesity and insulin resistance in the Polynesian cohort in this study, as this may relate to improvements in diabetes health outcomes
- 3) Alter gene expression of specific energy balance markers (such as leptin receptor, uncoupling proteins type 2 and 3) in skeletal muscle that may result from PRT exercise in the cohort of Polynesians in this study, as this may lead to improved metabolic outcomes in diabetes.

**Results:** Twenty-six adults of self-described Polynesian descent were randomised to either PRT or AER, three times per week, for 16 weeks. Body mass index (BMI) for this group of participants was  $42.7 \pm 9.0 \text{ kg/m}^2$ . This study is the first to have a cohort of such high BMI. Body mass, waist circumference, blood pressure, blood samples, urine samples and muscle biopsies were collected pre- and post intervention.

Associated haematological indices (such as HbA1c, fasting glucose, insulin and insulin resistance measured *via* HOMA, CBG, SHBG and leptin) were evaluated pre- and post intervention from blood samples obtained. Cortisol levels were evaluated pre- and post intervention from urine analysis. Eighteen patients (9/group) completed the study and were included in the per protocol analyses. Compliance to the intervention was low, with only eight

patients completing >75% of either regimen. HbA1c remained elevated after 16 weeks within both PRT and AER (ANOVA)(10.7±2.1 to 10.6±2.4%, p=0.86; 8.9±1.9 to 8.8±2.1 %, p=0.61, respectively). AER resulted in a reduction in systolic (p=0.006) and diastolic blood pressure (p=0.02), and a trend toward a reduction in hyperinsulinaemia (177±83 to 134±64pmol/L, p=0.09). AER also resulted in a trend toward a reduction in CBG levels (652±152 to 519±123pmol/L, p=0.09). PRT resulted in an increase in SHBG levels (p=0.016) and a trend towards a reduction in CBG levels (712±137 to 622±145pmol/L, p=0.07). No other physiological changes were observed, and no effects were significantly different between exercise groups and between males and females for leptin and urinary cortisol. Investigation of leptin receptor, uncoupling proteins type 2 and 3 gene expression levels in the skeletal muscle pre- and post biopsy samples is currently still in progress.

**Conclusion and future directions:** To date, there has been minimal advocacy for PRT exercise as an essential therapeutic adjunct for the medical management of Pacific Islanders diagnosed with type 2 diabetes. Results from the SPIRIT study, investigation of endocrine and physiological responses of this ethnic population to PRT, will potentially improve medical advocacy, community awareness and participation to aid in the reversal of the diabetes epidemic within the Pacific Islander community of New Zealand.

# University of Otago Wellington, School of Medicine and Health Sciences

## Autonomic regulation of cardiovascular variability in the anaesthetized rat

PYW Sin, YC Tzeng and DC Galletly  
Department of Surgery and Anaesthesia

**Background:** Respiratory sinus arrhythmia (RSA) is the beat-to-beat change in heart rate that occurs in synchrony with breathing. Traditionally, it is believed that RSA is purely mediated by the parasympathetic nervous system and many researchers have applied measures of RSA amplitude as non-invasive surrogate of parasympathetic neural tone. However, recent studies from this laboratory challenge this assumption; we have shown that under general anaesthesia RSA may be mediated by mechanical processes that are unrelated to cardiac parasympathetic regulation.

In addition, recent studies have suggested that sympathetic nervous activity, which is also a major component of the autonomic nervous system, may also contribute to the generation of RSA. On this background, we sought to determine whether sympathetic activity in additional mechanical mechanisms, also play a role in modulating RSA in the anaesthetised rat. This was achieved by an applying novel analysis techniques developed in our laboratory called 'pattern analysis' in rats under four different common anaesthetic regimes (Halothane, Pentobarbital, Chloral Hydrate and Ketamine-Xylazine). Using the pattern analysis technique we assessed not only amplitude changes, but also document for the first time alterations in RSA patterns occurring within the breathing cycle under each anaesthetic regime.

**Key Outcomes:** In summary, we found that while the removal the sympathetic nervous activity  $\beta_1$ -adrenergic blockade resulted in the enhancement of RSA amplitude, the patterns of RSA distinct to each anaesthetic agent was not significantly altered. First, these findings suggest that the distinct RSA patterns found uniquely to each anaesthetic agent are mediated by neither parasympathetic nor sympathetic activity. This leaves the possibility that they are primarily mediated by the mechanical chest excursions of respiration with some possible unknown influence from the each anaesthetic agent.

Second, our finding that sympathetic activity attenuates RSA amplitude is consistent with the established literature in both human and rat studies. However, it challenges the notion that the sympathetic influence of RSA is mediated via the antagonism of acetylcholine fluctuations at the sino-atrial node as widely considered. The absence of parasympathetic outflow, and therefore acetylcholine supply, suggest noradrenaline directly affects the sino-atrial node to attenuate RSA. This study reveals new insight into RSA in the anaesthetised rat and reiterates the caution that should be taken when using

RSA as simple estimate of parasympathetic activity. In the rat, RSA is markedly affected by mechanical, drug and sympathetic effects.

### **Characterization of an activity found in portal vein serum which increases glucose uptake in the HepG2 hepatoma cell line**

MT Hayes<sup>1</sup>, RS Stubbs<sup>1</sup> and C Buchanan<sup>2</sup>

<sup>1</sup> Department of Pathology and Molecular Medicine

<sup>2</sup> Centre for Molecular Biodiscovery, University of Auckland.

The obesity and diabetes group at Wakefield has been studying the mechanisms by which the majority of morbidly obese type 2 diabetics who undertake Roux-en-Y gastric bypass surgery (GBS) resolve their diabetes. This resolution usually occurs within 6 days of the operation which is before any significant weight loss could contribute to improved glycaemic control. A previous grant in aid from the foundation has allowed us to establish that reduced energy intake does not resolve diabetes. This work is further supported by evidence in the literature which shows that GBS on a non-obese diabetic rat resolved diabetes whereas energy deprivation on sham operated and non operated animals did not resolve diabetes. Further, diabetes returned when the operation was reversed in these animals strongly indicating that the area of gut isolated from the passage of food in the operation is producing some form of signaling which leads to a rapid and marked change in glycaemic control which allows resolution of type 2 diabetes in many cases.

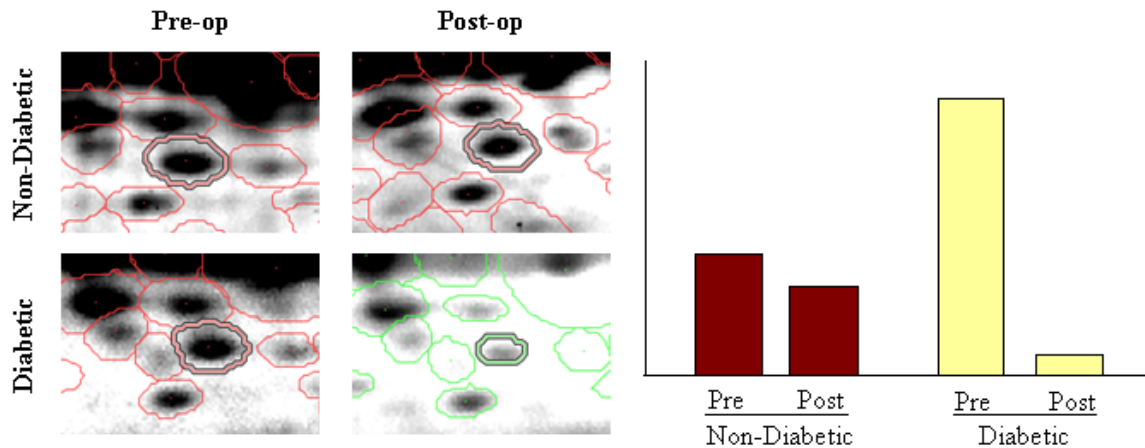
We believe that these signals from the gut are carried in the blood. Further, we have reasoned that as the blood from the gut drains through the portal vein to the liver and is subsequently diluted within the liver, proteins and peptides originating in the gut will be at their highest concentration in the portal vein. Therefore an assessment of the levels of such molecules in portal blood and peripheral blood taken at the same time will allow identification of molecules which originate from the gut. In order to isolate and identify active molecule/s from blood, it is essential to have a means of testing blood fractions and individual proteins identified by proteomic analysis for an affect on glycaemic control in response to insulin. We have developed an *in vitro* assay based on HepG2 human hepatoma cells to act as an *in vitro* screen for appropriate activity. The assay was initially used to identify that post operative serum from diabetics who resolve their diabetes after GBS significantly improves total glucose uptake in these cells in response to insulin. This grant in aid was provided to confirm that glycogen synthesis also improved, thereby lending additional support for our hypothesis. The grant also allowed us to purchase an Invitrogen IEF fractionator to fractionate the proteins in our serum samples into isoelectric point ranges for testing with the *in vitro* assay and to continue working on identifying active molecular weight range fractions.

To date we have collected 42 sets of pre and post operative portal and peripheral serum, plasma and P700 tube (BD plasma tube with a DPPIV inhibitor) samples.

We have confirmed that glycogen synthesis increases in our assay in parallel with total glucose uptake indicating that there are signals present in blood before or after surgery which have a significant effect on insulin responses within the cells. We have screened individual patients and chosen those with the greatest response in the *in vitro* assay for serum partitioning. Serum from individual patients has been partitioned by molecular weight interval and by pI interval but initial attempts to use these fractions in our *in vitro* assay were unsuccessful because the carrier solutions we used to isolate or re-solubilise the proteins were incompatible with cell viability. We have subsequently dialysed the fractions and are currently working through the fractions to identify activity.

We have identified twelve differentially expressed spots in gels and used MALDI TOF to identify four of these (Figure 1 below). We have extended this work comparing the peripheral serum with the portal serum and have identified eight protein spots on the gels which are differentially expressed between the pre and post operative portal blood but are not detected in peripheral serum suggesting that they originate in the gut. We are currently working to identify these proteins using MALDI TOF mass spectrometry. This work has the potential to identify molecules which contribute to the resolution of type 2 diabetes seen post GBS and provide new targets for better treating this disease.

**Figure 1. A differentially expressed protein identified from 2D gel analysis of diabetic pre and post operative (spot 8)**



## Characterisation of the Invasive Front of Metastatic Colorectal Tumours

K Hood and RS Stubbs  
Department of Pathology and Molecular Medicine

The cells within any particular colorectal cancer are heterogeneous and there is reason to believe that the cells at the invasive front (IF) of colorectal cancer

(the interface between the cancer and the surrounding normal tissue) are of particular importance in the process of metastasis. We have been interested in exploring the proteins in the cancer cells at the IF and have identified 91 protein spots on 2D gels which are differentially expressed in the IF versus the main tumour body (MTB). The current task is to identify these proteins by mass spectrometry and then to validate that they are indeed differentially expressed in the IF compared with the MTB. Previous work has completed work on 4 of these spots and the present project sought to identify and validate a further 6 proteins.

**Results:** We were unable to identify most of the protein spots by MALD-TOF mass spectrometry and needed to utilize MS/MS techniques performed for us under contract by Auckland University. 42 proteins have now been identified by this means, many of which have a known role in other types of cancers and some have a known role in cellular metabolism. We are now in a position to select proteins from within this pool of 42 proteins for validation using Western blotting techniques and immunohistochemistry (IHC).

At this stage we have identified and validated the following proteins as being differentially expressed; fibrinogen, ferritin, tropomyosins, and collagen VI. Further funding has recently been confirmed for a collaborative project with ESR to validate a further 10 of the identified proteins and to undertake microarray analysis of mRNA taken from the IF, MTB and LM from the same patients as have been used for the proteomic analysis. This will provide complementary data to support our findings. We are also proposing an extension of the present work. In this a selection of the proteins we have identified will be examined, using IHC, in an expanded set of 50 patients, half of whom are known to have developed recurrence. This should help determine the potential relevance of these proteins to the metastatic process.

### **Differential effect of anaesthetic agents on respiratory sinus arrhythmia in the rat**

YC Tzeng, PYW Sin and DC Galletly  
Department of Surgery and Anaesthesia

**Background:** Autonomic nervous system (ANS) dysfunction increases the risk of sudden death from cardiovascular disease, the leading cause of mortality in developed countries. In prognostic settings such as post-myocardial infarction, a low level of RSA is an independent mortality risk factor. However, despite clear associations to poor prognostic outcomes, there is still much uncertainty over fundamental questions concerning the information content revealed by RSA.

Experimental protocols that are deemed invasive, but which may otherwise bring important insight to the mechanism of RSA, necessitate the use of anaesthetic animal models; however, anaesthesia itself affects the ANS. Therefore a thorough understanding of anaesthetic-related effects on RSA is vital when interpreting results obtained under the influence of anaesthesia. Although many studies have assessed the effect of anaesthesia on

parasympathetic nervous activity, there is a paucity of data on how anaesthesia influences autonomic indices derived from RSA (eg spontaneous baroreflex sensitivity).

The current project conducts a systematic assessment of four commonly applied anaesthetic regimes on RSA and spontaneous indices of baroreflex function.

**Summary of Key Outcomes:** In brief, we found that with the exception of Ketamine-Xylazine, most anaesthetic regimes (Halothane, Pentobarbital, Choral hydrate) cause marked alterations in autonomic function. Only Ketamine-Xylazine was associated with RSA that comports with pattern of RSA reported in conscious animals. These findings indicate that Halothane, pentobarbital and chloral hydrate severely inhibits parasympathetic activity in the rat and that RSA under these circumstances reflect mechanical, rather than neural activity.

A second significant finding was that spontaneous baroreflex sensitivity measures could only be calculated with some techniques. For example, we were able to determine the  $\alpha$ -index based on power spectral analysis but not with the sequence index. This finding is novel as it clearly highlights a potential pitfall for research using power spectral indices of baroreflex function. Importantly we showed that given RSA under most modes of anaesthesia reflect mechanical activity, as a whole spontaneous indices have no place in the assessment of baroreflex function under anaesthesia. We recommend that where anaesthesia is applied, baroreflex function should be assessed using established pharmacological techniques.

### **Early Vascular Disease in Children with Epilepsy Receiving Anticonvulsants**

NF Keenan, LG Sadleir and EJ Wiltshire

Mortality from cardiovascular and cerebrovascular disease in patients with epilepsy is up to five times that seen in the general population. It is postulated that this elevation in cardiovascular disease is partly due to elevation of the cardiovascular risk factor Total Plasma Homocyst(e)ine (tHey). Elevated tHey is frequently elevated in adolescents and adults with epilepsy as a result of Antiepileptic Drug (AED) induced B-vitamin deficiencies. It has been recommended by previous investigators that all children with epilepsy should receive vitamin supplementation to reduce their cardiovascular risk. Other cardiovascular risk factors, such as oxidative stress, hyperinsulinaemia and hyperlipidaemia are also frequently reported in patients with epilepsy treated with AEDs.

Using ultrasound techniques and the measurement of biochemical risk factors, we investigated the cardiovascular risk in children with epilepsy receiving anticonvulsants. Thirty children with epilepsy aged between the ages of eight and eighteen years who had received unchanged AED therapy for greater than twelve months were recruited from the Wellington region in

New Zealand. Each child with epilepsy was age, sex and BMI matched with a healthy control. Vascular endothelial health was assessed using Flow-Mediated Dilatation (FMD) and the Intima-Media Thickness (IMT) of the carotid and aortic arteries. Each child also had a fasting blood sample taken measuring plasma glucose, lipids, tHcy, serum folate, red cell folate, vitamin B 12 and pyridoxal-5-phosphate (Vitamin B6) levels.

We were unable to demonstrate elevated tHcy in our children with epilepsy and so not surprisingly their endothelial function and structure was also unremarkable. Given our findings we concluded that vitamin supplementation in all children with epilepsy would appear unnecessary. It is likely that our population had diets with vitamin intakes adequate to compensate for any loss of vitamins induced by anticonvulsant therapy and subsequently the threshold needed to produce elevated tHcy was not reached. Therefore, vitamin supplementation may only be indicated in populations with lower nutritional intakes and adults who naturally have lower B-vitamin levels compared to children. We concluded that recommendations of diets high B-vitamins by paediatricians and neurologists would be of benefit.

### **Grant-in-aid for Purchase of Laser Capture Microscope (LCM)**

K Hood and RS Stubbs  
Department of Pathology and Molecular Medicine

In March of 2008 The Wellington Medical Research Foundation contributed \$23,000 to the purchase of a Laser Capture Microscope by the Wakefield Biomedical Research Unit. Lotteries Health had contributed \$75,000 and the Wellington Division of the Cancer Society had also contributed \$25,000. This allowed the establishment in Wellington of a Laser Dissection Facility to take place, for use by Wellington based researchers. Initially the facility was housed at the Wakefield Gastroenterology Research Institute at Wakefield Hospital, but relocated to the Wellington School of Medicine when the Wakefield Group moved to become part of the Dept of Pathology and Molecular Medicine at the School of Medicine. A workshop for interested researchers was held at Wakefield in April 2008 and included attendees from ESR, Wellington, ESR, Auckland, Victoria University, Malaghan Institute and Wellington School of Medicine.

This facility allows for analysis of discrete areas of cells and or stroma within cancer tissue (or other non-cancerous tissue) to be undertaken and provides an important new dimension to the research being undertaken by a number of the research Groups in Wellington. The accompanying figure shows the microscope and demonstrates how an area shown under the microscope can be “cut out” for subsequent analysis.



**Figure:** Laser capture microscope (left). The particular area of cancer cells shown in the right upper image has been “cut out” by laser microdissection for analysis (right lower image).

The Cancer Group within the Wakefield Biomedical Research Unit has been making good use of this capability and has already published two papers in the international Biomedical literature which have reported work involving this technology. In addition, some six poster and oral presentations have been made to scientific meetings in New Zealand on work utilising the LCM.

A collaboration has also begun between the Wakefield Biomedical Research Unit Cancer Group and the Envirogenomics Group from ESR (Donia Macartney) which will utilise the LCM to obtain tissue for a study involving mRNA in colorectal cancer. This study will run alongside and complement the proteomic studies being undertaken by the Wakefield Group.

### **Travel related infectious diseases in New Zealand 1997 – 2006: describing the epidemiology and assessing the risk**

S Gray, M Baker and R Edwards  
Department of Public Health

**Introduction:** Overseas travel increases an individual’s risk of some infectious diseases. It also poses a public health risk to the wider New Zealand population due to importation of these diseases. Despite these risks there is very little published in the literature regarding frequency with which New Zealand travellers acquire infectious diseases whilst overseas. In particular mention of the risks of travel to countries in the Pacific region which are all relatively popular travel destinations for New Zealanders is infrequent in the published international literature.

This study aimed to fill these knowledge gaps by describing the epidemiology of selected travel-related infectious diseases in the New Zealand setting. The goal of this study was to inform relevant policies and practices within New Zealand and thereby to contribute to reducing the amount of travel-related infectious disease in our population.

**Methods:** Six travel-related infectious diseases were selected to analyse - malaria and dengue fever (both vector-borne) and hepatitis A, typhoid, paratyphoid and shigellosis (all predominantly food and water-borne).

Using the research grant awarded by the Wellington Medical Research Foundation, numerator data was acquired from ESR on the numbers of notified cases of these diseases that were imported into New Zealand from 1997-2006 and denominator data was obtained from Statistics New Zealand on total arrivals into New Zealand and numbers of New Zealand travellers to various regions worldwide for the same period.

A quantitative assessment was carried out of the risk of acquiring these diseases whilst travelling. Trends in incidence risks (cases per 100,000 travellers) over time were analysed with incidence risks by region and country of destination, and age specific incidence risks also calculated for malaria, dengue fever and hepatitis A. For typhoid, paratyphoid and shigellosis the proportions of cases acquired in different regions and countries were calculated. Characteristics of travel-related cases, selected outcome measures, and the prevalence of recorded preventative measures were analysed for all six diseases.

**Results:** For the six infectious diseases studied, the mean incidence risk (cases per 100,000) for total arrivals into New Zealand was highest for shigellosis at 1.40 and lowest for typhoid at 0.45, for the period 1997 to 2006. When the analysis was restricted to New Zealand travellers, the incidence risk was 1.14 for malaria, 1.50 for dengue fever and 1.45 for hepatitis A.

When the incidence risk of travel-related infection for 1997 – 2001 was compared with that for 2002 – 2006 a statistically significant decline in incidence risk between these two five year periods was demonstrated for hepatitis A, shigellosis, paratyphoid and malaria ( $p < 0.05$ ). The incidence risk of dengue fever was very variable from year to year. The incidence risk of typhoid was fairly stable over time.

Regional destinations of highest risk were Africa for malaria and the Pacific region for dengue fever and hepatitis A. Papua New Guinea and the Solomon Islands were particularly high risk countries for malaria (with risks more than ten times greater than any other country) at 266.0 and 147.3 per 100,000 New Zealand travellers respectively. For dengue fever the highest country specific incidence risks per 100,000 New Zealand travellers were found for Samoa (31.8), Tahiti (20.0) and The Cook Islands (17.2). For hepatitis A, Tonga and Samoa stood out as the highest risk countries with incidence risks of 27.7 and 24.3 per 100,000 New Zealand travellers respectively. For the enteric diseases the Pacific and Asia were the regions to which most cases had travelled. India and Samoa together were responsible for almost 64% of travel-related typhoid, Indonesia and India were the most common sources of imported paratyphoid and India, Fiji, Indonesia and Samoa were the sources of over 40% of imported shigellosis.

Regarding characteristics of cases, there were statistically significant differences ( $p < 0.05$ ) in the proportions of cases of each gender for malaria (male to female ratio 2.7:1) and shigellosis (male to female ratio 0.8:1). The incidence risk of hepatitis A was highest in children (aged 0-9 years) whereas for malaria the highest incidence risk was in the 20 – 29 year old group closely followed by 30 – 39 year olds. Dengue was also significantly more common in the middle years, with significantly lower risks in 0-9 year olds and those aged over 60 years compared with other age groups. Regarding ethnicity, the proportion of travel-related cases of hepatitis A that identified as being of Pacific Peoples ethnicity was particularly high however, this finding was difficult to interpret as denominator data on travel by ethnicity was not available, so ethnicity-specific incidence risks could not be calculated.

**Conclusions:** By some measures, the overall risk of travel-related disease could be seen as low. The risk generally declined over the ten year period and there were no fatal cases. However disease risk varied enormously from destination to destination and for some destinations the risk of acquiring diseases was relatively high. Some diseases were more common in travellers of certain age, gender and ethnicity. These findings have implications for prevention as they enable the targeting of pre-travel advice.

# Victoria University of Wellington

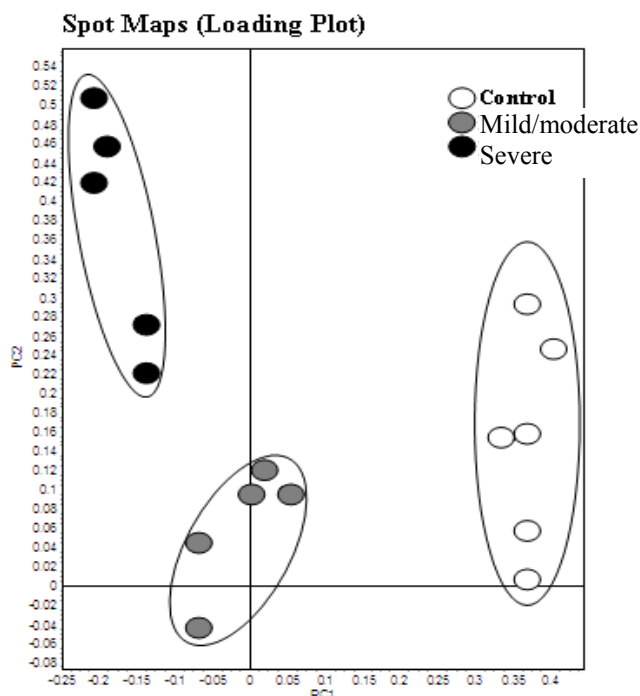
## Diagnostic Markers of Hepatosplenic Schistosomiasis

A. C. La Flamme<sup>1</sup>, W. E. Secor<sup>2</sup>, and T. W. Jordan<sup>1</sup>

<sup>1</sup>School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand and <sup>2</sup>Centers for Disease Control and Prevention, Atlanta, GA USA

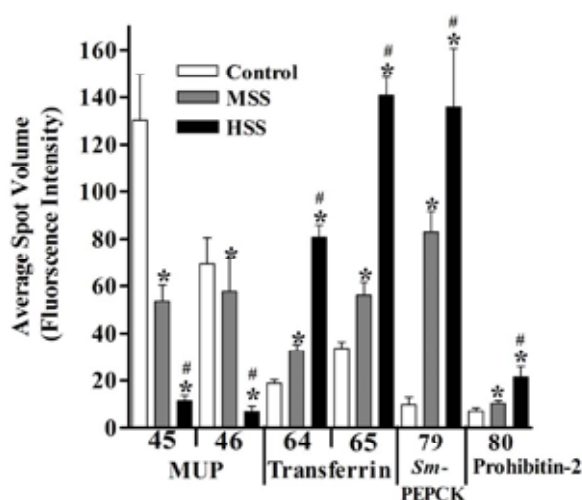
Schistosomiasis is a parasitic disease that afflicts more than 200 million people worldwide. Immune responses to *Schistosoma mansoni* eggs in the liver result in the formation of granulomas, and while granuloma formation is important for protection of the host from proteolytic enzymes, too strong of an immune response can result in life-threatening liver fibrosis. Recent findings suggest that the earlier fibrosis is detected, the more likely it can be reversed upon treatment of schistosome infection. In contrast, persons not treated until later stages of disease are less likely to improve after treatment. Current methods of assessing liver fibrosis are not sensitive enough to detect the initial stages of disease. Thus, development of a serology-based diagnostic tool for persons likely to develop severe pathology would be a valuable asset for improving the public health of persons at risk of schistosomiasis.

To address this issue, this project had two goals: to determine the impact of schistosomiasis on protein expression in the liver and to develop assays to detect these proteins in serum to enable early detection of infected individuals at high risk of developing severe hepatosplenic disease. We used a proteomic approach to investigate and identify the candidate proteins involved in schistosome-mediated liver disease in a mouse model. Comparison of the liver protein expression at 20 weeks of infection demonstrated significant differences in the expression of multiple proteins. A principle component analysis of the changes between uninfected mice and mice with either mild or severe disease revealed that these groups were distinguishable by their protein expression (Figure 1) as all animals within the groups clustered together. This finding supports our original hypothesis that we could distinguish between mild and severe disease by liver protein expression.



**Figure 1: Principle component analysis plot for pH (4-7) spots with 2-fold change and ANOVA  $\leq 0.01$ . Spot maps (2D-DIGE images) show a clear separation of the moderate and severe disease from the control (uninfected) CBA/J mice.**

An in-depth analysis of the individual protein changes that correlated with disease form led to the identification of several candidate markers for schistosome infection and for the hepatosplenic form of disease. Annexin5, glutathione S-transferase Pi class, and *S. mansoni* phosphoenolpyruvate carboxykinase were found to be good markers for schistosome infection (ie significantly changed during either mild or severe disease) while prohibitin 2, transferrin, and major urinary protein changes were unique to severe disease (Figure 2). These changes were verified by Western blotting and we have begun to investigate how the expression of these candidate proteins changes over time. Additionally, our studies will start to correlate the observed changes in liver expression to the presence or absence of these candidates in the serum.



**Figure 2: The abundances of major urinary protein isoforms, transferrin isoforms, *Sm*-PEPCK and prohibitin-2 are significantly altered in severe livers. One way-ANOVA for all spots  $p \leq 0.01$ . \*  $p < 0.05$  compared to control, #  $p < 0.05$  compared to mild by Newman-Keuls Multiple Comparison Test. The numbers on X-axis correspond to the numbers (No.) of protein spots.**

In summary, our proteomic studies revealed a unique picture of protein patterns expressed between mild and severe forms of schistosomiasis and revealed an increased abundance of proteins associated with the energy metabolism, choline metabolism and xenobiotic metabolism. We believe that these proteins may be valuable as potential diagnostic biomarkers that may assist in early detection and treatment of schistosomiasis patients in the future.

### Differential patterns of liver proteins in hepatosplenic schistosomiasis

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*Schistosoma mansoni* eggs produced by adult worms in the mesenteric vasculature become trapped in the liver where they induce granulomatous lesions and strong immune responses. Infected individuals suffer from intestinal schistosomiasis in 90% of cases whereas the remaining 10% present with severe hepatosplenic schistosomiasis. The CBA/J mouse model

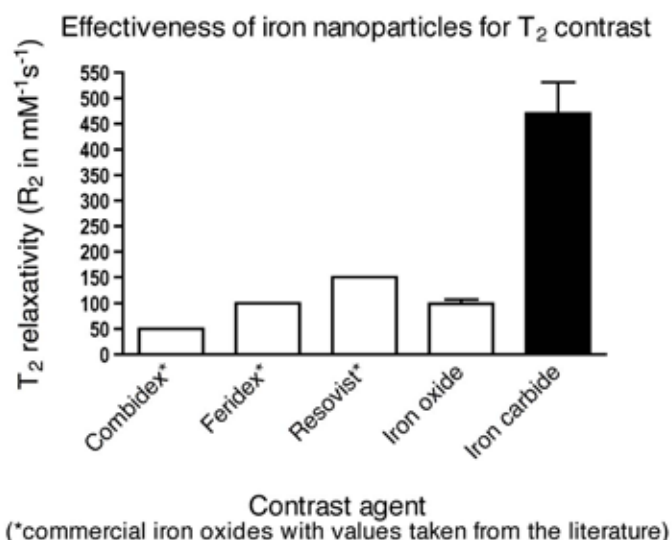
mimics human disease with 20% of infected mice developing hypersplenomegaly syndrome (HSS) that resembles hepatosplenic schistosomiasis and 80% developing moderate splenomegaly syndrome (MSS) similar to intestinal schistosomiasis. We studied differential patterns of protein expression in livers of 20-week infected CBA/J mice with MSS or HSS to understand the molecular changes that underlie these two disease forms. Using Differential In Gel Electrophoresis to identify differentially expressed protein spots, we found 80 protein spots significantly changed with infection and 35 changes specific to severe disease. In particular, the abundance of prohibitin 2, transferrin isoforms and major urinary protein isoforms were significantly altered in HSS mice. Furthermore, annexin5, glutathione S-transferase Pi class, and *S. mansoni* phosphoenolpyruvate carboxykinase expression changed significantly with schistosome infection. These findings indicate that the liver protein abundance differs between MSS and HSS mice and may be used in the development of diagnostic markers for early detection and treatment of hepatosplenic schistosomiasis.

### Iron Carbide Nanoparticles as MRI Contrast Agents

P Ferguson, I Hermans and R Tilley

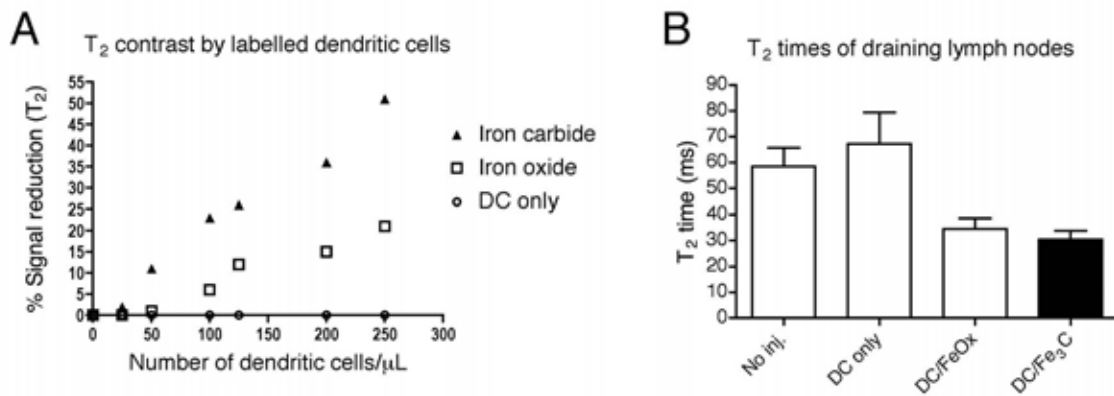
Superparamagnetic iron oxide nanoparticles have been used for two decades as  $T_2$  contrast agents in clinical magnetic resonance imaging (MRI). A novel synthesis has been developed at VUW to produce superparamagnetic nanoparticles with an iron carbide core encased in a shell of iron oxide. The presence of iron carbide produces a dipole moment four times greater than the commercial agents consisting of pure iron oxide.

The first objective of this project was to investigate whether iron carbide nanoparticles could produce better  $T_2$  contrast than iron oxides without an increase in cell toxicity. The iron carbide nanoparticles were conjugated to an organic molecule (DMSA) to produce an aqueous dispersion that is non-toxic in the concentration ranges required for in vitro cell labelling. Our results show that this preparation of iron carbide nanoparticles produces greater  $T_2$  contrast on MRI than a similar preparation of iron oxides (figure 1).



**Figure 1:** Iron carbide nanoparticles produce greater  $T_2$  contrast per unit concentration than iron oxide nanoparticles.

The second objective of this project was to determine the threshold for detecting cells labelled with iron carbide nanoparticles by MRI. We used a mouse dendritic cell model where bone marrow derived dendritic cells stimulated *in vitro* are known to migrate to the draining lymph nodes after subcutaneous injection. We have shown that dendritic cells take up iron carbide nanoparticles *in vitro* with no decrease in cell viability. After labelling, cells can be detected by MRI at a lower concentration than when using iron oxides (figure 2a). Furthermore, we have been able to track the migration of iron carbide labelled immune cells to draining lymph nodes in mice using MRI (figure 2b). This study has been confirmed by a tracking assay using cell labelling with fluorophores and detection by flow cytometry.



**Figure 2:** Labelling DCs with iron carbide nanoparticles produces greater change on  $T_2$  weighted MRI than labelling with iron oxides. (a) Dendritic cells were cultured in 20μg/mL of iron carbides or iron oxides and either dispersed in 1% agar or (b) injected into the forelimbs of mice where the draining lymph nodes were removed after 48 hours. MR Scanning was performed using a Bruker microimaging set at 9.4 Tesla.

The third and major objective of this project is to employ iron carbide nanoparticles to improve the sensitivity of MRI in detecting melanoma. The nanoparticles' specificity for melanoma may be improved by attaching an antibody which binds to melanoma cells. Mouse monoclonal antibody to human melanosomal antigen TRP-1 (Tyrosinase Related Protein 1) has been chosen for its restricted expression in normal tissues but near universal expression in human pigmented melanomas. Our results from flow cytometry confirm anti-TRP-1 also targets a murine melanoma cell line (B16) *in vitro*. Using the B16 cell line in a mouse model, we will determine whether iron carbide nanoparticles can improve the early detection of melanoma *in vivo*.

## **The Cellular Effects of Nicotine and Smoking – Investigating New Cellular Targets for Cessation Therapy**

BM Kivell<sup>1</sup> and P Truman<sup>2</sup>

<sup>1</sup>School of Biological Sciences, Victoria University of Wellington and

<sup>2</sup>Environmental Scientific Research (ESR)

Smoking releases neurotransmitters such as dopamine, serotonin and nor-epinephrine. Dopamine activates the brains reward system, whereas serotonin acts as a mood elevator and nor-epinephrine influences both of these systems. Monoamine transporters function to remove these neurotransmitters from the synapse where they are active, thus terminating the actions of neurotransmitters. Pharmacotherapies that modulate monoamine transporter function may aid the development of more effective anti-addiction therapies.

Very few studies have investigated the effects of smoking on the monoamine transporters. In this study we investigated the effects of nicotine and other constituents of tobacco smoke on the expression and function of monoamine transporters. Using real-time PCR we measured the mRNA levels of serotonin (SERT), dopamine (DAT), and nor-epinephrine (NET) monoamine transporters after chronic (10 days, 0.35 or 3 mg/kg/day) and acute (0.35 mg/kg single injection, euthanized one hour later) exposure to nicotine and total particulate matter (TPM) extracted from cigarette smoke in various regions of the brain. Results have shown that there are no changes in mRNA expression levels of NET in the locus coeruleus between control and nicotine treated rats (n=8), however, there is a significant decrease in expression in animals treated chronically with TPM (n=7, p<0.01). This change was not seen in animals treated acutely with TPM. No changes in SERT or DAT expression levels are seen in the Dorsal Raphe, Substantia Nigra or Ventral Tegmental Area between control, TPM and nicotine treated groups with acute or chronic treatment.

To further our understanding of the effects of nicotine and cigarette smoke on isolated brain regions, protein expression in various regions was measured using Western blotting. Results have shown no change in protein expression of SERT, DAT or NET in response to acute or chronic treatment with nicotine or TPM. In addition to quantifying monoamine transporter mRNA and protein expression, the function of monoamine transporters was also measured in isolated tissue following acute nicotine and TPM treatment. The neurochemical technique called Rotating Disk Electrode Voltammetry (RDEV) has recently been established in my laboratory by PhD student Bridget Simonson. Characterisation and validation of this technique has been completed and uptake kinetics for monoamine transporters in cultured cells and in tissue from control animals has been performed. Preliminary experiments have shown a trend towards an increase in dopamine uptake by DAT in the striatum in response to both TPM and nicotine. However additional replicates will need to be tested before any significant conclusions can be made.

These results show that NET mRNA expression may be altered on exposure to TPM. Interestingly there were no observed changes in expression following nicotine exposure. No significant changes in protein expression have been seen in Western blot experiments, however preliminary experiments suggest DAT function may be increased following acute nicotine and TPM exposure. These findings are important as they increase our understanding of addiction to smoking. Traditional cessation treatments focus on nicotine, but other components of cigarette smoke have addictive properties and may alter brain neurochemistry leading to addiction. Further experiments investigating changes in monoamine transporter function following chronic nicotine and TPM exposure will determine if these proteins are desirable targets for the development of anti-smoking pharmacotherapies.

## **Project Grants 2009**

The following projects were approved for funding in May 2009 and will be reported on in subsequent Annual Reports of the Foundation.

### **Mike Berridge**

Malaghan Institute of Medical Research

#### **Investigating immunotherapy targeted at tumour stem cells within brain tumours**

This grant will facilitate the purchase of stereotaxic equipment to inject tumour cells. This in turn will enable the researches to model immunotherapy of brain tumours and leukaemias with CNS involvement.

### **Vinko Besic**

The Wakefield Biomedical Research Unit, Department of Pathology and Molecular Medicine, University of Otago, Wellington.

#### **A study of hepatic insulin receptor signalling using si RNA-based gene silencing**

The information expected to come from this series of experiments will advance knowledge concerning the defects in hepatic insulin signalling which may be associated with the type 2 diabetes mellitus.

### **Alan Clark**

Victoria University of Wellington

#### **Kinetic and fluorometric studies on mechanisms of inhibition of human prostaglandin D synthase**

Prostaglandins (PG) are a family of structurally related eicosanoids that have regulatory roles in both normal physiological and pathological contexts. The outcomes of this work are expected to provide a substantial platform from which structure-based drug design initiatives can be developed.

### **Joyce Colussi-Mas**

Victoria University of Wellington

#### **Use of in vivo microdialysis to measure neurochemical consequences of MDMA self-administration**

The aim of this research is to determine neurochemical correlates of drug self administration. The information will ultimately lead to a greater understanding of the neurochemistry of compulsive drug use and will help to inform effective therapeutic interventions.

### **Sally Evers**

University of Otago, Wellington

#### **Effect of regular paracetamol on asthma control in mild to moderate asthma**

The aims of this study are to determine whether the regular use of paracetamol in subjects with mild to moderate asthma leads to an increase in asthma severity, and to determine whether the regular use of paracetamol influences the key physiological, clinical and immunological characteristics of asthma.

**Chandra Kirana**

The Wakefield Biomedical Research Unit, Department of Pathology and Molecular Medicine, University of Otago, Wellington.

**Protein Biomarkers for Predicting Colorectal Cancer Spread.**

This project aims to identify biomarkers which can stratify CRC patients according to their risk of subsequent spread.

**Bronwyn Kivell**

Victoria University of Wellington

**Investigating Novel Compounds to Treat Addiction**

The long term goal of this study is to identify the cellular mechanism by which novel and traditional components modulate the function of the dopamine transporter. Benefits may also include the development of therapeutic drugs to treat addiction.

**Jeremy Krebs**

Diabetes Research Centre, Wellington Hospital

**The effects of caffeine and coffee on glucose tolerance and insulin sensitivity in healthy and diabetic subjects.**

The objective of this study is to determine the effect of a single dose of caffeine versus placebo, and caffeinated coffee versus decaffeinated coffee, on insulin sensitivity and glucose tolerance in men and women with and without type 2 diabetes.

**Jason Low**

Victoria University Wellington

**Can children with autism use inner speech to support working memory?**

The results of this study will provide an accurate picture of the interface between language and executive functioning amongst children with autism. Information from the study will aid in the design of more targeted and effective cognitive interventions for the population at a clinical and grassroots level.

**Trols Petersen**

Malaghan Institute of Medical Research

**Characterisation of cross-presenting langerin-positive dendritic cells in the spleen**

The potential significance of this project is that because of the range of the functions attributed to dendritic cells as a whole, it is important to establish whether all effector functions are carried out by the same DCs or whether

different functions are carried out by different subsets. It then becomes possible to specifically target and manipulate individual DC subsets clinically in a range of diseases.

**Lynette Sadler**

University of Otago, Wellington

**Genetics of Epilepsy**

The objectives of this study are to describe the clinical and genetic features of epilepsies in families with many affected individuals. Finding new epilepsy syndromes and the genes responsible for these will allow more accurate diagnosis and prognosis, and lead to novel improved therapies.

**Penelope Truman**

Institute of Environmental Science and Research Ltd.

**Tobacco smoke as an addictive substance - more than just the devil we know**

This study will assist in the overall objective of developing improved pharmacological aids to smoking cessation and thus in helping people to quit smoking.

**Shieak Tzeng**

University of Otago, Wellington

**Baroreflex Sensitivity in Heart Failure**

The objective of this project is to determine whether breathing frequency modulates baroreflex sensitivity in healthy subjects and patients with congestive heart failure.

**Ellen Woodcock**

University of Otago, Wellington

**Characteristics of Ventricular Fibrillation in an isolated perfused rabbit heart**

Sudden cardiac death due to ventricular fibrillation is a leading cause of mortality in western countries. This project will establish and validate an isolated perfused heart model in which ventricular fibrillation can be induced and studied.

## **Travel Grants**

### **2008 Gordon Research Conference "Immunochemistry and Immunobiology"**

H Simkins

Funding from the WMRF enabled me to attend the Gordon Research conference "Immunochemistry and Immunobiology" held at the Magdalen College in Oxford, England, from 17 – 22 August of this year. The conference was made up of only approximately 130 people from all over the world making it quite an international meeting. The small number of attendees along with the wide range of people encouraged many interesting discussions with top international scientists. It also allowed me to meet and get to know leading immunologists as well as other students and post-docs who have become valuable contacts for the future. The conference also encouraged speakers to present unpublished data, thus allowing us to hear about the latest developments in the field of immunology.

The conference was filled with a series of demanding but fascinating lectures on many different aspects of immunology, from lymphocyte activation and signal transduction to innate and mucosal immunity as well as a session on autoimmunity. Throughout the week each afternoon there was a poster session. The posters being presented were placed up for two sessions each. This allowed plenty of time for everyone to see all the posters and facilitated great discussion. I presented a poster on my project, entitled "Reduced numbers of splenic CD8<sup>+</sup> DC after  $\alpha$ -GalCer administration". After attending the conference I also had the opportunity to visit a number of prestigious laboratories in the United Kingdom and Europe. Whilst visiting these laboratories I presented my work in the form of an oral presentation. I also had many one on one discussions with PhD students, post docs and principle investigators about their research. This enabled me to further widen my contacts that I had made at the conference and identify a possible laboratory to undertake a postdoctoral position.

### **7<sup>th</sup> Annual Conference Louis Pasteur**

L Green

On the 11 - 13 November I attended the 7<sup>th</sup> Annual Conference Louis Pasteur hosted by the Institut Pasteur in Paris, France. Being the 120th anniversary of The Institut, the meeting, entitled "Understand and Controlling Infectious Diseases: an agenda for the 21<sup>st</sup> century", aimed to discuss how current research methodologies might advance our ability to understand, monitor, and combat infectious disease with particular reference to the Millennium Development Goals. The meeting included a wide range of presentations from renowned researchers in fields ranging from epidemiology and public health to immunology and microbiology.

Highlights include a thought-provoking lecture given by Robert, Professor Lord May, of Oxford University, England. With a background in ecology, his

presentation focused on current public health initiatives related to the United Nations Millennium Development Goals. He boldly challenges the United Nations omission of "population control" from its directives and offered his own very controversial opinion as to why this would be the case. Another entertaining presentation, delivered by Dr Richard Hatchet of the National Institute of Health, USA, discussed how policy makers dissect and relay scientific information derived from biological modelling systems.

More related to my own topic of research was a presentation by Dr Arturo Zychlinsky of the Max Planck Institute for Infection Biology, Germany. Dr Zychlinsky has identified an additional mechanism leukocytes use to clear bacterial pathogens in combination with ROS. According to his research, a specific population of leukocytes "discharge" a neutrophil extracellular trap (NET) to "capture" and break-down bacterial threats. Information from this particular seminar has proven extremely valuable in setting specific parameters for my own research.

My poster "*Pseudomonas aeruginosa* Pathogenesis: assessing the role of soluble nitro- and quinone reductases", was well received and generated good discussion on the topic. Since my return, a conference report has been presented to the laboratory and shared within the Microbiology Department here at Victoria University.

### **10th International Congress of Behavioural Medicine, Tokyo, 2008, 27 – 30 August 2008**

W Levack

At this conference I had the opportunity to share some findings from my recently completed PhD in an oral presentation entitled: 'Navigating the borderlands of patient-centered goal planning: a grounded theory investigation'. I also had the opportunity to attend several sessions on health psychology research at this conference, which have since significantly informed my post-doctoral research.

One noteworthy observation from the conference was how much emphasis has been placed on theory development in health psychology in comparison to the more pragmatic discipline of rehabilitation. Correspondingly, there is still much work to do in health psychology to investigate the applications of such theories in applied settings such as rehabilitation. The disciplines thus appear to have much to gain from collaborative work with each other.

From my perspective, as a rehabilitation researcher, this conference strengthened an opinion that theory development will be an important part of the future growth of rehabilitation as a science. It was this conference that first drew my attention to the United Kingdom's Medical Research Council's recently revised guidelines for "*Developing and Evaluating Complex Interventions*" ([www.mrc.ac.uk/complexinterventionsguidance](http://www.mrc.ac.uk/complexinterventionsguidance)). These guidelines highlight theory development as an essential component of any research programme into a complex intervention and consequentially provide strong support for the future funding of such research.

Attending international-level conferences and having the opportunity to meet and exchange ideas with leading academics from around the world is very important for the advancement of emerging researchers from New Zealand. I have certainly gained a lot from the experience and the new knowledge and networks arising from this conference continue to influence my work. Many thanks for your support.

### **Keystone Symposia Conference and Laboratory Tour USA, February - March 2009**

L Goldsack

On 8 – 13 February this year I attended the Keystone Symposia Conference "Immunologic Memory and Host Defense" held at Keystone, Colorado, USA. This meeting had a fantastic attendance including many leaders in the field of immunological memory, who were available to discuss ideas and scientific data with. Following on from the conference I carried out a laboratory tour across the United States, visiting six scientists that are well known for their outstanding research in the field of immunological memory. I had the opportunity to share and discuss both my own research and the research conducted in the different laboratories. This experience has been very rewarding for me and I wish to acknowledge the financial support provided by the Wellington Medical Research Foundation that made this possible.

The Keystone Symposia series are prestigious forums, which focus on specific topics, therefore the plenary speakers at the meeting were at the top-of-the field and gave highly relevant presentations. One of the great benefits of attending this meeting was that the majority of data presented was unpublished, providing an amazing source of information and an overall sense of the current and future directions of the field.

During my visit to the USA I visited several prominent scientists and their laboratories which are well known for ground breaking research, including: Dr David Woodland, President of the Trudeau Institute, Saranac Lake, New York, Professor Marc Jenkins, University of Minnesota, Dr Robert Seder, Vaccine Research Centre, National Institutes of Health, Professor Seven Reiner, University of Pennsylvania, Professor Donna Farber, University of Maryland and Dr John Wherry, Wistar Institute, Philadelphia, Pennsylvania. At each of the laboratories I visited, I gave a presentation on the role that memory CD4<sup>+</sup> T cells play in protection against Tuberculosis. This was a fantastic opportunity to receive feedback, suggestions and professional critique from the leaders in the field.

My visit to the Trudeau Institute was very rewarding for our laboratory, as we have gained a new collaboration with one of their scientist, Dr Andrea Cooper, who has agreed to send us a very valuable transgenic mouse strain, which contain T cells that are specific for a protein from the BCG vaccine for Tuberculosis. This mouse model will be an amazing tool for our laboratory and will allow us to conduct research in the field of Tuberculosis and

immunological memory to a standard that is equal and beyond the research that is carried out overseas.

Lastly, as a result of this trip I have been given a very exciting opportunity, as I have been offered post-doctoral positions in several of the laboratories I visited. Working in any of these laboratories for a period of time will be an amazing opportunity to create and strengthen networks with other international scientists as well as learn invaluable skills in the laboratory that I can bring back to New Zealand.

## Summer Student Research Reports



### **Health professionals perception of team work in the management of chronic conditions in primary health care**

Christopher Badenhorst

The purpose of this study was to gain an understanding of the current perception which health professionals have with regards to team work in primary care. This involved interviewing 4 health professionals, one nurse and one doctor at two very different medical practices.

The main findings of this study were:

1. At present health professionals have a good understanding of the “concept of team work” and believe that they are working well together as a team. Compared to other studies, what health professionals described could be seen more as collaboration as opposed to “teamwork.”
2. All health professionals agreed that approaching chronic conditions as a team was the only way to go.
3. Doctors and Nurses have different perceptions of where the patient fits into the team but both agree the patient is essential to the team.
4. Doctors today have started to recognise the expertise of nurses and appreciate how much of an asset they are.
5. Effective communication seemed to be one of the most important elements necessary if any form of team work is to be achieved.

Effective team work has undisputedly been shown as the best way to address chronic health care problems. For this reason it is vital that health professional’s practices are in line with their perception that team work is important.

## Opportunities for alcohol and other drug advice in the GP consultation



Laura Chen

The aim of this project was to identify opportunities for advice on alcohol and other drugs (AOD) in the GP consultation, to analyse the AOD discussion that took place and to identify things that might help or hinder the discussion of AOD.

For this we studied a subset of 27 video recorded naturally occurring GP consultations from the ARCH corpus was selected for this study as they contained topics where AOD discussion was likely to take place. 26 recordings were complete and suitable for studies through conversational analysis of the AOD discussion in the consultation. Conversation patterns were identified. There were eight GPs with consultations in this study and three of them were interviewed to gain further insight into the AOD discussion in the clinical setting.

Results were that AOD conversation does not happen as often as might be expected. Only 69% (18/27) of these preselected consultations contained any mention of AOD. AOD advice was only given in 56% of those (10/18). Discomfort was noticed in both the patient and GP when discussing AOD. The patients expressed their discomfort with their body language and by getting defensive when asked about their AOD use. GPs expressed their discomfort by stuttering, pausing, and rephrasing things and not mentioning AOD again even in the consultation summary. Discussion about smoking was handled better than alcohol. The GPs themselves identified time pressure and sensitivity of the topic as the main barriers to talking about AOD.

The uncomfortable AOD interactions can be explained by mutual face work. GPs give and accept understatement about AOD to help the patient save face and avoid discussing a potentially threatening topic with the patient. The patient keeps face by giving socially acceptable answers and providing the GP with reason to end the AOD discussion. Not only are opportunities missed, but also both the GP and patient actively work to avoid opportunities to discuss AOD in the consultation.



## Human serum extracellular superoxide dismutase variation in a gout patient population

Neal Kerr

Gout is a common and intensely painful form of arthritis affecting approximately 10% of New Zealand males during some stage of adult life. The inflammation characteristic of gout is in response to the deposition of Monosodium Urate crystals (MSU) within synovial joints. Associated with this inflammatory condition is the elevation of the damaging superoxide anion ( $O_2^{\cdot-}$ ). Biological defence against superoxide anion toxicity is through superoxide dismutase (SOD), an enzyme that converts superoxide into hydrogen peroxide and water. The objective of the present study was to determine the relationship between serum SOD activity and the clinical occurrence of gout.

Serum samples from non-gouty (n=45) and gouty (n=46) subjects were analysed for SOD activity using a superoxide inhibition assay. Results showed mean serum SOD activity was significantly greater for gout subjects compared with non-gouty controls (95.69 vs 59.43 ng/ml,  $P < 0.05$ ). A trend towards increasing SOD activity with disease progression was observed, however, this was not statistically significant. Correlations between SOD and IL-1 $\beta$ , TNF- $\alpha$  and MCP-1 were also found in the non-gouty population only. Associations between serum SOD and other biological parameters i.e. IL-1 $\beta$ , MCP-1, TNF- $\alpha$ , IL-8, NO $^{\cdot-}$ , Uric Acid, IL-6 or CRP were not observed in the gout group. SOD demonstrated an association with NO $^{\cdot-}$  specific to the asymptomatic hyperuricaemic group alone identifying a possible avenue for research.

In summary the higher level of SOD activity demonstrated in the gout population may indicate a protective mechanism by which SOD activity is elevated in response to inflammation to prevent superoxide anion toxicity.



### **Defining core knowledge: exploring expectations of teachers and students in medical education**

Sarah Lucas

In the fourth year Surgical and Clinical Skills module at the Wellington School of Medicine and Health Sciences in 2008, medical students were required to create a brief summary “core topic” of an important surgical condition. These core topics were required to be aimed at the level of a graduating medical student. It was thought that these topics may prove a useful educational resource for the developing learner based curriculum. Each of the topics were reviewed by other students, a student editing committee and a specialist in that field (usually a surgical registrar).

To assess the core topics for appropriate level, quality and usefulness as a resource; they were sent to General Practitioners (GPs) and Surgical Specialists throughout New Zealand for evaluation using a standardised survey template.

The survey revealed that the core topics may make useful educational resources provided they are reviewed [but they were reviewed], referenced, current and evidence based. They were generally rated positively in the quality measures surveyed, with results suggesting that on average the core topics were “at the correct level” of core knowledge required for a graduating medical student. GPs rated the topics higher than the surgeons in all areas studied, suggesting that presented with the same information; doctors of different specialities have different expectations of the core knowledge required of students. The expectations of GPs and students were also much more closely aligned than that of surgeons and students. This has important implications for curriculum development as well as for the future possibility of defining the knowledge required of a medical school graduate using an outcomes database or other means.



### **Is the clinician a barrier to ICD referral in New Zealand?**

Ben McHale

Sudden cardiac death (SCD) is the leading mechanism of death in New Zealand (NZ). For patients identified at high risk of SCD, implantable cardioverter defibrillators (ICDs) have been demonstrated to be the most effective therapy at reducing mortality, and are therefore considered the standard of care.

Despite our high rate of SCD, the rate at which we implant ICDs to prevent SCD is low by international standards. This implies that patients who are at

high risk, and should have an ICD, are not receiving these devices and may therefore die prematurely. This study aimed to investigate the role of the primary clinician's knowledge of and attitudes towards ICDs as a potential barrier to patients receiving ICDs in NZ. To do this, we surveyed 100 cardiologists and general physicians throughout NZ who look after patients with heart disease.

We found that perceived lack of funding and concerns about device cost and cost effectiveness, are likely to act as important barriers to ICD referral. Other important issues are; uncertainty and lack of transparency within the referral process, perceived lack of expertise and inadequate knowledge of indications for ICD referral. The lack of published NZ guidelines may contribute to many of these concerns. We also found that doctors caring for potential ICD candidates in rural hospitals were more likely to be general physicians, have a poorer knowledge of the indications for referral, be less familiar with implantation guidelines and have restricted access to important investigations required for ICD referral. This may limit access to ICD therapy for rural New Zealanders.

Overall, our study suggests that the clinician can be an important barrier to ICD referral and has highlighted areas that need addressing to improve New Zealand's ICD service.



**Do emergency doctors calculate a risk score before requesting D-dimer assays in patients with possible thromboembolic disease?**

Chani Tomop van Dalen

Venous Thromboembolism (VTE) occurs when blood clots in the deep veins of the leg (DVT) or in the arteries of the lung (PE). The Wells score is an internationally recognised method of assessing a patient's risk for VTE before any other tests are performed. At Wellington Hospital the Suspected VTE guidelines recommend that the Wells score is performed on all patients suspected of VTE prior to undergoing a D-dimer blood test. A negative D-dimer test can be used to rule out the presence of VTE in those patients who have a low Wells score, limiting the need for imaging studies which would otherwise consume hospital resources.

An audit of all D-dimer tests requested through the Emergency Department (ED) during January 2008 was performed using patient notes. Compliance with the Suspected VTE guideline was found to be 9%. Separate educational presentations were then given to the ED doctors and nurses, which provided them with information about the Wells score, D-dimers and appropriate use of the Suspected VTE guideline. A follow-up audit was then performed and compliance with the guideline was found to have increased to 20%.

Improving knowledge made some difference to compliance with the guidelines but there is still much scope for improvement. It is likely that there is a systems problem in the ED, which is resulting in low use of the Suspected VTE guidelines. Further research into reasons for low compliance with the Suspected VTE guideline and a review of the system in place for authorising D-dimer testing would be valuable.

