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Editorial

If there was to be a patron saint for researchers, then one of the Princes of Serendip must be a strong contender. These heroes of the Ceylonese fairytale *The Three Princes of Serendip* were always making discoveries which, as Horace Walpole noted, were by 'accidents and sagacity of things they were not in quest off'. It was from this fairy tale that Walpole, in 1754, coined the word serendipity to signify a faculty for making happy chance findings.

While the basic tenet of science is the reasoned testing of hypothesis, it is well recognised that a chance finding may take a project down a path different from that intended. The results of any such diversion, should themselves, be subjected to appropriate scrutiny and evaluation, and often the value of a serendipitous discovery is only realised after many years of labour.

Medical science has in its history many examples of significant chance findings that have led to important advances. Röntgen's discovery of X-rays was, at the time, unexpected; however, he was able to develop this into an important tool which now has a wide application in science, industry and medicine. Perhaps the most famous piece of medical serendipity was Fleming's discovery of penicillin, although this took years of dedication by Florey and Chain before penicillin could be produced in clinically useful amounts.

Much research is now funded on a contract basis with defined outputs and tight budgets. Of necessity, interesting and potentially valuable avenues of investigation may be overlooked which limits the opportunity for the development of parallel studies. While serendipity is an unreliable form of progress, its occurrence often provides a significant advance in scientific knowledge. In recognising this, the Foundation is pleased to foster research that is based on sound scientific principles and encourages researchers to pursue their activities in novel directions.

The current educational and research funding environment has led to declining opportunities for medical research. The introduction of tuition fees by universities, in response to reduced Government funding, has meant that students are driven to complete their degrees in minimum time, qualify in a specialist vocation and enter the workforce as a clinician. The necessity to commence clinical practice at the earliest opportunity is a consequence of the high levels of debt that students accrue during their undergraduate careers, with many new graduates owing an amount equivalent to a home mortgage.

In the past many medical students opted for a gap-year in their training to undertake a one-year research degree and it is recognised that many successful academic careers developed from the work undertaken during this period. The concern that an extra year as a student will produce additional debt has led to a decline in interest in these degrees. Similarly, fewer medical graduates

undertake training towards higher degrees and as a result many potentially excellent researchers are lost to the profession. In some cases academic careers are commenced without the possession of a higher degree and with little research experience, which compounds the difficulty these staff have in obtaining research funding in an already highly competitive environment.

The Wellington Medical Research Foundation acknowledges the difficulties faced by junior academics and for this reason priority is given for funding researchers in the early stages of their careers. On several occasions this has provided seeding to allow the development of a line of investigation to the stage that major project funding has been obtained. In this way the Foundation serves to foster research beyond the provision of financial support by assisting promising junior academics to develop a research career pathway.

Professor Brett Delahunt
Editor

Research Advisory Committee Membership

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

Malaghan Institute of Medical Research

Epicutaneous immunisation as a new cancer immunotherapy?

P Stoitzner

Background

Dendritic cells (DC) are immune cells that are very important in initiating immune responses. Immunotherapy using dendritic cells loaded with tumour material has proved to be effective in some patients, leading to a decrease in tumour burden and prolonged survival. Nonetheless, the need for fully autologous vaccines that must be generated from patient's blood, loaded with tumour antigens and reinjected into patients makes this procedure laborious and expensive. Targeting skin DC by immunisation through the skin may overcome some of these limitations. Immunisation strategies through the skin have proven to be feasible and elicit both systemic and mucosal immunity. For effective cancer immunotherapy it is desirable to activate both the CD4+ and CD8+ T cell subsets, so that CD4+T cell can provide help for CD8+T cell effector function and memory development. Furthermore, the relative contribution of individual populations of skin DC, the epidermal Langerhans cells (LC) and the dermal DC, to the induction of immune responses through the skin needs to be understood to improve the cancer treatment.

Aims

In this study we asked whether epicutaneous immunisation with a tumour model antigen is effective at inducing anti-tumour responses, and whether Langerhans cells are the main players in the induction of these cellular immune responses.

Results

T cells become activated after immunisation through the skin

Dendritic cells capture, process and present antigen to T cells in lymphoid tissue. CD4+T cells differentiate into T helper cells and contribute to the productive activation of CD8+ T cells. Activated CD8+T cells become cytotoxic and are able to kill tumour cells. Both the CD4+ and CD8+ T cell subsets are likely to be needed for the destruction of tumour cells. When we performed immunisation through the skin both T cell subsets proliferated vigorously and to a similar extent. The CD8+T cells developed cytotoxic activity, produced the cytokine IFN-γ and developed into memory T cells. When we challenged immunised mice with melanoma cells, they were protected from tumour growth. Unfortunately we could not determine if CD4 T cell help is required for the development of effector

CD8+T cells since MHC II knock-out mice, which lack CD4+ T cells, rejected tumours spontaneously.

Langerhans cells are major players in skin immunisation

In all the reports about skin immunisation, the precise role of skin dendritic cells, Langerhans cells versus dermal dendritic cells was not defined. However, understanding the contribution of both skin dendritic cell subsets to the anti-tumour response is important to improve the efficiency of this immunisation strategy. We addressed this question by using transgenic mice that express the human diphtheria toxin receptor under the control of a promoter specifically expressed in Langerhans cells, the Langerin promoter. When mice are injected with diphtheria toxin, the Langerhans cells undergo apoptosis within 24h and no Langerhans cells can be demonstrated in the epidermis for days to weeks. We used diphtheria toxin injections to deplete Langerhans cells in transgenic mice, and immunised them through the skin. In contrast to control mice, mice lacking Langerhans cells were no longer protected from tumour growth indicating a major role for Langerhans cells in stimulating the anti-tumour response.

Skin immunisation can be improved with adjuvants

For most of our experiments we injected mice with antigen-specific CD8+T cells to facilitate the measurement of T cell responses. When these mice were challenged with tumour cells after epicutanous immunisation they were completely protected from tumour growth. However, these mice had an artificially high frequency of antigen-specific CD8+T cells. Thus, we performed experiments in mice with a normal frequency of effector T cells and found that immunisation through the skin delayed but did not prevent tumour growth in these mice. It has been previously described that an adjuvant is required to achieve potent cytotoxic T cell responses with immunisation strategies through the skin. We chose two different ways to activate the skin dendritic cells during immunisation. The skin barrier can be disturbed by tape stripping which is a repeated application of sticky tape to the skin. We found that tape stripping prior to antigen application on the skin was pivotal for potent and long-lasting T cell responses. Additionally, we tested the toll-like receptor ligand imiquimod since it can give a danger signal to different cells and induce Langerhans cell migration. We could further delay tumour growth when we applied the tumour model antigen in a cream containing imiquimod on tape stripped skin, suggesting that the anti-tumour effects we can generate in a “more-patient like” situation can be further improved.

Conclusion

Immunisation through the skin to treat skin cancer is a very attractive approach since it is easy to perform and not as cost intensive as other immunotherapies. The results we obtained in this study demonstrate that immunizing with a tumour

model antigen through barrier-disrupted skin with the addition of a danger signal, like a toll-like receptor ligand, can stimulate potent CD4+ and CD8+T cell responses. The T cells develop into effector cells and acquire cytotoxic activity and produce cytokines. Immunisation through the skin with a tumour model antigen leads to control of tumour growth and this requires the presence of Langerhans cells, one of the skin dendritic cell subsets. The knowledge we gained in this study will help us to further develop this vaccination strategy and may lead to a new form of cancer immunotherapy.

Immune Inflammation in Neutrophilic disease – A study of Gouty Arthritis

R Grainger, JL Harper

A gout attack occurs as a result of the deposition of mono-sodium urate (MSU) crystals in the joints triggering cellular infiltration, swelling of the joint and intense pain. Gout is reported to be increasing worldwide and the prevalence in New Zealand is especially high, owing to the predilection of Maori and Pacific Island people towards elevated levels of uric acid in the blood (hyperuricaemia). Interestingly, only 20% of hyperuricaemics develop gout and it is unclear why the other 80% remain asymptomatic.

This project aims to determine whether immune cells from hyperuricaemic gouty and non-gouty individuals respond differently to exposure to MSU and if these cells produce inflammatory markers that might indicate susceptibility to developing gout.

Monocyte and neutrophils are recognised as key immune cells involved in the inflammatory response to MSU in gout patients. For this reason we have made them the target cells for our clinical study. Monocytes and neutrophils can be readily isolated from human blood for testing. Using both mixed and isolated immune cells we have now established robust screening assays to measure the activation status of monocytes and neutrophils. The parameters to be investigated include cell surface markers, the production of inflammatory cytokines including IL-1□, TNF□, IL-6, IL-8 and release of damage-causing reactive oxygen species such as superoxide.

Recruitment of participants, blood collection and testing is now underway and cells and sera are being collected from acute and chronic gout patients, hyperuricaemic non-gouty patients and healthy volunteers. The results of this study will used to determine if differences in cell immune function are responsible for susceptibility towards or protection from developing gouty arthritis and whether these trends are useful prognostic markers for gout.

Investigating the interaction between regulatory T cells and antigen presenting cells: Lessons to learn for potential therapeutics of Multiple Sclerosis

Y Bai, E Spittle, S Mirmoeini, BT Bäckström

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease, in which the immune response is directed against the myelin sheath that surrounds axons of the central nervous system. The disease usually starts between 20 to 40 years of age for humans and leads to substantial disability through deficits of motor and sensory nerve function. In New Zealand, more than 4,000 patients have MS and approximately 200 people are diagnosed with the disease every year. Experimental autoimmune encephalomyelitis (EAE) is the best-defined experimental model of MS in mice and resembles the immunopathology in human MS. EAE has been utilized extensively to study the cause and potential treatments of MS.

In a previous study, we have found that a modified superantigen (mSag) coupled to a central nervous system-specific peptide (myelin oligodendrocyte glycoprotein 35-55, MOG_{35-55}) protects from disease, when administered into mice. The mechanism for this protection is not known, but we have proposed that it involves the expansion and/or activation of a population of MOG_{35-55}-specific T regulatory cells (Tregs).

The main feature of Tregs is their ability to suppress immune responses. Tregs from peripheral blood of MS patients have been found to have a significant deficiency in their suppressive function compared with healthy donors. It is proposed that the deficiency of these Tregs leads to a failure to inhibit self-reactive T cells, which results in MS. Therefore, a method which restores the suppressive function of Tregs in patients with MS has potential to be used clinically to help MS patients.

Aim

It is known that antigen presenting cells (APCs) are important for Tregs to acquire their suppressive function. The overall aim of the proposed research is to investigate the cellular and molecular mechanisms by which Tregs inhibit autoreactive effector T cells in EAE with the focus on the interactions between Tregs and APCs during immune suppression.

Results

There is a large number of APCs in the body. However, only a few APCs have the ability to present antigens to Tregs. It has been well documented that Sags

bind to major histocompatibility complex (MHC) class II molecules on APCs and we have showed that the mSag could deliver MOG_{35-55} to Tregs and induce Tregs activation. To identify which specific subpopulation of APCs the mSag binds to, we labelled the mSag with a fluorescent dye, Alexa 488, and injected mSag-Alexa 488 into mice to enable it to bind to APCs.

Shortly thereafter, mice were bled and the cells that had specifically taken up the mSag were identified using flow cytometry. The labelled mSag was detected on MHC class II+ APCs. More importantly, the mSag was also found to bind to a distinct subpopulation (Figure 1). This cell population showed a phenotype of CD11b+MHCII-F4/80^{intermediate} and was prominent in the blood. In the spleen, we found that the same population of cells had bound mSag, although fewer than what we found in blood. mSag-binding cells were absent in the lymph nodes. In order to account for non-receptor mediated uptake of protein molecules in the blood, ovalbumin (OVA) protein and a control version of mSag were labelled and used as negative controls. The control mSag (DM) has a mutated MHC class II binding site, and therefore cannot bind to the MHC class II molecules on cells. Negative staining of OVA-Alexa 488 indicated the staining was not caused by non-specific general uptake of proteins, while positive staining of DM-Alexa 488 on the same population suggested that a specific second receptor (distinct from MHC class II) for superantigen binding to APCs exists. To verify this, genetically modified mice lacking MHC class II molecules were tested for binding. Results showed that the DM-mSag in fact could bind APCs from MHC class II deficient mice (Figure 1). Therefore, the presence of MHC class II molecules is not necessary for binding of the mSag to this distinct newly identified cell subset.

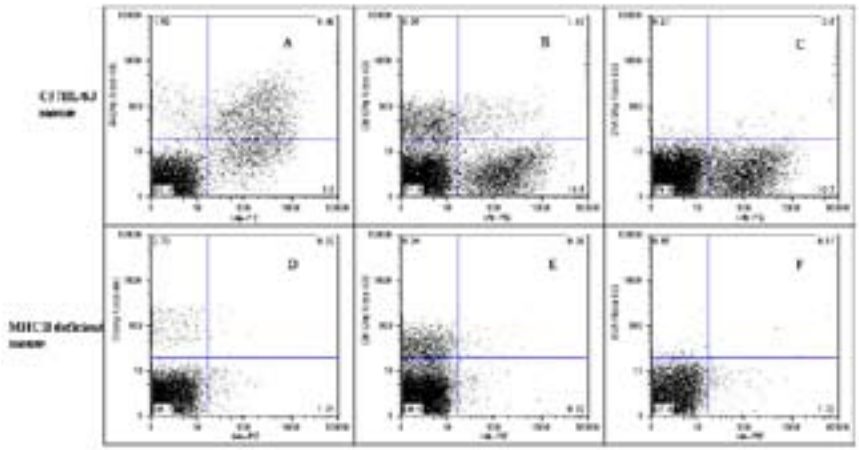

Figure 1. A distinct blood-borne cell population showed positive staining for mSag. Mice were injected with 50 µg Alexa 488 labelled mSag, DM Sag or OVA respectively. Three hours later, blood cells were collected and analysed. A-C, Blood cells from C57BL/6 mice injected with Alexa 488 labelled mSag, DM Sag or OVA respectively; D-F, Blood cells from MHCII deficient transgenic mice injected with Alexa 488 labelled Sag, DM Sag or OVA respectively. The plots shown were gated on live cells.

The newly discovered receptor may be expressed on cells involved in disease suppression.

To test whether the binding to the blood-borne cells has therapeutic value, DM SAg-MOG_{35-55} was injected subcutaneously into C57BL/6 mice 5 days after disease induction. As shown in Figure 2, the onset of the disease in mice received DM Sag- MOG_{35-55} treatment was delayed and the symptoms were ameliorated.

Since the only DM Sag-binding cells discovered so far are F4/80^{intermediate}CD11b+ blood cells, these cells may be responsible for the suppression observed (Figure 2). Although we have previously found that mSag generates suppressor CD25+ T cells, our new evidence shows that another cell population also participates in the disease suppression. It is possible that the blood-borne cells binding to mSag may be the cells delivering the stimulating peptide MOG_{35-55} to Tregs and causing disease-suppression.

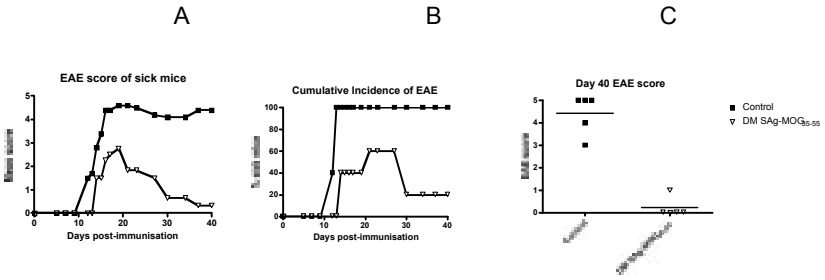

Figure 2. DM SAg-MOG_{35-55} is able to suppress EAE. Disease was induced in two groups of mice (n=5). Five days later, 1 group was injected with 1 μg DM SAg-MOG_{35-55} subcutaneously, and the other group was not treated as controls. A. EAE scores of sick mice. DM SAg-MOG_{35-55} treated mice have ameliorated symptoms of EAE. B. Cumulative incidence of EAE. DM Sag- MOG_{35-55} treated mice developed EAE later and the percentage of sick mice was reduced. C. EAE scores 40 days after EAE induction.

Conclusions and future directions

The animal model has been established to investigate the mechanisms by which mSAg-MOG_{35-55} suppresses EAE, various experimental methods have been validated and the target cells have been identified. We are now focusing on the cellular and molecular mechanisms by which mSag suppress EAE. We hypothesize that mSags binds to and change certain properties of the specific blood-borne cells identified in this study. Such changes endow these cells with the ability to suppress EAE. Identification of the underlying mechanisms is

important to develop an immuno-therapy to prevent and treat MS and other autoimmune diseases.

Signalling Lipid Compounds as a Novel Therapy for Tuberculosis

J Kirman, M Vyssotski, M Denis

Tuberculosis (Tb) was declared a global emergency in 1993 by the World Health Organization (WHO), and every year it still claims 1.8 million lives and newly infects >8 million people. With this project grant we proposed to undertake preliminary research to investigate the efficacy of a potential novel class of Tb therapeutics that had shown promise in a preliminary study. N-acylethanolamines (NAEs) are naturally occurring derivatives of fatty acids that are linked to ethanolamine by an amide or ester linkage.

In a pilot study we had found that compared to controls, 2 of 3 NAEs tested resulted in statistically significant accelerated bacterial clearance from the lungs at week 3 post-infection (>90% reduction in bacterial burden compared with saline treated mice) and at week 5 post-infection.

For this project we aimed to repeat this study testing 3 NAE preparations against a saline only negative control, a combination of NAE 1 (the most effective in the preliminary studies) with an effective antimycobacterial, isoniazid (INH), and INH as the positive control. Mice were infected with BCG intranasally, then treated twice weekly with the NAE preparations. Weight change was monitored at varying times after infection and bacterial growth in the lungs of infected animals measured at 3, 6 and 10 weeks after infection.

Weight gain after infection was greatest in mice treated with NAE1 and INH; compared with INH or NAE1 alone or saline controls. Unfortunately, the 90% reduction in bacterial burden in the lungs of NAE-treated mice that we had seen in the pilot study was not observed in any of the NAE-treated animals tested in this repeat study, when compared to the saline only controls. Mice treated with NAE1 and INH also had no greater reduction in lung bacterial burden compared with animals treated with INH alone. As the animals were of the same sex, age and strain as used in the pilot study and the NAE preparations were made in the same way as in the pilot study it is unclear as to why we were unable to repeat this finding.

The 3 NAE compounds were also tested *in vitro* for their direct and indirect anti-mycobacterial activities against BCG French (Pasteur strain) and virulent strains of *Mycobacterium bovis* and *Mycobacterium tuberculosis*, using a radiometric assay and an *in vitro* macrophage culture assay, respectively.

None had significant direct activity on a variety of mycobacterial strains, nor did they impact significantly on the interaction of macrophages with mycobacteria, under the experimental conditions tested. One NAE variant (OO-NAE) diminished nitric oxide (NO) production by macrophages infected with mycobacteria, with no significant impact on mycobacterial intracellular growth.

Together, these results suggest that NAEs will not be reliably effective as a novel chemotherapeutic agent against Tb; however we thank the Wellington Medical Research Foundation for the opportunity to explore this avenue of research given our initial exciting findings.

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

J Kirman, F Rich, M Camberis, K Eckert

Tuberculosis (Tb) claims 1.7 million lives and newly infects 8.9 million people each year. The currently available vaccine, BCG, has been given to >3 billion people yet fails to consistently protect against Tb with an overall protective efficacy of just 50%. BCG is least effective in countries closer to the equator, that also have the highest incidence of Tb. A number of hypotheses have been put forward to account for the observed vaccine failure. The reasons behind BCG's failure need to be taken into account when designing new, more effective Tb vaccines, to ensure they will work in situations where BCG fails. One possible cause for BCG's variable efficacy is that worm infection impairs the ability of BCG to protect, due to the opposing or suppressive immune responses induced by each organism. Importantly, countries in which BCG is least effective also have high parasitic worm burdens.

For this project 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 3 weeks to grow on agar.

- Initiated studies investigating whether regulatory T cells induced by helminth infection suppress the development of Th1 memory by the BCG vaccine. A Masters student in the laboratory has shown that after *Nippostrongylus brasiliensis* infection, regulatory T cells increase in number in the lungs, but not in the spleen or lymph nodes. T reg depletion studies are currently underway to determine whether removing these cells abrogrates the effect of helminth infection on BCG's efficacy.

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 *Nippostrongylus brasiliensis* or not.

Conclusions

We have found that regulatory T cells are induced in the lungs of *Nippostrongylus brasiliensis* infected mice. Whether these cells suppress the immune response induced by BCG vaccination remains to be determined, and the effect of *Nippostrongylus brasiliensis* on Th1 cytokine production in the lungs of mice remains to be studied. However, we know that Th1 cytokine production is not decreased in the spleen or lymph nodes of BCG-vaccinated *Nippostrongylus brasiliensis* infected mice, suggesting that the local Th2 response induced in these tissues does not inhibit the development of a Th1 response. Further studies using STAT6-/- mice will be conducted to confirm this. We anticipate having all studies completed by November 2007.

Victoria University of Wellington

Haemangioma proliferation and TRAIL receptor expression.

DJ Day, A Vishvanath, S Tan
School of Biological Sciences

Haemangioma is a benign tumour of the microvasculature, characterized by active angiogenesis. In the proliferative phase the tumour is composed of small densely packed microvessels, lined by mitotically active endothelial cells. Tumours spontaneously undergo involution in early childhood, leading to deposition of fibro-fatty tissue. As aberrant expression and activation of STAT3 via VEGFR-2 has been reported in similar tumours, we examined the changes in mRNA and protein expression for STAT3 and VEGFR-2 in 20 haemangioma biopsies at different stages of development. We found high expression of STAT3 and VEGFR-2 mRNA in proliferating haemangioma with mRNA levels for both these genes decreasing proportionately in the involuting and involuted specimens ($p < 0.05$). In contrast, the abundance of STAT3 protein (relative to the total protein) did not vary between proliferating, involuting and involuted lesions. In addition, the amount of STAT3 activated by phosphorylation at tyrosine-705 was less in involuted lesions as compared with proliferating lesions ($p < 0.05$) but did not vary significantly between proliferating and involuting lesions. Immunohistochemical staining showed that STAT3 and VEGFR-2 immunoreactivity was highest in proliferating lesions that stained strongly for the proliferation marker PCNA, but was lower in involuting and involuted specimens that contained few PCNA-positive nuclei. Culture of tissue removed during surgical treatment showed increased microvessel growth when treated with VEGF, but cell cycle arrest when treated with the JAK/STAT inhibitor AG490, supporting a role for VEGF signalling through STAT proteins in regulating haemangioma growth.
Isolation and characterization of the cells forming the microvessels in tissue explants show them to be mesenchymal stem cells (MSC) on the basis of markers they expressed and the ability to differentiate into adipocytes and osteoblasts. The MSC expressed and secreted high levels of osteoprotegerin (OPG), which as well as having potent osteoclastogenesis inhibitory activity, functions as a survival factor for endothelial cells by binding TRAIL (TNF related apoptosis inducing ligand) preventing TRAIL induced apoptosis Characterization of the active and decoy TRAIL receptors expressed by the MSC compared with the tissue they grew from and other human cell lines (HUVEC and Hela) showed that the MSCs expressed high levels of TRAIL decoy receptor 1, as well as OPG. To determine whether this difference in expression was of functional significance we examined the ability of TRAIL to induce apoptosis in tissue explants, and in the MSC derived from the same tissue. The tissue explants were sensitive to low doses of both TRAIL and interferon, which when used in a combined treatment acted synergistically to induce apoptosis. By contrast, MSC showed similar sensitivity to interferon but were resistant to TRAIL induced apoptosis. The role

of MSC, OPG and TRAIL receptors in haemangioma progression, remains to be elucidated.

Long-Term Cellular Effects of Ecstasy - Adaptation or Degeneration?

JH Miller, DJ Day, BM Kivell, S Schenk, B Lake, J Colussi-Mas, K Danielson, B Simonson
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MDMA (ecstasy) is a popular recreational drug that produces feelings of euphoria as a result of an acute, rapid release of serotonin and dopamine from nerve terminals. The long-term detrimental effects of MDMA are not very well known, but may include learning deficits and psychiatric disorders. We are using an animal model of drug abuse to investigate the changes in brain neurochemistry that occur after experimenter-administered or self-administered (lever pressing) MDMA. Understanding the neurochemical changes will help us determine whether brain function can recover following long-term use of MDMA.

One aspect of MDMA use being studied in the School of Psychology is the initial deficits and recovery following exposure to the drug. We are also correlating cFos expression to behavioural responses to the drug. cFos is a transcription factor that is turned on when neurons are activated. We can also monitor serotonin levels in specific brain regions by high-pressure liquid chromatography to give us a clearer picture of the neurotransmitter changes associated with the drug. In the School of Biological Sciences, analysis of brain tissue from treated animals has shown that a decrease in serotonin transporter (SERT) density in some brain regions is not a result of a decrease in SERT mRNA, nor is it due to a decrease in the amount of SERT protein. This has been shown by others as well and suggests that SERT is functionally modified, either by undergoing MDMA-induced posttranscriptional or translational changes, or due to loss of its plasma membrane location in the neurons. Internalisation of SERT away from the cell surface into endosomes would remove it from the site of serotonin transport and make the transporter protein non-functional.

As it is difficult to study the kinetics of serotonin transport in the intact brain, we are also using two novel *in vitro* approaches to monitor changes in SERT function. One makes use of cells that have been transfected with SERT DNA and examination of transporter function in the cells using a live-cell confocal microscope. Victoria University has recently acquired a new Olympus confocal microscope. The transport of a fluorescent SERT substrate, ASP+, into the transfected cells is being assessed in the presence and absence of MDMA. These experiments will help us determine if MDMA works at the individual cell level or requires more complex interactions between different brain regions. Our second approach involves measuring changes in voltage in minced brain tissues. These voltage changes are proportional to monoamine uptake

kinetics. This technique is called rotating disc electrode voltametry and can be used in tissues dissected from specific brain regions taken from animals that were treated with MDMA.

The answers we obtain to the above questions on the type of neurochemical changes being induced by MDMA and their associated behaviours will improve our understanding and lead to a rationale for treating chronic MDMA use in humans.

Regulation of susceptibility to autoimmunity by interleukin-4 receptor α (IL-4Rα)

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Multiple sclerosis (MS) is a debilitating neurological disease caused by the development of an autoimmune response to normal proteins in the central nervous system (CNS). Current immunological beliefs hold that pro-inflammatory, T helper cell type 1 (Th1) responses promote autoimmune diseases like MS; whereas, anti-inflammatory, Th2 responses suppress disease. The studies supported by this grant investigated how a receptor of Th2 signaling (interleukin-4 receptor α; IL-4Rα) alters macrophage (Mϕ) function to prevent damage in the CNS by self-reactive T cells. We found that contrary to the current belief that Th2 responses suppress EAE, these responses are actually disease-inducing in the Balb/c mouse strain. Furthermore, Mϕ appear to be critical to this process.

Our laboratory investigated the mechanism by which Th2 responses promote disease and found that IFN-γ production by cells from the draining lymph node peaked earlier in IL-4Rα-/- compared to Balb/c animals and suggests that high levels of IFN-γ early in disease may initiate a down-regulatory immune pathway that resolves the self-reactive response before clinical disease manifests. Our studies suggest that in Balb/c mice, Th2 cytokines, signaling through IL-4Rα, may regulate this process. Indeed, we found that altering the Th1-inducing stimulus changed the susceptibility of mice to development of disease such that with a lower Th1 stimulus the susceptibility of IL-4Rα-deficient animals increased and vice versa. Additionally, we examined whether regulatory T (Tregs) cells were involved in the regulation of susceptibility but did not find any significant correlation between Treg numbers and disease incidence or severity. These studies support the belief that increased pro-inflammatory responses in the absence of IL-4Rα signaling induces a down-regulatory pathway that resolves the autoreactive response before overt disease occurs.

Using mice in which the capability to respond to IL-4 and IL-13, through IL-4Rα, has been specifically eliminated from Mϕ but not other cells in the mouse, moIL-4Rα-/-, we found that it is the ability of Mϕ to respond to these Th2 cytokines that

promotes disease. Compared to their littermate controls, these mice can produce similar Th2 responses. Therefore, the disease regulating effects are mediated by IL-4/IL-13-regulated Mϕ effector functions. We examined the involvement of nitric oxide synthase-2 and indoleamine 2,3-dioxygenase in Mϕ-mediated T cell inhibition. While these two enzymes have been shown to be effective at inhibiting T cell function in other systems, they do not appear to be the main mechanism by which Mϕ are preventing the development of EAE in Balb/c mice.

Through identifying factors that suppress disease progression in our unique mouse model of MS, we have expanded our understanding of the pathological progression of MS in the human population. Because individuals and genetically distinct mice (i.e. different inbred strains) manifest different MS disease patterns (i.e. relapsing-remitting, primary progressive), our novel finding that Th2 responses promote disease in Balb/c mice, suggests that the immune pathways causing autoimmune-mediated damage may differ between individuals. Thus this enhancement our understanding of the pathways involved in regulating MS development will help in the development of alternative therapeutic strategies to provide effective treatment of patients who do not respond to currently available therapies.

Targeting multiple sclerosis with anti-mitotic drugs

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

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

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

The third part of the study was to determine the effectiveness of paclitaxel and peloruside at treating MS, using the EAE mouse model. These studies indicated that both paclitaxel and peloruside significantly delayed the onset of EAE. However, treatment did not have any observable effect on autoreactive T cell expansion or responses suggesting that the protection afforded by paclitaxel is not due to a specific deletion of auto-reactive T cells. Additionally, despite the effectiveness of paclitaxel at delaying disease, the treatment regime was not well tolerated, possibly due to the ability of paclitaxel to directly induce pro-inflammatory mediator production. In contrast, peloruside was well-tolerated indicating that this novel drug shows great promise as a possible treatment for MS. Future studies will focus on the mechanism of action of these drugs and optimization of the treatment regime.

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

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[1]School of Biological Sciences, [2]Environmental Scientific Research Institute

Smoking is one of the leading causes of preventable illness in the world. The Ministry of Health estimates that over 4300 deaths in New Zealand each year are attributed to cigarette smoking. Current cessation treatments based on nicotine replacement therapy are ineffective, with a success rate of less than 20% after 1 year. Smoking releases neurotransmitters such as dopamine and serotonin. Dopamine activates the brains reward system, whereas serotonin acts as a mood elevator. Monoamine transporters function to remove these neurotransmitters from the synapse where they are active, thus terminating the actions of neurotransmitters.

Very few studies have investigated the effects of smoking on the monoamine transporters. In this study we are investigating the effects of smoking and nicotine on the function of monoamine transporters. MSc Student Kirsty Danielson is currently investigating the effects of smoking and nicotine and its effects on the mRNA levels of the serotonin (SERT), dopamine (DAT), and nor-epinephrine (NET) monoamine transporters. Various brain regions from the rat are removed and analysed for changes in mRNA expression using real-time PCR in drug exposed and drug control animals. Design and testing of the primers have been carried out and preliminary experiments have identified key areas of the brain known to be involved in addiction pathways that also express high levels of monoamine transporter mRNA. Areas not expressing SERT, DAT and NET mRNA have also been identified and will be used as negative controls.

Collection of all experimental tissue samples from drug exposed and control animals is scheduled for July with the real-time PCR results estimated to be complete by September.

SBS has recently established a live-cell confocal microscopy facility. This facility enables the function of monoamine transporters to be measured in real-time in live-cells. Preliminary experiments have been carried out optimising the sensitivity and determining the experimental parameters required to perform the transporter function studies in transfected cells expressing SERT, DAT and NET using a fluorescent substrate for monoamine transporters.

The function of monoamine transporters in isolated tissue and in cells is currently being established using a neurochemical technique called Rotating Disk Electrode Voltammetry. Hons student Bridget Simonson is optimising this technique in my laboratory and is currently determining the uptake kinetics for DAT in tissue from control animals. This technique allows the kinetics of monoamine transporters to be determined and will soon be compared to animals exposed to nicotine and other components of cigarette smoke.

Establishing these techniques allows us to determine the effects of nicotine and smoking in the whole animal, tissue, and cellular levels, an essential combination when investigating the effects of drugs of abuse.

Wakefield Hospital

Examination of the effects of serum from pre and post gastric bypass patients on insulin binding to its receptor.

MT Hayes, F Kwan, D Hutchins, RS Stubbs
Obesity and Diabetes Program, Wakefield Gastroenterology Research Institute.

Type 2 Diabetes has reached epidemic proportions in New Zealand and other developed countries. The global estimate of the incidence of diabetes in 2003 was 194 million people and it is estimated that this figure will double by 2030. The current information for New Zealand is based on the incidence measured in 1996 (diagnosed 81,496) and forecasts an approximate doubling of this incidence by 2011. Maori and Pacific peoples have an incidence rate three times greater than that of the European population and they are five times more likely to die from the disease. Co-morbidities of this disease include increased risk of dyslipidaemia, hypertension, neuropathy, cardiovascular disease, renal failure, blindness, amputation of limbs and shortened life expectancy.

We and others have reported that the majority of obese type 2 diabetics who undergo gastric bypass surgery experience a return to normal glycaemic control within six days of surgery and no longer need diabetes medication. We suspect that a humoral factor or factors derived from the stomach duodenum or jejunum released by contact with food, contributes to or causes type 2 diabetes. After surgery these structures are isolated from contact with food and diabetes is rapidly resolved. Blood samples taken from diabetics before and after surgery should thus contain different levels of such factors and have been collected from over 120 diabetic patients over the past decade.

One potential way that such a humoral factor could act is by modulating the affinity of insulin for its receptor. Reduced affinity would require higher levels of insulin in the blood stream to produce the same level of response from the receptor on the cells. Receptor binding can be influenced by 1) an alternate ligand competing for the binding site, 2) changes in the chemical environment which make binding less favorable and 3) the binding of other molecules to the extra cellular part of the receptor which can change the binging constant.

This research aims to study whether the pre and post operative serum environment changes the affinity of insulin for its receptor. The first stage of the research involves isolation of the intact human receptor from red blood cells using affinity chromatography then binding the purified receptor to plasmon resonance plates for use in a Biacore machine (Auckland University). This instrument measures interactions between receptors and ligands allowing us to study the effect of pre and post operative serum on both the rate and extent of insulin receptor interactions. Additionally, the intact receptor will also be bound to magnetic beads (Dynabeads) which can be agitated in pre and post operative

serum or plasma to concentrate molecules which interact with the receptor. The beads are then isolated with magnets and the adhering molecules eluted off and analyzed by mass spectroscopy. Individual molecules identified in this approach can then be tested using the Biacore to ascertain whether they influence insulin binding.

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

RS Stubbs, J Krebs, MT Hayes, F Kwan, D Hutchins
Wakefield Gastroenterology Centre and Research Institute

We and others have reported that Type 2 diabetes is resolved within 6 days in the majority of obese patients who undertake gastric bypass surgery (GBS). In previous work we have shown that this is accounted for by an almost immediate resolution of insulin resistance following this operation. Our work has led us to believe that insulin resistance (IR) is due to a factor released from that portion of the stomach, duodenum or jejunum which is bypassed by the operation. The overall aim of our diabetes research programme is to identify that factor, and to develop an antagonist to the factor which might become a highly effective new treatment for type 2 diabetes.

This project originally set out to determine whether this improvement in insulin resistance might be related to the dietary restriction imposed by the operation. This was to be achieved by comparison in the changes in insulin resistance achieved by six days of very low calorie diet (VLCD), and those achieved 6 days after gastric bypass. The method of measurement of insulin resistance was an insulin tolerance test, which is a measure of "whole-body" insulin resistance (as opposed to hepatic insulin resistance which had been measured in previous studies by us). To our surprise insulin resistance by this method deteriorated after both VLCD and gastric bypass, which taken in conjunction with our earlier findings means that diabetes is primarily a disease of hepatic insulin resistance. With this important and new revelation, we chose not to complete the study as originally conceived, although enough studies have been completed to provide sufficient data for publication in the international literature. The last of this data is being compiled and analysed in preparation for a manuscript.

Interestingly studies from others involving selective knockout of the insulin receptor in mice support this conclusion since selective knockouts of the insulin receptor in fat and/or muscle fail to produce diabetes. Whereas, knockout of the insulin receptor in the liver does produce type 2 diabetes suggesting that this organ is key in the development of type 2 diabetes.

Our work to date has led us to believe that IR occurs as a result of the presence of an as yet unidentified substance carried in the blood, released from that part of

the stomach, duodenum or jejunum which is bypassed by the gastric bypass operation. Recent work on diabetic rat models using variations of GBS has shown that isolating the jejunum and duodenum from contact with food reverses diabetes and that re-establishing normal contact with ingested food to these structures restores the disease. Thus the source of our proposed signal is likely to be the jejunum and/or duodenum while the principal target organ for the signal is likely to be the liver.

Our unexpected finding that both VLCD and gastric bypass deteriorates "whole-body" IR has fundamentally changed the direction of this research. We are proceeding by examining the gene and protein expression profiles of liver and jejunal tissues obtained from patients undergoing gastric bypass (unfortunately it is not practical to regularly obtain duodenal tissue from our patients). We have chosen to examine protein and total RNA from these tissues and compare profiles from the two extremes of the IR spectrum (ie insulin sensitive and diabetic patients) using cDNA arrays and 2D DIGE electrophoresis coupled with mass spectrometry. In addition we continue with our efforts to identify and purify the putative factor causing insulin resistance from the blood of those undergoing gastric bypass.

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

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

D Bell, N Smith, J Krebs
Endocrinology, Department of Medicine

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. Since November we have screened 238 people, and have randomized 63 in to the study, we have another 65 people to undergo consent. We have conducted baseline visits on 65 people, and currently have 46 undergoing the dietary intervention.

From the perspective of the sub study we have purchased the two continuous glucose monitors, and the related software. Our staff have all assisted the diabetes nurses in placing the sensors and educating the patient on the operation of these devices. This was all done via the use of hospital CGMS machines and sensors as these patients were put forward to have these as part of there ongoing care. Our nurses and Dr Smith and myself are now well versed in the application of the CGMS and Dr Smith, Dr Krebs and myself versed in the interpretation of the results.

Due to the intensive initiation period, and need for the Wellington staff to coordinate the training and initiation of the other two sites for the DEWL study, we as yet have not initiated any patients on the sub study, but currently have a cohort waiting to start, with dates for initiation starting from 23rd of July.

We will be able to perform 4 CGMS and OGTT's each week, the baseline tests will initiate after the consent meeting of the 23rd of July, and we anticipate we will be performing these baseline tests over the 4 months from late July to mid November. We have delayed starting as of June, as the six month visits would fall around the Christmas period for a number of people, and we believed compliance with them would have been reduced. See time line.

Timeline for the sub study.

Baseline visits	Late July 07 – early November 07.
Six month visits	Late January 08 – early May 08
One year visits	Late July 08 – early November 08

During the consent meetings we have had so far, we asked people if the sub study was something they were interested in, and we have had around an 80% positive response rate. We anticipate there will be ample time to enrol the 50 participants we are aiming for. The 16 week time periods for performing the visits will correspond well with the monthly initiation of dietary cohorts. Our ability to perform 4 patients visits per week builds in a reserve time which will aid flexibility in ensuring the visits are done.

Fine Particulate Pollution

PR Edwards, N Wilson, G Thomson
Department of Public Health

This is the first report for an equipment grant to purchase a *TSI SidePak AM510 Personal Aerosol Monitor.* The Aerosol Monitor will be used to support a programme of research investigating the level of hazardous fine particulate pollution from second-hand smoke in a variety of settings including private cars, semi-enclosed outdoor areas and homes.

The equipment was required for use in research projects and as a component of research grant applications to support work investigating levels of second-hand tobacco smoke (SHS) in a variety of settings.

Funds were received in November 2006, and the equipment purchase and supply was completed in January 2007.

Activity to date

1. Investigation of levels of SHS in outdoor areas of pubs and bars in small towns in the Wairarapa

   An initial study was conducted to assess fine particulate levels in five pubs in small towns in the Wairarapa. The areas were all semi-enclosed, but occupancy and smoking levels were low. The mean particulate levels varied between 5.6 and 25.1$\mu g/m^3$. These levels are close to those observed in ambient air, and are well below the levels that are seen in indoor areas of pubs where smoking is allowed (typical values from about 100-1000$\mu g/m^3$, with a mean of around 300$\mu g/m^3$).

   This suggests that levels of SHS particulate pollution are not of great significance to non-smokers exposed (eg bar staff entering the smoking areas) where occupancy and smoking rates are low, such as in these rural and small town pubs.

2. Investigation of levels of SHS during simultaneous measurements in semi-enclosed outdoor smoking areas and adjacent indoor areas of pubs and bars in Wellington CBD

   We have so far carried out monitoring of particulate levels (PM2.5) in outdoor smoking areas with varying degrees of occupancy and enclosure, and simultaneous measurements of indoor non-smoking areas in seven pubs in Wellington CBD on two busy weekend evenings. This work has been made possible by the temporary acquisition of another Sidepak monitor on loan from the Roswell Park Cancer Institute in Buffalo, USA.

   Mean ambient PM2.5 levels were 7.9$\mu g/m^3$ on the first night for Pub 2_1, Pub 2_2, and Pub 2_3; and 21$\mu g/m^3$ during the second night for Pub 1_1, Pub 1_2, Pub 1_3, and Pub 1_4.

   The results show:
   - that very high levels of particulate pollution can occur in smoking areas, particularly where they are highly enclosed
   - overall the levels in semi-enclosed areas were mostly not very high, but occupancy and smoking rates were generally low
   - there is some evidence that drift of SHS particulate pollution occurs into adjacent indoor areas.

3. Grant application for work on SHS levels in homes with varying smoking policies
   We have applied for a major HRC grant to investigate influences on SHS exposure and smoking uptake among children. As part of this we have included a sub-project to investigate the impact of different smoking policies in the home (no smoking allowed in the home, smoking allowed in designated area in the house, and smoking not restricted in the house) on SHS levels in living areas and in children's bedrooms.

Is a mite allergic individual more or less Allegic to their own mattress house dust when compared to a mite solution containing equivalent quantities of mite allergen? Do the other constituents of house dust aggravate or mitigate its allergenicity?

TV Stanley
Department of Paediatrics

The original research project involving patch testing has been modified due to the original research not being possible on the basis of the sums available. As a result the project now involves looking at patch test responsiveness to house dust mite solutions and dust solutions collected from house dust mite sensitive

individuals' own mattresses. The plan is to see if there are other components in the mattress dust that may alter the size of patch and prick test results in individuals. As a result we have done some preliminary studies looking at how much prick test dilution alters the size of the prick test response. We have done this using house dust mite solution. We demonstrated that at least 3 serial dilutions do not significantly alter the size of the prick test. We are now in the process of collecting house dust samples from allergic individuals and generating a prick test solution and a patch test solution from these samples. We then plan to prick and patch test individuals against a theoretically equipotent dose of house dust extract from a commercial supplier. If the reactions differ it would suggest there is something in the house dust, apart from the mites, which has an immunological effect. It is our theory that endotoxin may be that substance and as a result we are going to include samples with endotoxin and with inactivated endotoxin. We also plan to test individuals with mattress dust samples from another individual's bed to see if responses differ. We have studied commercial dust skin prick samples and shown these are of reduced potency compared to commercial mite solutions, but they contain considerably less mite particles.

The study involves adults at this stage as it is a pilot study. We are in the process of fine tuning the extraction process for the house dust mite and dust extracts. The scientist involved in this work has been on leave although is due to return in the next few days and we expect to move on to the next exciting phase of this study. The study has been made more complex by the need to sterilise the samples of dust without in any way altering their immunological characteristics. As mentioned this is a pilot study and should it show some differences between the allergenicity of an individual's own dust and that of commercial dust mite extract, we will proceed to a larger study.

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

Optimising EEG studies for epilepsy syndrome diagnosis in children with new onset seizures

LG Sadleir, IE Scheffer
Department of Paediatrics

An early epilepsy syndrome diagnosis is essential for the optimal management of children with new onset seizures. An electroencephalogram (EEG) is an essential component of the epilepsy syndrome diagnosis of the child, however the best way of performing this is debated. Ideally, an EEG would show

epileptiform discharges in all children with epilepsy. Our aims were to ascertain how many children can be classified into an epilepsy syndrome at presentation and to establish whether an early postictal EEG is a more useful diagnostic tool than a late sleep-deprived (SD) EEG.

Children aged 2 to 16 years presenting acutely to hospital with new onset seizures were recruited. Between Jan 1 2002 and Dec 31 2005, we recruited all eligible children. Each child had 2 EEG studies: an early routine EEG within 24 hours of the seizure (early post-ictal EEG) and a later sleep-deprived EEG at least 48 hours after a seizure (SD EEG). Two paediatric neurologists, who were blinded to both the patient's history and the type of EEG, read the EEGs independently. As sleep was captured in a significant proportion of the early EEGs and a proportion of the children did not sleep in the SD EEGs, blinding was effective.

Of 92 children, 59% had a single seizure; 41% had had 2 or more seizures. Seizures were focal in 66% and generalised in 21%. Eighty-seven percent of the children were diagnosed with a broad epilepsy syndrome; idiopathic generalised epilepsy (17), idiopathic focal epilepsy (57), symptomatic generalised epilepsy (2) and symptomatic focal epilepsy (4). Fifty-five percent of these children were diagnosed with a specific epilepsy syndrome.

Epileptiform abnormalities were seen in 57% of the early EEGs and 61% of the SD EEG's. This was a difference of 4% (95% CI –0.3% to 14%), which is neither clinically or statistically significant. McNemar's test of observed versus expected confirmed this (p=0.27). Epileptiform abnormalities were seen in at least one EEG in 65% of the children. There appeared to be a trend for SD EEG being more informative than the early EEG in the subgroup of children with two or more seizure and those with generalised seizures although the numbers in these groups was small. Epileptiform discharges were least likely to be seen in the children with seizures that were unable to be classified.

Background abnormalities were seen in 46% of the early EEGs and 28% of the SD EEG's. This was a difference of 18% (95% confidence interval 7% to 28%). McNemar's test of observed versus expected confirmed that there was more likely to be slowing in the early EEG than the SD EEG (p=0.001).

Parents preferred the early EEG (71%) to SD EEG (15%) as they preferred the EEG as soon as possible after the seizure.

An important aspect of the management of children with seizures is the empowerment of the family to deal with the disorder. This empowerment comes when a family understands why the seizures have occurred and how they are going to affect their child in the short and long term. Physicians have tended to regard first and second seizures differently as children are generally not treated with AED's until the second seizure and the diagnosis of "epilepsy" still has significant social implications and stigma. Although this is true, from the families

perspective the arbitrary distinction between one or more seizures is irrelevant, as they need information as soon as possible. An early epilepsy syndrome diagnosis allows this information to be given to the best of our present scientific knowledge. In this study we have shown that the majority of children with new onset seizures can be diagnosed into a broad epilepsy syndrome diagnosis with over half also able to be diagnosed with a specific epilepsy syndrome.

This is the first study to directly compare early EEG's with SD EEG's. We found both sleep deprived and early EEG's showed high epileptiform rates with a similar yield however the early EEG's were significantly more likely to have background abnormalities. This background slowing appears to generally result from the seizure itself rather than represent a persistent abnormality.

The size of an EEG department and the way it is organized will impact on whether an early EEG or a SD EEG is easier for a particular department. In smaller departments it can be difficult to fit in an extra EEG that isn't actually a clinical emergency at short notice. For these departments being able to schedule a SD EEG some time within the next four weeks allows better use of their resources. Larger departments with more technicians may have more capacity to fit in an extra EEG at short notice. Our findings suggest that either an early EEG's or a SD EEG's is better than a routine EEG, and that patient, service and economic preference should guide the choice of EEG protocol.

Randomised Adolescent Pedometer Trial

EJ Wiltshire
Department of Paediatrics

This study is a randomised blind trial of use of pedometers and text messaging to improve exercise and adherence in adolescents with type 1 diabetes.

The study commenced in 2005. Subjects have baseline data collected, which include step count with a closed pedometer over 4 days, questionnaires that assess exercise and quality of life and clinical data relating to diabetes. Subjects are then randomly allocated to control or intervention groups for a 12 week period (height, weight, blood pressure, HbA1c). The intervention group use a pedometer in an open fashion and are texted regularly about their step counts. Both groups then repeat the baseline measures.

Progress/Results

We have so far recruited 76 subjects, in Capital and Coast DHB, Hutt Valley DHB, Mid-Central DHB and Canterbury DHB. Of these 72 have completed the study and the final 4 subjects will have their end of study data collected during the next 2 weeks. Four subjects have dropped out, a much lower proportion than expected. As the study is a blinded randomised trial, data analysis will occur

once the final four subjects have completed their follow-up measures. We hope to have analysis completed to present the results at the Australasian Pediatric Endocrine Group Annual Scientific Meeting in October 2007.

Project Grants 2007

The following projects were approved for funding in May 2007 and will be reported on in subsequent Annual Reports of the Foundation.

Anne Burston
Kapiti Health Centre
Does the time of day affect the outcome of the Dix-Hallpike Manoeuvre, used in diagnosis of Benign Paroxysmal Positional Vertigo

Benign Paroxysmal Positional Vertigo is the most common cause of vertigo due to peripheral vestibular disorder. Clinical diagnosis is most-commonly based on clinical history and objective examination using the Dix-Hallpike manoeuvre. Clinical experience suggests there may be a variation in the results depending on the time of day the manouvre is performed. The aim of this study is to establish if there is a variation in the results if the manoeuvre is performed at different times of the day.

Julian Crane
Wellington School of Medicine
TLR gene polymorphisms, endotoxin exposure and asthma and allergy in a NZ birth cohort

This project aims to examine the interactions between toll-like receptor (TLR) Gene polymorphisms and domestic endotoxin exposure on the early development of wheezing and eczema and on the later development of atopy and asthma in New Zealand children.

Scott Harding
Cardiology, Wellington Hospital
Investigation of the effect of avorvastatin on lipids and inflammation in chronic failure

This project aims to assess the effect of atorvastatin on a range of proinflammatory and prothrombotlc factors which are thought to drive atherothrombosls in patients with advanced renal failure.

Kylie Hood
Wakefield Gastroenterology Research Institute
Evaluation of metastasis - associated proteins in colorectal cancer

This grant was for the purchase of a research grade microscope with epifluorescence and image capture and analysis capabilities. The research addresses important and highly relevant areas of colorectal cancer and diabetes.

Amanda Kvalsvig
Wellington School of Medicine
The effect of pre-hospital parenteral antibiotics on mortality meningococcal disease

This projects aims to elucidate an important an important and controversial topic in the early management of meningococcal disease.

Kavita Kumareswaran
Capital and Coast Health
The use of Metformin in patients commencing atypical antipsychotic therapy

The object of this project is to assess whether initiating Metformin therapy in patients commencing an atypical antipsychotic results in prevention of weight gain, and improvement of other metabolic parameters, including fasting lipids, fasting glucose and blood pressure compared with placebo.

Joanna MacKichan
Institute of Environmental Science and Research
Mucosal Immunity Implications for Meningococcal carriage and spread

This project will allow the researchers to assess the effect of nationwide serogroup B vaccination program on meningococcal carriage strains in New Zealand.

Bridget Stocker
Malaghan Institute of Medical Research
A sweet solution to the Th 2 bias in Asthma

The results of this study will provide the first detailed carbohydrates and Th 1/Th 2 immune response bias. In the long term this may lead to the identification of specific Th 2 enhancing or inhibiting enzymatic targets for drug therapy and asthma medications.

Mattie Timmer
Victoria University of Wellington
Mycobacterium Tuberculosis: The fat behind the cough

The overall aim of this research is to gain understanding bout the role that mycolic acid, the main constituent of the outer cell wall of mycobacteria, plays in the immune response to mycobacterial infection.

John Miller
Victoria University of Wellington
The effects of tobacco smoke components on monoamine oxidase enzymes and mu-opioid receptors

The aim of this project is to determine if there are components of tobacco smoke, other than nicotine, that can influence the addictiveness of smoking and/or mu-opioid activity.

Travel Grants

Conference Report
S Dean

Dr Sarah Dean was awarded a Wellington Medical Research Foundation Travel Grant to give three research presentations abroad during June and July 2007. Her trip was also supported by the University of Otago School of Medicine, Wellington.

The first oral paper was given at the World Congress of Physical Therapy, held in Vancouver, Canada on 2nd to 6th June. This congress is a highly respected international conference that is held every four years and is regarded as the top conference for the physiotherapy profession. Sarah presented work undertaken with her colleague Dr Jean Hay-Smith, from their University of Otago funded qualitative research project: "The special specialist: exploring how health professionals facilitate understanding and adherence to conservative treatment for women with urinary incontinence." The presentation was attended by over 70 women's health specialists and was well received; an extended version of this talk was given at the Wellington School on the 1st of August.

The second presentation was at the invitation of Professor John Weinman, who had visited the Wellington School in March 2007. Sarah presented this second paper, in a seminar format, to members of the Health Psychology Section, Institute of Psychiatry (based at the Guy's Hospital campus of Kings College, London). This was an extended version of the conference paper Sarah went on to present at the Society of Rehabilitation Research, University of Leeds on the 3rd and 4th July. These papers were a collaborative venture with Professors John Weinman and Alan Tennant as well as with RTRU colleague Dr Will Taylor. The presented work used a specific statistical analysis for the development of measurement scales in rehabilitation, in particular the: "The Coherence subscale of the Illness Perception Questionnaire adapted for low back pain - a Rasch analysis".

Collaboration with these colleagues has continued: John Weinman has undertaken an advisory role in a recent University of Otago grant application being led by Dr Jean Hay-Smith and there are also plans for further Rasch analysis work. The support from Wellington Medical Research Foundation has therefore been greatly appreciated as the travel grant has enabled Sarah to not only disseminate her completed research findings in international arenas but to also promote further international research collaboration.

Report on the 2006 FASEB "Lymphocytes and Antibodies" Summer Conference

R Perret

Thanks to funding by the WMRF I was able to attend a FASEB Summer Conference in Indian Wells, California, from the 12th - 17th August of this year. It was a worthwhile and enriching experience and allowed me to gain a much more in depth knowledge of the broader field of immunology as well as allowing me to meet with and get to know leading immunologists as well as other students and post-docs who have become valuable contacts for the future.

The title of the course was "Lymphocytes and Antibodies" and the week was filled with a series of demanding but fascinating sessions on many different aspects of immunology, from the development of stem cells into different arms of the immune system to B cell activation, regulation and mechanisms of antibody production; T cell generation, signalling, cytokines, migration and memory as well as many other aspects of immunology and specifically cancer immunotherapy, which is of particular interest to me. It was an invaluable experience to learn from prestigious immunologists who were presenting the latest developments in their fields and speaking in depth about T cell trafficking, development of immune memory, antigen presentation and tumour immunology.

The format of the course was very effective in that each day was made up of a series of afternoon and evening lectures, with free time in the mornings to prepare for the coming sessions and to network with other students and scientists. Meals were taken communally, allowing interaction with the speakers in a less formal and intimidating environment. During the week there were 3 poster sessions and as many of the posters presented were by members of the research groups of the speakers, this was a great opportunity to learn more about the topics they were presenting and to discuss experimental procedures with the people directly involved with doing the research. I was able to present a poster on my own project, entitled "Tracking Tumour Antigen-Specific CD8+ T cells by Adoptive Transfer". My poster was well received and I enjoyed the opportunity to discuss my work with others working in my field of interest. I received many helpful suggestions for continuing my research.

The summer conference was an excellent experience and I feel that it taught me a great deal as well as giving me the benefit of meeting with top American immunologists and getting to know other young scientists from all around the world. I greatly appreciate having had the opportunity to attend this course. I feel it has helped to prepare me for my future career in scientific research and I have gained confidence in presenting my research and in interacting with other immunologists.

Short report on the "9th International Conference on Dendritic Cells"

P Stoitzner

I am very grateful for the financial support from the Wellington Medical Research Foundation that enabled me to attend the "9th International Conference on Dendritic Cells" in Edinburgh, Scotland this year in September. This prestigious meeting takes place every second year and is the most relevant one in my area of research. Scientists from all over the world meet on these conferences to discuss the newest findings in the field of dendritic cell biology so it was a great chance to present my work currently undertaken at the Malaghan Institute of Medical Research.

The dendritic cell conferences are thought as an occasion to bring together prominent well-known researchers working on the origin, subsets and localization of dendritic cells as well as their role in innate and adoptive immunity. Dendritic cells are antigen-presenting cells that are mandatory to induce and immune response. They are distributed all over the body and survey tissue for invasion of pathogens. When an infection happens dendritic cells induce a T cell response to fight the invaders. On the contrary dendritic cells are also involved in autoimmunity, meaning the attack of self-tissue by the immune system. Understanding the interaction of dendritic cells with other cells of the immune system will help to further develop immunotherapy against cancer and autoimmunity. Dendritic cells are already widely used in cancer treatment and they are currently further explored for use in other diseases.

The "9th International Conference on Dendritic Cells" this year attracted 800 scientists from all around the world. The program was divided into symposia covering different topics from dendritic cells origin, biology to function and their use in immunotherapy. The evenings belonged to poster sessions in which everyone had a chance to present their own work and mix and mingle with leading scientists and get their opinion on their work. It was a great mixture of students, post docs and group/institute leaders and there was a lively discussion going on over these 4 days.

This meeting was invaluable for me since I was able to discuss the work I have undertaken at the Malaghan Institute of Medical Research in the last one and a half years. I present a poster entitled “Epicutaneous immunization a new anti-cancer therapy?” during the meeting. My poster was well received and I was given several helpful suggestions and ideas to continue my research. I also enjoyed the opportunity to discuss my work with others who are studying similar topics and I gathered lots of new important information for my future work on the interaction of dendritic cells with tumours. I hope that with these new inspirations I will be able to move on with my projects and will be able to develop new means and techniques how to use dendritic cells to treat skin cancer. The conference

was an excellent experience and my work will benefit from what I have learned about these fascinating cells and their role in the immune system.

9th International Dendritic Cell conference in Edinburgh

J Walton

I would like to thank you for the opportunity to attend the 9th International Dendritic Cell conference in Edinburgh. The conference lasted 4 days, from the 17th -20th September; in which time 80 talks were given and 515 posters were displayed, including a poster of my own.

Research presented in my poster was performed at the Malaghan Institute of Medical Research and was from a recent phase VII trial discussing the use of 'Autologous dendritic cells pulsed with eluted peptide as immunotherapy for advanced B-cell malignancies'.

A number of speakers focused on the functional differences of distinct DC subsets and their ability to induce a variety of different humoral and cellular immune responses. Jacques Banchereau, a leader in the field of dendritic cell in therapeutic vaccines, presented data on how interstitial-DCs, DCs found in the dermis, activated humoral immunity. Langerhans cells, DCs found in the epidermis, were found to preferentially induced cellular immunity. Jacques talk highlighted functional specialisation of DC subsets and their importance in vaccine design. Hans-Christian Reinecker reiterated this point with his discussion on the differences between functionally defined DC subsets. He discussed the uptake and presentation of antigens on DCs, the cells ability to distinguish between self and pathogenic antigens and the cooperative regulation of immune function.

Several clinical trials were discussed, most relating to melanoma patients, such as Gerold Schuler's work. Gerold reviewed clinical trial work involving 200 patients receiving DC vaccines loaded with tumour RNA and mixed peptides. He discussed several aspects of vaccine design in relation to clinical and measurable immune responses observed in the trial patients. He also reported T cell reactivity in 10% of his patients to human serum albumin, which is used in vaccine production, resulting in a rash. However, overall survival time for patients increased with each consecutive trial performed even though objective regressions were rare. The conference meeting was summarised in the final talk by Ralph Steinman, the discoverer of dendritic cells. Ralph also discussed the in vivo functions of dendritic cells and their relevance to vaccine design. Ralph concluded the meeting by emphasising the need for researchers to continue with and increase the number of dendritic cell therapeutic trials being conducted in order to validate DC therapy as an effective treatment for disease.

Clearly the research presented at the conference was of a very high level and in incredibly large volume of knowledge was shared amongst the group. Exposure to new ideas and current research is limited and challenging to maintain in New Zealand due to our physical isolation from other scientists working in the same field. Attending international conferences of this sort is invaluable for assisting the Malaghan Institute in furthering our research and for providing the most effective clinical trial programs to New Zealand patients.

Advanced Optical Imaging Workshop, November 2006, Melbourne, Australia

L Green

The Travel grant from the Wellington Medical Research Foundation allowed me to attend the first *"Advanced Optical Imaging Workshop"* in Melbourne, Australia this past November. Representing leaders in the imaging field, scientists from all over the world gathered to showcase some of the latest developments in fluorescence lifetime imaging microscopy (FLIM) and fluorescence resonance energy transfer (FRET).

For many fluorescent molecules that are widely used in research, the spectra are poorly defined. Using FLIM, one can distinguish between different fluorescent molecules in multi-stained samples due to well-defined fluorescent lifetimes. FLIM is a novel development in the field of fluorescence spectroscopy utilising fluorescent decay rates to characterise the molecular microenvironment of molecules. Factors that influence decay rate can include "hydrophobicity, ionic strength, oxygen concentration, and proximity of molecules that can deplete the excited state," thus making fluorescence lifetime measurements an indicator of such parameters (www.locLwisc.edu). FRET, being a measurement of energy transfer between two fluorescence molecules, can be used to describe intramolecular distances. FLIM and FRET measurements can be combined and used to determine distances between cellular subunits, to indicate the spatial distribution of fluorescent molecules, and to describe the microenvironment.

The Advanced Optical Imaging Workshop attracted over 90 participants representing 14 research institutions from five countries. The meeting involved two days of talks highlighting advancements in statistical analysis for FLIM and FRET measurements, developments of more user-friendly software, and identifications of more photostable tracking technologies for a variety of biological applications. Attendees were also invited to laboratory tours, hands-on software and equipment demonstrations, and social gatherings.

This meeting was a wonderful opportunity to present my work using fluorescent microscopy for the "Characterization of Leukocyte Infiltrate in Mouse Melanoma" to an imaging community. I had the chance to speak with researchers using

novel techniques to address similar issues concerning melanoma research that provided me with several helpful recommendations. This was a unique occasion to introduce Wellington and New Zealand imaging research to an elite group of scientists as many groups indicated interest in pursuing international collaborations with New Zealand research initiatives. Attending this workshop proved to be an invaluable experience.

National Institute of Health (NIB) in Washington DC

K Price

With support from the Wellington Medical Research Foundation J was able to attend a laser safety course and to meet with world-renowned flow cytometrists at the National Institute of Health (NIB) in Washington DC in April this year.

The flow cytometry suite at the Malaghan Institute has three Class 3B lasers, which are hazardous for direct eye exposure (causing blindness) and can cause minor skin damage. We have recently purchased a I00m W UV laser and the long-term effects of UV laser exposure are the formation of cataracts and the potential to develop melanoma. The course provided comprehensive training in manual calculations of laser hazards as well as providing software able to compute intrabeam nominal ocular hazard distances (NOHD) and nominal hazard zones (NHZ) for lasers of any specification. After attending the course and becoming a certified laser safety officer, I feel confident that I can assess the potential for hazard from our lasers and will be implementing new safety protocols to prevent injury.

At the NIH I met with several flow cytometrists and not only gained valuable contacts but also received input that will be of great importance with upcoming flow cytometric applications. The new UV laser was purchased to enable sorting of Side Population (SP) cells, which we will be sorting from primary human tissue to investigate the hypothesis that there are cancer stem cells. I was fortunate enough to meet with the Barbara Taylor, the facility manager of the National Cancer Institute at the NIH who routinely performs SP cell sorts. Barbara imparted valuable information to me regarding the complex set-up and experimental design for successful SP ce11 sorting.

I greatly appreciate the opportunity I had to increase my knowledge and network base and believe that this experience is going to benefit not only the staff at the Malaghan Institute but also the wider scientific community.

Summer Student Research Reports

The Relationship between Standard Response Criteria and Patient Perception of Response in Rheumatoid Arthritis Following Inpatient Treatment

Neil Avery

Rheumatoid Arthritis (RA) is a chronic inflammatory disorder of unknown cause which occurs with varying frequency throughout the world. It primarily affects the joints and can show a highly variable often fluctuating course. If uncontrolled, it can lead to significant joint deformity and significantly impair quality of life. The variable nature of RA causes problems when attempting to define improvement after treatment. Several groups have come together to attempt to define improvement criteria. The two main groups are the European League Against Rheumatism (EULAR), and the American College of Rheumatologists (ACR). This study attempts to address the accuracy of these criteria in predicting response to treatment compared with the patient's perception of improvement as measured by a transition questionnaire.

In order to achieve this, consecutive patients admitted electively to Hutt Hospital rheumatology ward for treatment of RA were selected and data collected at four time points. This data was then analysed for response criteria and the results compared to the patient's response on a transition questionnaire. There were between 48 and 66 patients analysed for each response criteria, with the EULAR moderate criteria being effective at detecting if a patient felt they had improved and the ACR70 most accurate at predicting if a patient felt they had not improved. Statistical analysis was then carried out and found the EULAR moderate criteria to be the most effective at predicting patient perception of response.

This study had some problems; firstly with the validity of the transition questionnaire. Statistical analysis found the transition questionnaire to be biased by current state. This may have resulted in the EULAR criteria appearing more accurate as they include a measure of current state.

Other problems included missing data (making some patients unable to be analysed for some criteria) and the lack of standardisation of treatment and hospital stay length. This study found the EULAR moderate criteria to most accurately predict patient perception of improvement; however it was not very effective at determining when patients felt they had not improved. These inaccuracies suggest that the current response criteria are not ideal, do not effectively predict patient perception of improvement and are in need of further development.

How Do Assessors Make Decisions on Scoring Observed Consultations?

Chris Ingham

The consultation between a medical practitioner and a patient is central to medical practice. At Wellington School of Medicine and Health Sciences, students are assessed in their consultation skills through observed consultations, where a student's consultation with a simulated or real patient is marked. The aim of this project is to find how assessors make decisions when marking an observed consultation.

Staff from WSM&HS and the Capital & Coast District Health Board, were shown video recordings of consultations between medical students and simulated patients played by actors. They marked the students on a checklist of history points, on five attributes, namely the student's history taking technique, formulation of a diagnosis, formulation of a management plan, explanation to the patient, and communication with the patient, and on an overall scale. They were also asked how confident they were of their mark. The staff assessors were then interviewed as to how they made decisions about the marks that they gave.

The results showed that assessors give a mark based on a prescribed marking schedule, as well as on their own personal impression of a student. Statistical analysis showed that diagnostic formulation and the explanation to the patient were the most important contributors to the overall scale mark, however when questioned, assessors said they thought all attributes were linked with each other, and as such all contributed to the mark. A student's ability to take a logical history that led to a broad diagnostic formulation was important in gaining a good mark. Although assessors gave similar reasons for giving a mark, they sometimes gave different marks to the same student.

Assessors were more confident of giving a student a mark if they felt the student was clearly above or clearly below the pass/fail threshold. They were more confident if the student was slightly above than slightly below the threshold. Assessors would have felt more confident giving the student a mark had they seen them in a consultation on more than one occasion. Assessors also felt less confident if they felt the simulated patient gave the student more information than was asked for.

Identification of respiratory pathogens in nasopharyngeal aspirate (NPA) samples from infants hospitalised with bronchiolitis (2003-2005)

Sophie Robinson

Bronchiolitis is inflammation of the small airways, a disease that most commonly affects children under 2 years of age, with a peak incidence between 3 and 6 months. Bronchiolitis is characterised by wheezing, cough, rapid breathing and shortness of breath, which develops over one to two days. Severe disease may require hospitalisation for supportive care or mechanical ventilation, with 8% of New Zealand infants being hospitalised with bronchiolitis each year (2003 NZHIS data). One virus, Respiratory Syncytial Virus (RSV), accounts for 60-90% of bronchiolitis cases. Other viruses that cause the remaining cases, as well as dual infection with RSV, include Human Metapneumovirus (hMPV), Parainfluenza (PIV), Influenza, Coronavirus, Adenovirus and Rhinovirus.

Our aim was to find out whether infection with other viruses that cause bronchiolitis, or dual infection with RSV and another virus, caused a more severe form of the disease than infection with RSV alone, and how viruses other than RSV were contributing to New Zealand's high rate of bronchiolitis requiring hospitalisation. We designed and optimised molecular tests that could detect Human metapneumovirus, PIV types 1, 2 and 3, and influenza A and B. I used these tests to begin screening the 252 nasopharyngeal aspirate samples collected from infants who had been hospitalised with bronchiolitis during the annual winter epidemics of 2003-2005.

I screened 64 samples for hMPV and found one positive case (1.56%). I also samples for PIV 1 and 2, and 40 samples for PIV 3 and found no positive cases among these.

This research is part of the ongoing analysis of the samples collected from infants from 2003-2005 and has begun to contribute information on circulating respiratory pathogens. When all the samples from 2003-2005 have been screened for their specific viral pathogen, the data from this study can be compared to severity data from the children who were hospitalised. This will provide valuable information on how respiratory viruses other than RSV are contributing to the high rate of bronchiolitis hospitalisations in New Zealand.

Epidemiology and Risk Factors for the Homicide of Media Workers (2002-2006)

L Riddick

Media workers are often threatened, abused and killed. These attacks aim to prevent news from being collected or shared.

We collected information about 371 media worker deaths during 2002-2006. We looked at individual factors in the homicides of media workers and compared national death rates with national data on corruption, political terror, and the how well the government works.

The number of media workers killed each year has risen over the last five years, but less than 25% or these murders result in either an arrest or prosecution. Most deaths were either known, or strongly suspected to be related to the journalists work.

The rate of media worker homicides in a country is closely related to the measure of how much a country is terrorised by armed groups (private and state sponsored).

Iraq has had the most media deaths in the last 5 years. In 2003 these were mostly foreign media workers, but since 2004 they have been Iraqis.

Lack of prosecution encourages murder as a way of silencing the media. In areas experiencing political change, media workers adjust to increased freedom of speech faster than those in power adjust to increased scrutiny. This increases attacks on the media as those in power seek to silence them.

Events Precipitating Pensioners Move Into Rest Homes Study 2007

Yuen Lung Choi

The study aimed to establish why people had moved into rest homes; and 66 rest home and 25 retirement village residents from the Wellington, Lower Hutt and Porirua area were interviewed.

For rest homes residents, the most frequently mentioned reason for moving was medical (66%) followed by falls (44%) and housing (32%). For village residents, the most frequently mentioned reason was housing (84%) followed by falls (28%) and medical (20%). Of the housing reasons, difficulty with steps and stairs was the most common for rest home residents; for village residents, it was house and garden maintenance.

The residents from both groups were similar in that 70% of the residents had always felt warm enough during winter in their old house and more than 70% of residents found that friends, public transport and shops were close enough. However, house ownership had been higher amongst village residents (V:76%; RH:48%) and rest home residents typically took less time to decide to move.

As rest home and retirement village residents frequently entered these facilities with housing as one of their reasons, future studies should investigate the cost effectiveness of subsidies for people to move into accommodation designed for older people.

Outcomes of Induction of Labour in Women of Advanced Age, at or Beyond Term

Jessica Robinson

Prolonged labour (greater than 42 weeks gestation) has long been associated with adverse maternal and neonatal outcomes. One way to reduce these adverse outcomes is with earlier induction of labour. Currently in Wellington Women's Hospital there is a recommendation that women are induced at 40 weeks + 10 days induction however this is not strictly followed.

This study looked at the outcomes of deliveries in women who were induced for exceeding their expected due date (40 weeks). We were particularly interested in women of advanced maternal age, defined as 35 years and older. The aim of the study was to see if there was a relationship between advanced maternal age, the timing of induction in pregnancies after 40 weeks gestation, and outcomes in both the mother and baby.

12,284 deliveries, which took place at Wellington Women's Hospital, between July 1996 and June 2006, were included in the study. Our results showed that induction of labour in women with uncomplicated pregnancies is associated with increased rates of meconium, fetal distress, primary post partum haemorrhage and caesarean deliveries regardless of the gestational week in which they are induced or the age of the patient.