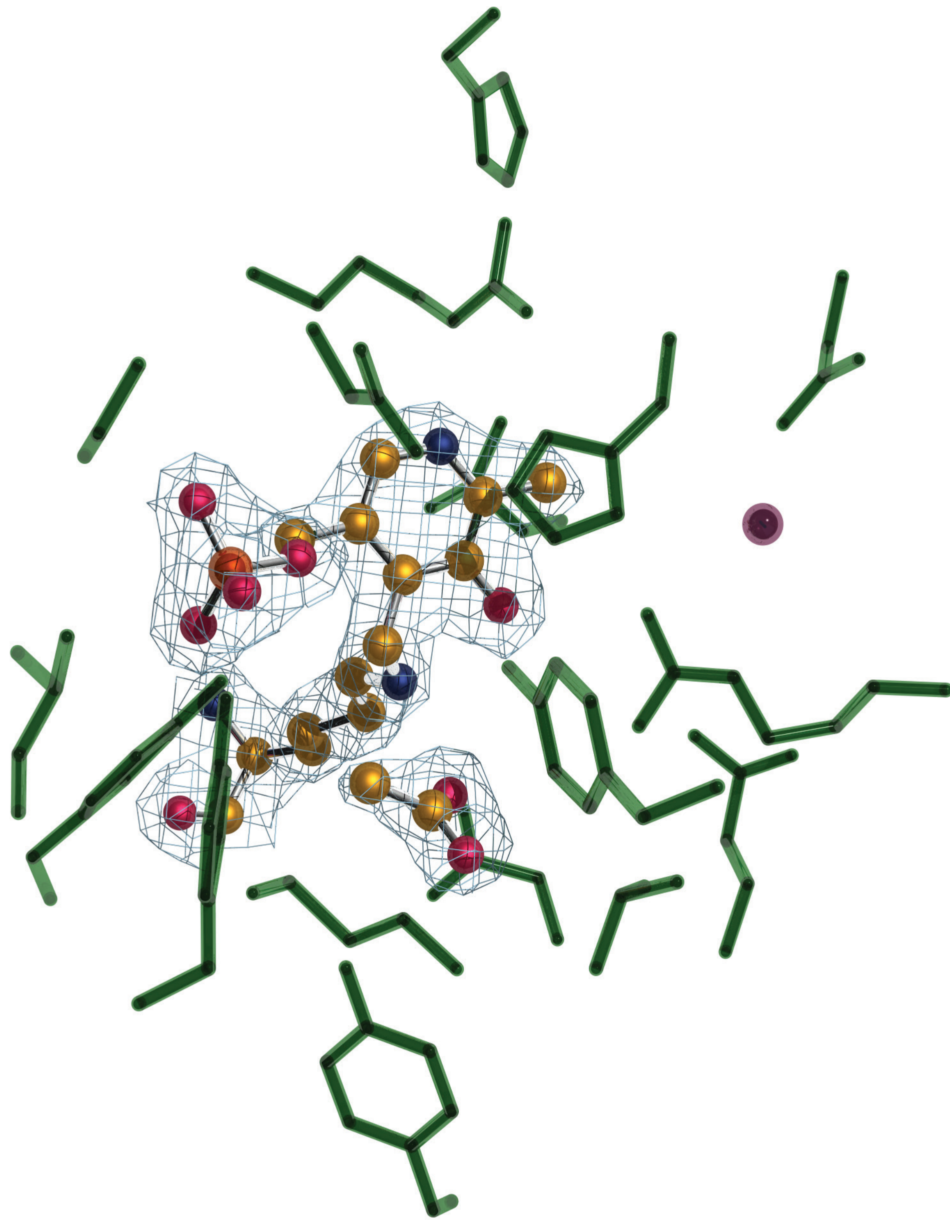
Webster Centre Symposium 2009

Webster Centre for Infectious Diseases



23-24 April 2009

St. Margaret's College

University of Otago

Dunedin, New Zealand

On the Cover:

An electron density map of LLP and an acetate molecule in the active site of alanine racemase from *Bacillus anthracis*.

Points of interest include the LLP residue, an acetate molecule found in the active site, and a Cl^- ion which is in close proximity to the active site. LLP is a modified lysine residue. Alanine racemase is an essential enzyme in prokayotes and is therefore a putative drug target.

Image modified by Rebecca Psutka and Bronwyn Carlisle using MegaPOV from a structure determined by Dr. Rafael Couñago (all department of Biochemistry, University of Otago).

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Symposium Programme

Thursday 23 April 2009

8:30 am – 8:50 am	Coffee/Tea
8:50 am – 9:00 am	Welcome, Introductory Comments (Kurt Krause, Director WCID and David Skegg, Vice-Chancellor)

Current Topics in Infectious Diseases I — Chair: Professor Kurt Krause

9:00 am – 9:20 am	**Warren Tate** University of Otago "The translational frameshift mechanism of HIV-1 as a drug target: can damage to the same mechanism in human gene peg10 be avoided?"
9:20 am – 9:40 am	**George Lees** University of Otago "Exploring the mechanisms for the Neurotoxicity of Efavirenz (EFV)
9:40 am – 10:00 am	**Russell Poulter** University of Otago "Genetics and genomics of the New Zealand isolates of the chytrid pathogen of amphibians, *Batrachochytrium dendrobatidis*"
10:00 am – 10:20 am	**Gary Evans** Industrial Research Ltd. "Transition State Analogues of 5'-Methylthioadenosine Nucleosidase Disrupt Quorum Sensing"
10:20 am – 10:40 am	Morning Tea
10:40 am – 11:00 am	***Anna Konings** University of Otago "Mechanisms of Iron Uptake by *Pseudomonas aeruginosa* in the Cystic Fibrosis Airway"
11:00 am - 11:20 am	**Sue Huang** ESR NCBID "Influenza surveillance, immunisation, antiviral susceptibility monitoring, zoonosis research in NZ"
11:20 am – 11:40 am	**Chris Brown** University of Otago "Analysis of virus and human genome sequences for RNA regulatory elements"
11:40 am – 12:00 noon	**Rafael Couñago** University of Otago "Structural characterization of alanine racemase from *Bacillus anthracis* (Ames)"
12:00 noon – 1:00 pm	Lunch

*eligible for student oral or poster presentation award

Tuberculosis and Mycobacterial Diseases — Chair: Professor Greg Cook

Time	Session
1:00 pm – 1:20 pm	**Vic Arcus** University of Waikato "The vapBC operon from *Mycobacterium smegmatis* is a toxin-antitoxin module which controls growth via inhibition of translation"
1:20 pm – 1:40 pm	***Mark Robinson** University of Otago "Gene expresion profiles differentiate pathological outcomes of Johne's Diseases: an infectious model of inflammatory bowel disease"
1:40 pm – 2:00 pm	**Fenella Rich** Malaghan Institute of Medical Research "Lung-resident memory lymphocytes are essential for protection against a Mycobacterial lung infectious challenge"
2:00pm – 2:10 pm	Short Break
2:10 pm- 2:30 pm	**Ronan O'Toole** Victoria University of Wellington "Absolutely, positively searching for new anti-tubercular drugs"
2:30 pm- 2:50 pm	**Colin Mackintosh** AgResearch "Are young red deer (*Cervus elaphus*) more susceptible to Johne's Disease than older deer?"
2:50 pm- 3:10 pm	**David Larsen** University of Otago "The Structure, Chemical Syntheses and Adjuvant Properties of Phosphatidylinositol Mannosides from Mycobacteria"
3:10 pm- 3:30 pm	Afternoon Tea
3:30 pm- 3:50 pm	**Frank Griffin** University of Otago "Paratuberculosis in deer: A large animal model for heritable resistance to pathogenic Mycobacteria"
3:50 pm- 4:10 pm	***Mridula Dogra** University of Auckland "Does reductive metabolism of PA-824, a novel anti-TB drug, occur in human liver S9?"
4:10 pm- 4:30 pm	**Stephen Chambers** University of Otago, at Christchurch "The scent of *Mycobacterium tuberculosis* – part II"
4:30 pm – 6:30 pm	Free time until dinner. Bus to depart from St. Margaret's College for Glenfalloch at 6:00pm
6:30 pm	Dinner at Glenfalloch Restaurants and Gardens
8:00 pm	**Clifton E. Barry, III** NIH, NIAID Keynote Address: "In vivo imaging of tuberculosis and its response to chemotherapy"

*eligible for student oral or poster presentation award

Friday 24 April 2009

8:30 am – 9:00 am	Coffee/Tea

Vaccine Discovery & Development — Chair: Professor Andy Mercer

9:00 am – 9:20 am	**Hannah Kelly** Malaghan Institute of Medical Research "Immunogenicity and protective capacity of DNA vaccines encoding tuberculosis latency antigens"
9:20 am – 9:40 am	**Desmond Collins** AgResearch NCBID "New live *Mycobacterium bovis* vaccines for possums"
9:40 am – 10:00 am	**Sarah Young** University of Otago "The use of Virus-like particles as vaccines and immunotherapies for cancer and infectious diseases"
10:00 am – 10:20 am	Morning Tea
10:20 am – 10:40 am	**Sarah Hook** University of Otago "Designing Sustained Release, Particulate, Sub-unit Vaccines"
10:40 am – 11:00 am	**Philip Hill** University of Otago "Introduction of Prevenar into Africa: The Gambian pneumococcal surveillance project"
11:00 am – 12:00 noon	**Cheryl Jo White** VGX Pharmaceuticals **Keynote Address: "How to get Prophylactic or Cancer Vaccines Licensed in 10 (Not So) Easy Steps"**
12:00 noon – 12:45 pm	Lunch
12:45 pm – 1:45 pm	Poster Session Michael Berney Tony Cardno *Genevieve Evans Shaun Lott *Joanna McKenzie *Ali Ruthe *Emma Summers
1:45pm – 2:00 pm	**Kurt Krause** University of Otago "Networking at the Webster Centre"

*eligible for student oral or poster presentation award

Current Topics in Infectious Diseases II — Chair: Professor Frank Griffin

2:00 pm – 2:20 pm	**John Tagg**	University of Otago
	“Something old and something new- the amazing repertoire of bacteriocins produced by *Streptococcus salivarius*”	
2:20 pm – 2:40 pm	**Margi Butler**	University of Otago
	“Chloramphenicol cures chytridiomycosis in frogs”	
2:40 pm – 3:00 pm	**Muriel Dufour**	ESR NCBID
	Surveillance of enteric pathogens in New Zealand	
3:00pm – 3:20 pm	**Michael Baker**	University of Otago, at Wellington
	“The epidemiology of Tuberculosis in New Zealand: Reinforcing the need to address national and global health inequalities”	
3:20 pm – 3:40 pm	**Clifton E. Barry, III**	NIH, NIAID
	TBA	
3:40 pm	Wrap up and Finish	

Who we are:

Welcome from Webster Centre Director Professor Krause

Welcome to the 2009 Webster Centre Symposium in Dunedin. This will be our second nationwide research meeting in infectious diseases and it promises to be an interesting gathering. We are fortunate to have some outstanding international keynote speakers and also fortunate to be hearing from some of the very best infectious diseases researchers in the country.

In addition to enjoying the talks this year, it is a good time to think about the future of the Webster Centre and of infectious diseases research in New Zealand. To that end the Webster Centre is seeking to help foster the development of research networks in New Zealand consisting of 4 or 5 laboratories working on a common theme. The establishment of these networks could be a key step in increasing visibility and funding for our research. If you have formed such a group we would be keen to feature it in a profile on our new web site, so please e-mail me at kurt.krause@otago.ac.nz and we will take it from there. Have a great conference!

Kurt Krause
Department of Biochemistry

Webster Centre Coordinator Rebecca Psutka

Rebecca has primarily been focused on ensuring the success of the Webster Centre Symposium 2009 - from preliminary advertising and marketing to venue scouting, coordinating registrations, preparing this Programme and attracting some corporate sponsorship. Since October 2008, Rebecca has also been responsible for coordinating the Webster Centre Summer Studentship Awards, Webster Centre Postgraduate Travel Stipends, and writting the monthly newsletter. Rebecca has coordinated the travel of our sponsored seminar speakers as well as the Keynote speakers at the Webster Centre Symposium 2009. Additionally, Rebecca was responsible for designing and developing the Webster Centre for Infectious Diseases website: www.webstercentre.otago.ac.nz. Rebecca is thankful for assistance from Bronwyn Carlisle, Teena Joyce, and Chelsea Ivey from the Department of Biochemistry.

Rebecca is also part of Professor Kurt Krause's research group as an Assistant Research Fellow and maintains/transfects tissue culture of mammalian cells to produce proteins for further study.

Rebecca Psutka has a BSc(Hons) in Immunology and Infectious Diseases from the University of Alberta, Canada, and is interested in infectious diseases from a public health and control standpoint. She is going to take a course-based MSc. in Control of Infectious Diseases from the London School of Hygiene and Tropical Medicine beginning September 2009.

Keynote Speakers

Clifton E. Barry, III, PhD

Clifton E. Barry, III, Ph.D. is currently Chief of the Tuberculosis Research Section at the National Institutes of Health in the Institute of Allergy and Infectious Disease. He received his Ph.D. in organic chemistry in 1989 from Cornell University with Tadhg Begley. Following postdoctoral research with Craig Townsend at Johns Hopkins University, Dr. Barry joined NIAID in 1991 and was tenured in 1998. His research group works on all aspects of tuberculosis drug discovery and genomics and includes a clinical trials program in patients with highly drug-resistant disease in South Korea. Dr. Barry has authored more than 100 research publications in tuberculosis since entering the field.

C. Jo White, M.D.

C. Jo White, M.D., has 21 years of clinical and product development experience in the pharmaceutical industry. Her experience has been focused in the areas of infectious diseases, primarily in vaccine development. Over the past 21 years she has designed and conducted over 40 Phase 1-4 clinical trials, filed 6 BLA/MAAs and has licensed 4 different vaccines: Certiva® (DTaP vaccine), Varivax® (live, attenuated varicella vaccine), VAQTA® (hepatitis A vaccine), NeisVax® (conjugated meningococcal B vaccine) in both the United States and Europe. She is presently the Chief Medical Officer of VGX Pharmaceuticals, Inc. and is developing a small molecule for use in treating rheumatoid arthritis (Phase I) and a DNA vaccine delivered by electroporation for treatment of cervical neoplasias due to human papilloma virus (Phase I).

Dr. White received her MD with honors (member of AOA) from Baylor College of Medicine, completed an internship and residency in internal medicine at North Carolina Baptist Hospital (Bowman Gray School of Medicine), and a fellowship in infectious diseases at the National Institutes of Health. She is board certified in Internal Medicine and Infectious Diseases. She is married with 3 boys in college/graduate school and runs, swims, bikes and plays golf for fun.

9:00 am - 9:20 am
Professor Warren Tate, University of Otago

The translational frameshift mechanism of HIV-1 as a drug target: Can damage to the same mechanism in human gene peg10 be avoided?

Warren Tate, Elizabeth Poole, Tina Edgar, Tony Cardno, Suneeth Mathew, Yosuke Shimaki, Sarah James, Caillan Crowe-McAuliffe, Julian Peat, & Nathalie Saurat

Department of Biochemistry, University of Otago

HIV-1 uses a rare genetic mechanism, translational frameshifting, to regulate the ratio of its structural and enzyme proteins. This backwards slippage event of the RNA on the translating ribosome is crucial to the virus' infectivity, and is mediated by an invariant slippery heptanucleotide, an 'intercodon', and a highly conserved stem-loop. PEG10 is a human gene that has arisen from 'molecular domestication' of a retroelement insertion into an early mammalian genome. It also uses backwards frameshifting for its expression. We have shown this gene is expressed into protein with frameshifting in proliferating placenta and amniotic membranes, but not in any form in adult mammalian tissues. Hence an antiviral strategy against HIV-1 that excludes pregnant women remains possible. We developed a new model for the HIV-1 frameshift mechanism, and have developed an inexpensive and fast bifluorophore cell-based assay for drug screening that is now being converted to high throughput screening in Melbourne at the WEHI High Throughput Chemical Screening facility. Currently, we are investigating how PEG10 expression is regulated negatively in adult mammalian tissues, having identified two prime candidate microRNAs, and/or regulated positively in placenta, and are developing new strategies to identify cellular proteins that we have shown in preliminary experiments to interact with the HIV-1 and PEG10 frameshift elements.

9:20 am - 9:40 am
Professor George Lees, University of Otago

Exploring the mechanisms for the Neurotoxicity of Efavirenz (EFV)

George Lees[1], Christine Dixon[1] and Joel Tyndall[2]

[1]Dept of Pharmacology & Toxicology , University of Otago, Dunedin
[2] School of Pharmacy, University of Otago, Dunedin

Drug management of HIV AIDS has significantly reduced patient mortality but the emergence of resistance profiles accentuates the need for new and better drugs (and drug combinations). Efavirenz (EFV) is an effective antiviral drug (non-nucleotide inhibitor of reverse transcriptase) but it exerts undesirable CNS toxicity in up to 40% of patients. Here we address the hypothesis that this toxicity may reflect an interaction with membrane ion channels. Primary cultures were prepared from the cortex of E18 rats (University Otago ethics approval ET27/07) and whole cell patch recording was used to examine both ligand- and voltage-gated ion channels. Currents through GABAa chloride channels were not significantly modulated by EFV at 10 μM but a significant depression of membrane excitability was noted in response to 10 μM EFV. EFV blocked secondary spikes in an evoked bursts of action potentials: effectively mimicking the effects of anticonvulsant drugs. Using in-sillico modeling techniques, EFV appears to overlay well with carbamazepine, phenytoin and lamotrigine (all bear aromatic and hydrogen bond donors and acceptors in similar steric locations which may indicate a common pharmacophore). The molecular identity of the voltage-gated ion channels sensing the actions of EFV will be the focus of ongoing studies in the Lees lab.

9:40 am - 10:00 am
Associate Professor Russell Poulter, University of Otago

Genetics and genomics of the New Zealand isolates of the chytrid pathogen of amphibians, *Batrachochytrium dendrobatidis*

Russell Poulter, Cynthia Huang, Annika Bokor, Margi Butler

University of Otago

A third of amphibian species are threatened with imminent extinction. The biggest threat is *Batrachochytrium dendrobatidis* (Bd), a pathogenic fungus that is rapidly fatal to susceptible frog species. First identified ten years ago, this recently emerged pathogen has driven frog species to extinction in North, Central and South America and Australia. We have recently isolated this chytrid from two frog species in New Zealand.

The origin of the pandemic is unclear. Genomic analysis indicates Bd is diploid. The complete sequence of strain JEL423 is publicly available. Our preliminary analysis revealed an unexpected feature, the genome displays a mosaic pattern; some sequence blocks show frequent heterozygous sites while the remainder of the genome consists of homozygous blocks. The mosaic pattern of heterozygosity and homozygosity suggests that the pandemic strain was formed by hybridisation between two dissimilar strains (generating general heterozygosity) and that this hybrid has undergone genetic recombination (giving homozygous blocks). We have performed a preliminary analysis of selected regions of a number of New Zealand and other isolates and found that this recombination is occurring at a very high frequency. Recombination gives rise to patterns of homozygosity that vary among strains and these patterns can be used in epidemiological studies.

10:00 am - 10:20 am
Dr. Gary Evans, Industrial Research Ltd.

Transition State Analogues of 5′-Methylthioadenosine Nucleosidase Disrupt Quorum Sensing

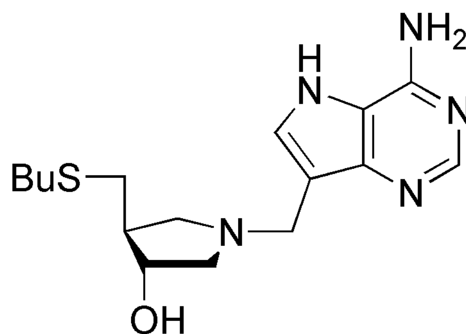
Gary B. Evans,[1] Richard H. Furneaux,[1] Vern L. Schramm,[2] and Peter C Tyler[1]

[1]Industrial Research Ltd
[2]Department of Biochemistry, Albert Einstein College of Medicine, Yeshiva University

Bacteria communicate with each other by a process known as quorum sensing. When the population density reaches critical levels, they produce and detect signaling molecules known as autoinducers (AIs) to coordinate gene expression and regulate processes beneficial to the microbial communities. Methylthioadenosine nucleosidases have a crucial role in maintaining homeostasis in bacteria. MTANs are directly involved in the biosynthesis of AIs thus MTAN inhibition has been proposed as a means of blocking AI production, thereby disrupting quorum sensing and hence inhibitors of MTANs are potential antibiotics.

Through utilising transition state analysis of a variety of bacterial MTANs we have designed and then synthesized a series of powerful inhibitors with dissociation constants as low as 47 fM. Three of these inhibitors have been shown to disrupt quorum sensing in a variety of bacteria and one inhibitor in particular, BuT-DADMe-ImmucillinA, inhibited AI production in *V. cholerae* and enterohemorrhagic *E. coli* O157:H7 which persisted for several generations and in the case of the *E. coli* strain caused reduction in biofilm formation (1).

Herein we will briefly describe the design and synthesis of these inhibitors and their associated biological results.



BuT-DADMe-Immucillin-A

1. Gutierrez, J.A. et al Nat. Chem. Biol., 2009, Mar 8 (Epub ahead of print)

10:40 am - 11:00 am
Anna Konings, University of Otago

Mechanisms of Iron Uptake by *Pseudomonas aeruginosa* in the Cystic Fibrosis Airway

Anna F. Konings[1], Lois W. Martin[1], David W. Reid[2] and Iain L. Lamont[1]

[1] Department of Biochemistry, University of Otago, Dunedin, NZ
[2] University of Tasmania School of Medicine, Hobart, Australia.

Pulmonary infections with the pathogenic bacterium *Pseudomonas aeruginosa* are a major cause of mortality for patients with the genetic disease cystic fibrosis (CF). Growth of *P. aeruginosa* depends on the ability to acquire iron from the iron-deplete environment of the lung. For this purpose, *P. aeruginosa* employs two iron-scavenging siderophores, pyoverdine and pyochelin. Pyoverdine is preferentially produced and is thought to be the mechanism through which iron is taken up in the CF airway.

Fluorometric assays of CF patient sputum indicate that pyoverdine is present in most but not all samples. We have developed a quantitative RT-PCR approach to correlate pyoverdine production with gene expression. Furthermore, we are extending this approach to determine whether bacteria that fail to make pyoverdine are instead producing pyochelin for iron uptake; or alternately whether they are utilising heme in the host lung as an iron source.

* Anna Konings is eligible for the Student Oral Presentation Award

11:00 am - 11:20am
Dr. Sue Huang, ESR NCBID

Influenza surveillance, immunisation, antiviral susceptibility monitoring, zoonosis research in New Zealand

Q. Sue Huang[1], Richard Hall[1], Liza D. Lopez[2], Lisa McCallum[2], Hurt A.C.[3], Ian Barr[3], Bruce Adlam[2], NCBID avian influenza project team[4]

[1]The WHO National Influenza Centre
[2]Population and Environmental Health[2], Institute of Environmental Science and Research.
[3]The WHO Collaborating Cente for Reference and Research on Influenza, Melbourne, Australia.
[4]Zoonosis study by a collaborative research team among MAF Biosecurity New Zealand, AgResearch and ESR.

We present the national influenza surveillance data collected during 1997-2006 in terms of the community disease burden, circulating viral strains, hospitalisations, mortality, and immunisation coverage. When the last 10 years were compared to the previous years, sentinel general practice surveillance recorded a decreasing trend of influenza-like illness rates in the community. Sentinel surveillance also showed that children aged 0-4 years were the most affected. Influenza-related hospitalisation surveillance reported an increasing trend of hospital admissions particularly in children aged 0-19 years. Introduction of routine influenza vaccination among the New Zealand elderly was associated with a significant decrease of influenza-related mortality.

In 2007, New Zealand became the first developed country in the world to allow Tamiflu® (oseltamivir) to be distributed over-the-counter in pharmacies for treatment of influenza in adults during the period when influenza was most prevalent. The WHO National Influenza Centre at ESR has established a national antiviral susceptibility surveillance system, to monitor oseltamivir-resistant influenza viruses. Results from 2006 to 2008 including the emergence of oseltamivir resistant influenza A(H1N1) viruses in New Zealand are presented.

The avian influenza zoonosis research was the first joint project undertaken by the National Centre for Biosecurity and Infectious Disease. The project involved collaboration between participants from the Ministry of Agriculture (MAF), Environmental Science and Research (ESR) and AgResearch and provided a model for multi-institutional collaborations on a topic of animal and public health importance. The goal of this two year study (2006-2008) was to understand the ecology, including the potential transmission pathways, of low pathogenic strains of avian influenza amongst wild birds, non-commercial back yard poultry flocks (chickens, ducks, turkeys, geese etc.) and humans in New Zealand. The findings from this project are presented.

11:20 am - 11:40am
Dr. Chris Brown, University of Otago

Analysis of virus and human genome sequences for RNA regulatory elements

Chris M Brown[1], Tracy Wilkinson[1], Nattanan Panjaworayan[1], Andrew E Firth[1], Augustine Chen[1], Stewart G Stevens[1], Angelina Wong[1], Mik Black[1], Paul Gardner[2] Stephan K Roessner[1]

[1] Biochemistry and Webster Centre for Infectious Diseases, University of Otago
[2] Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, CB10 1SA, UK

There are a large number of viral genome sequences available (>500,000), which provide a rich resource for genome mining. However, most of the publicly available sequences have limited annotation, usually restricted to identification of the protein coding regions on the genome. While NCBI has recently annotated a set of RefSeq viral genomes to a higher standard, this mainly involves the protein coding regions (3,032 sequences).

We wish to discover novel RNA regulatory elements or proteins that are conserved across viral genomes. We have developed tools to analyse conserved novel coding regions (MLOGD) and elements (CDS-Plotcon), and included comparisons to published RNA structure tools implemented locally (e.g. RNAAlifold). This is published as a complete HBV genome resource, that includes comparisons to all other viral and non-viral sequences (HBVRegDB). This data is visualised through a GBrowse browser and available via the KAREN network.

When putative RNA elements are discovered they are compared to known regulatory elements, particularly those in from the human genome. To increase the repertoire of known regulatory elements with computational models we have cross-referenced these with known cellular elements (our Transterm data) and collaborated with Rfam (Wellcome) to increase the coverage of known 'cis regulatory RNA elements' in that database.

To facilitate the discovery of RNA regulatory elements, we have implemented a publically available integrated human mRNA 3' UTR analysis platform (bioanalysis.otago.ac.nz/galaxy).

11:40am - 12:00noon
Dr. Rafael Couñago, University of Otago

Structural characterization of alanine racemase from *Bacillus anthracis* (Ames)

Rafael M. Couñago[1], Ryan E. Hill[1], Mylia Davlieva[2], Ulrich Strych[2] and Kurt L. Krause[1].

[1]Department of Biochemistry, University of Otago, PO Box 56, Dunedin, New Zealand
[2]Department of Biology and Biochemistry, U. of Houston, Houston, Texas, 77204, USA

Bacillus anthracis is the causative agent of anthrax and a potential bioterrorism threat. Here we report the structural characterization of *B. anthracis* (Ames) alanine racemase (Dal1Bax). Alanine racemase is an essential enzyme in prokaryotes. Moreover, this enzyme's activity plays a major role during sporulation and germination of *B. anthracis*. The crystal structure of native Dal1Bax was solved to 1.95Å and reveals a homodimer formed by a head-to-tail-association of two monomers. The enzyme's PLP cofactor forms a covalent bond to Lys41 and points at the center of the α/β-barrel. Despite the overall fold similarity to other alanine racemases, Dal1Bax makes use of a structural chloride ion to position key active site residues for catalysis, a feature not yet observed for other alanine racemases and that may represent a novel strategy for the design of Dal1Bax inhibitors. We also compare the structures of native Dal1 with the one obtained following reductive lysine methylation. Despite differences in space group and crystal form, the two Dal1Bax structures are very similar and supports the case that reductive methylation is a valid rescue strategy for proteins recalcitrant to crystallization. Inhibitors of Dal1Bax may represent a viable strategy for spore remediation.

1:00pm - 1:20pm
Vic Arcus, University of Waikato

The vapBC operon from *Mycobacterium smegmatis* is a toxin-antitoxin module which controls growth via inhibition of translation

Jennifer Robson[1], Joanna L. McKenzie[2], Ray Cursons[2], Gregory M. Cook[1] and **Vickery L. Arcus[2]**

[1]Department of Microbiology and Immunology, Otago School of Medical Sciences, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand.
[2]Department of Biological Sciences, University of Waikato, Private Bag 3105, Hamilton 3240, New Zealand.

The largest family of bacterial toxin-antitoxin (TA) modules are the vapBC operons and these are grouped together by virtue of their toxin components belonging to the PilT N-terminal domain (PIN-domain) family of proteins, which are thought to function as ribonucleases. We have identified a single vapBC operon in the genome of *Mycobacterium smegmatis* which is transcribed as a leaderless mRNA that is constitutively synthesised throughout the growth cycle. The vapBC operon is autoregulated by the VapBC protein complex which binds to inverted repeat DNA sequences in the vapBC promoter region that overlap the -35 and 10 promoter elements. Neither a ΔvapBC nor ΔvapB mutant strain exhibited any phenotypic deviation to that of the isogenic wild-type parent strain under normal laboratory growth conditions, but conditional overexpression of VapC in *M. smegmatis* inhibited growth by a bacteriostatic mechanism and this phenotype is exacerbated in a ΔvapBC mutant. This effect is mediated through VapC-dependent inhibition of translation, and not inhibition of DNA replication or transcription. Taken together these data demonstrate that VapBC from *M. smegmatis* has all the hallmarks of a toxin-antitoxin module with the capacity to cause growth inhibition by regulating translation.

1:20pm - 1:40pm
Mark Robinson, University of Otago

Gene expression profiles differentiate pathological outcomes of Johne's Disease: an infectious model of inflammatory bowel disease

Robinson, M.W., O'Brien, R., Griffin, J.F.T.

Disease Research Laboratory, University of Otago, New Zealand.

The gastrointestinal immune response is unique in its opposing goals of tolerating food antigens and commensal microflora versus the need to activate strong immune responses to control pathogenic infections. When this immunological balance is upset chronic diseases such as inflammatory bowel disease (IBD) can develop. Using an experimental infection model of IBD in red deer (*Cervus elaphus)*, we studied the immune responses during the different pathological states of disease. Johne's disease is a chronic IBD of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). A broad spectrum of pathological outcomes are observed following experimental infection with MAP ranging from clearance or containment of bacteria through to excessive inflammation and unrestrained bacterial growth causing death in clinically diseased animals. Gene expression profiling of peripheral blood mononuclear cells and gut-associated lymphatic tissues has shown an association between uncontrolled Th1 and Th17 responses and gut immunopathology. In animals with contained infection an association of Treg and Th2 immune responses in balancing the immune responses has been observed, challenging the dogma that Th2 responses in Johne's disease are characteristic of severe disease. These immune profiles provide novel diagnostic markers for the different pathological states of Johne's disease and other inflammatory bowel diseases.

* Mark Robinson is eligible for the Student Oral Presentation Award

1:40pm - 2:00pm
Ms. Fenella Rich, Malaghan Institute of Medical Research

Lung-resident memory lymphocytes are essential for protection against a Mycobacterial lung infectious challenge

Fenella Rich, Lisa Goldsack, Marina Harvie, Kylie Quinn, Volker Brinkmann[1], Graham Le Gros and Joanna Kirman.

Malaghan Institute of Medical Research, Wellington, New Zealand
[1]Novartis Institutes for Biomedical Research, Basel, Switzerland

The mechanisms orchestrating the protection against tuberculosis that follows BCG vaccination are not fully resolved. We explore the hypothesis that the location of memory T cells (Tmem) at the site of infection is paramount for immunological protection. We gave the drug fingolimod (FTY720), which prevents egress of lymphocytes from the lymph node, to mice either during BCG vaccination or a subsequent mycobacterial lung challenge to block lymph node effector and Tmem from entering the periphery during priming, or secondary exposure.

Treatment with fingolimod during vaccination resulted in no protection from a subsequent mycobacterial lung challenge. By contrast, BCG-vaccinated mice were protected when fingolimod was given during challenge, suggesting that migration of T cells to peripheral tissue following vaccination is essential for protection. Furthermore, Tmem residing in the lung during secondary exposure are sufficient for protection and do not require the help of Tmem recruited from the lymph node. Notably, the number of IFNγ- or multicytokine-producing CD4+ T cells in the lung after mycobacterial challenge did not correlate with protection. However, increased expression of MHC class II on macrophages isolated from lungs post challenge did correlate with protection. We conclude that protection conferred by BCG vaccination is dependent on memory lymphocytes residing in the lung and that an important quality of these cells might relate to the activation of alveolar macrophages.

2:10pm - 2:30pm
Dr. Ronan O'Toole, Victoria University of Wellington

Absolutely, positively searching for new anti-tubercular drugs

Ronan O'Toole, Chris Miller, Mudassar Altaf, Ian Bassett, Shahista Nisa, Sandi Dempsey, Nathaniel Dasysam, Emma Earl.

Victoria University of Wellington

The most recent data from the World Health Organisation demonstrate that over 9 million new cases of tuberculosis (Tb) occur each year. To meet the WHO's goal of halving the incidence of the disease, new drugs are needed to treat MDR and XDR forms of Tb, shorten the treatment period and cure latent Tb infections. The primary objective of our work is the identification of new inhibitors of mycobacteria and their cellular targets.

We have developed a fluorescence-based whole cell protocol for the detection of compounds with anti-mycobacterial activity. The assay has been validated for sensitivity and reproducibility using existing first- and second-line anti-tubercular drugs. We have applied the assay to high-throughput screening of multiple synthetic and natural product libraries and have identified a series of previously-unknown mycobacterial inhibitors. To facilitate targeted screens for inhibitors of specific mycobacterial cellular processes or molecules, we have also integrated reverse genetics into our assay. For the detection of compounds which kill non-replicating mycobacteria, we have recently established a metabolism-based high-throughput assay.

The anti-mycobacterial protocols developed and data on the inhibitors identified will be presented. Funding for this work has been provided by the NZ Health Research Council and the Wellington Medical Research Foundation.

2:30pm - 2:50pm
Dr. Colin Mackintosh, AgResearch

Are young red deer (*Cervus elaphus*) more susceptible to Johne's Disease than older deer?

CG Mackintosh, RG Clark, B Thompson, B Tolentino, G de Lisle.

AgResearch, Invermay

Aim: to measure the relative susceptibility of three age classes of red deer to Johne's disease, using experimental challenge with *Mycobacterium avium* subsp. *paratuberculosis* (MAP).

Three groups of sero-negative female deer (30 three-month-old weaners, 20 fifteen-month-old yearlings and 20 adults) received four oral doses of ~109 colony forming units of a bovine MAP. They were monitored for 50 weeks, clinically affected animals were promptly euthanised and the remaining deer were killed at the end. Necropsies were carried out and samples of intestine and associated lymph nodes were taken for culture and histopathology from all deer.

Ten weaners developed clinical Johne's disease and were euthanased 20-28 weeks pi. No clinical cases occurred in the yearlings or adults. All 10 clinically affected weaners had severe gross and histopathological lesions of Johne's disease. At slaughter, gross lesions were seen in the jejunal lymph nodes of 8/17 weaners, 2/19 yearlings, and 0/20 adults. *M. ptb* was cultured from samples of the intestine and/or lymph nodes from all 10 clinical cases and from 16/17 weaners, 19/19 yearlings and 18/20 adult hinds at slaughter.

There is a strong age-related resistance against clinical disease and subclinical disease, but not to infection, after heavy challenge.

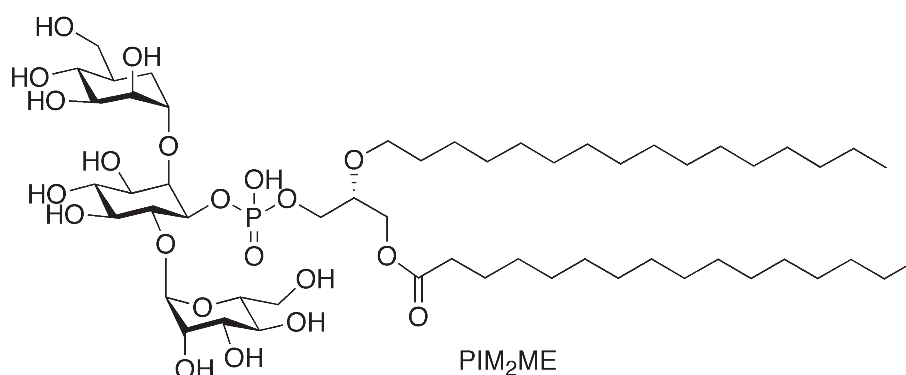
2:50pm -3:10pm
Associate Professor David Larsen, University of Otago

The Structure, Chemical Syntheses and Adjuvant Properties of Phosphatidylinositol Mannosides from Mycobacteria

D. S. Larsen,[1] G. D. Ainge,[2] B. S. Dyer,[1] M. Denis,[3] P. Rendle[2] and G. F. Painter[2]

[1]Department of Chemistry, University of Otago, Dunedin
[2]Industrial Research Ltd, , PO Box 31310, Lower Hutt, New Zealand.
[3]AgResearch, Hopkirk Research Institute, AgResearch Grasslands, Palmerston North, New Zealand.

Phosphatidylinositol mannosides (PIMs), present in the cell walls of mycobacteria, are attracting a great deal of attention as they elicit a range of immune responses and constitute the biosynthetic precursors of lipomannan and lipoarabinomannan. PIMs act as agonists of Toll-like receptor 2 (TLR2) which is involved with innate immunity. 1,2 Structure-activity relationships of PIMs are complicated by the fact that these molecules exist in nature in multi-acylated forms and obtaining single entities from cell wall material is challenging. Chemical synthesis of PIMs has allowed us to overcome many of these problems and resulted in the development of a new adjuvant candidate PIM2ME. This paper will focus on the key aspects of the syntheses and report preliminary structure-activity data.



1) Gilleron, M.; Ronet, C.; Mempel, M.; Monsarrat, B.; Gachelin, G.; Puzo, G. J. Biol. Chem. 2001, 276, 34896-34904.
(2) Gilleron, M.; Quesniaux, V. F. J.; Puzo, G. J. Biol. Chem. 2003, 278, 29880-29889.

3:30pm - 3:50pm
Professor Frank Griffin, University of Otago

Paratuberculosis in deer: A large animal model for heritable resistance to pathogenic Mycobacteria

Frank Griffin, Simon Liggett, Rory O'Brien & Colin Mackintosh[1]

Disease Research Laboratory, Department of Microbiology and Immunology, University of Otago.
[1]AgResearch, Invermay.

Disease outcomes following exposure to pathogenic mycobacteria are considered to be influenced significantly by the host genotype. Red deer (*Cervus elaphus*) represent a unique animal model for the study mycobacterial diseases such Johne's disease caused by *Mycobacterium avium* subsp *paratuberculosis* (Map). They exhibit polarized phenotypes for susceptibility, manifest by early onset and clearly identifiable clinical signs of disease. Animals with a susceptible phenotype can be clearly confirmed by culture, histopathology, excretion of the organism and serological assays. By contrast, animals that display a resistant phenotype do not develop pathology and remain free from Map infection following natural or experimental exposure to Map. It is possible to carry out detailed genetic studies on these animals because individual breeds have been maintained purebred through assisted breeding programmes, involving AI and embryo transfer. Our earlier finding (Mackintosh et al, 2001) that resistance TB, caused by *M. bovis*, is highly heritable has been confirmed for Map. We will discuss the results from studies designed to chart the immune profiles in susceptible or resistant deer breeds, to identify gene markers for functional immunity. Other variables (sex, nutrition, production traits) which appear to influence susceptible phenotype will also be discussed.

3:50pm - 4:10pm
Mridula Dogra, University of Auckland

Does reductive metabolism of PA-824, a novel anti-TB drug, occur in human liver S9?

Mridula Dogra[1], Nuala A. Helsby[1, 2], Brian D. Palmer[2], Malcolm D. Tingle[3] and William A. Denny[2]

[1] Department of Molecular Medicine and Pathology,
[2] Auckland Cancer Society Research Centre
[3] Department of Pharmacology, University of Auckland, Auckland, New Zealand

PA-824, a 2-nitroimidazooxazine, is a novel prodrug currently in Phase II clinical trial for tuberculosis therapy. The prodrug is bio-activated by susceptible strains of *Mycobacterium tuberculosis* to a series of polar metabolites which include products of reduction of the imidazole ring. Whilst reduction of the imidazole ring of PA-824 in preference to the nitro moiety has been demonstrated previously using radiolytic reduction chemistry, there have been no reports on the human liver metabolism of this prodrug. We have investigated the metabolic profile of PA-824 in human liver S9 and identified a number of metabolic products (M1-M4). In contrast to deazaflavin (F420)-dependent nitroreductase (Ddn) catalysed metabolism of PA-824 in *M. tuberculosis*, there was no apparent des-nitrification of PA-824 by human liver S9. However, a reduction product (M3), previously proposed as an intermediate in the Ddn catalysed des-nitrification of PA-824, was observed. Product (M2) was tentatively identified as a hydrated reduction product as proposed previously following radiolytic reduction of PA-824. Hence, human liver reductive metabolism of PA-824 may occur following imidazole ring reduction by either of the two mechanisms proposed previously (i.e. hydride transfer or radical anion intermediate formation).

* Mridula Dogra is eligible for the Student Oral Presentation Award

4:10pm - 4:30pm
Professor Steve Chambers, University of Otago at Christchurch

The scent of Mycobacterium tuberculosis – part II

Stephen T Chambers[1], Mona Syhre[1], Laurens Manning[2]

[1]Otago University, Christchurch School of Medicine and Health Sciences, New Zealand
[2]Papua New Guinea Institute of Medical Research, Yagaum, Madang, Papua New Guinea

We have demonstrated that specific volatile compounds are present in the head space of cultures of *M. tuberculosis*, but not other respiratory pathogens, making these a potential diagnostic target for a breath test. One of these, methyl nicotinate was selected for a pilot study to determine whether this could be detected in the breath of patients with open tuberculosis.

Non-smoking patients with smear positive tuberculosis attending clinics at Modilon Hospital, Madang, Papua New Guinea, and age and sex matched controls were recruited. Breath samples were collected by forced expiration into a deactivated 1 L gas sampling bulb. Volatiles were absorbed onto a SPME fibre and trimethyl sulfonium hydroxide used as an in situ methylating agent for conversion of nicotinic acid into methyl nicotinate. GC/MS analysis was performed on a Saturn 2100 system instrument.

Significant amounts of methyl nicotinate was detectable in the breath of all 10 patients with tuberculosis in the low femto mol/mol range. Much lower amounts were found in the breath of 10 control subjects ($p<0.003$).

M. tuberculosis is well known to produce nicotinic acid (niacin) in vitro and this is routinely used as a diagnostic test. A derivative is also detectable in the breath of patients with open tuberculosis demonstrating that a breath test may be feasible.

Symposium Oral Presentations: Abstracts

Symposium Keynote Address

8:00 pm - Glenfalloch Restaurants and Gardens

Clifton E. Barry, III
Chief, TB Research Section
NIAID/NIH

In vivo imaging of tuberculosis and its response to chemotherapy

9:00 am - 9:20 am
Miss Hannah Kelly, Malaghan Institute of Medical Research

Immunogenicity and protective capacity of DNA vaccines encoding tuberculosis latency antigens

Hannah Kelly[1], Fenella Rich[1], Kylie Quinn[1], Shaun Lott[2], Bryce Buddle[3], and Joanna Kirman[1]

[1]Malaghan Institute of Medical Research, Wellington
[2]School of Biological Sciences, University of Auckland
[3]AgResearch Hopkirk Research Institute, Palmerston North

Despite extensive use of the bacille Calmette-Guérin *Mycobacterium bovis* (BCG) vaccine, tuberculosis is still responsible for the death of approximately 2 million people each year. Therefore a new vaccine is critically required. The causative agent, *Mycobacterium tuberculosis,* enters a latent phase a few weeks into infection. During this phase, protein expression changes and BCG may not prime the immune response sufficiently to react against latently-expressed antigens. DNA vaccines offer a novel approach for eliciting a protective immune response against these antigens. This study investigated the immunogenicity and protective capacity of plasmid DNA vaccines encoding one of four selected tuberculosis latency antigens. Three of the four vaccines tested resulted in detectable immune responses, and DNA encoding Rv2626c was the most immunogenic. In contrast to previously published reports, we observed that BCG vaccination did elicit an immune response against latency antigens. Vaccination with DNA encoding latency antigen Rv2624c protected mice against virulent *M. tuberculosis* to the same extent as BCG, as measured by bacterial burden in the lungs 10 weeks after infection. These results suggest there is potential to combine latency antigens with antigens expressed during active growth and reactivation, thus protecting against all stages of *M. tuberculosis* infection.

9:20 am - 9:40 am
Dr. Desmond Collins, AgResearch NCBID

New live *Mycobacterium bovis* vaccines for possums

Desmond M Collins, Geoffrey W de Lisle

AgResearch NCBID Wallaceville, Upper Hutt

Bovine tuberculosis continues to cost New Zealand more than $80 million per year, mostly because extensive areas of the country are occupied by possums infected with *Mycobacterium bovis*. At AgResearch NCBID Wallaceville, we have a major programme aimed at producing a new live tuberculosis vaccine that can be delivered to possums. We have made many mutants of *M. bovis* which are avirulent and have tested their vaccine efficacy in guinea pigs compared to BCG. A number of these candidate vaccines that have given at least comparable protection to that provided by BCG, have subsequently been tested as vaccines in possums. We have found that while the protective efficacy of a vaccine in guinea pigs is a good indication that the vaccine will provide some protection in possums, the most efficacious vaccine in guinea pigs is not the same as that in possums. This illustrates the importance of testing in the target species as part of the development process for any new vaccine.

9:40 am - 10:00 am
Dr. Sarah Young, University of Otago

The use of Virus-like particles as vaccines and immunotherapies for cancer and infectious diseases

Sarah Young, Stephanie Win, Michelle Wilson, Vivienne Young, Vernon Ward, Margaret Baird

Department of Microbiology and Immunology, University of Otago

The development of vaccines is a significant strategy in the war against infectious diseases and cancer. The way in which antigens are delivered to the immune system plays a crucial role in the immune response that ensues. Virus-like particles (VLP) which are empty viral protein shells formed from protein derived from the parent virus, are highly immunogenic and are currently used as vaccines against Hepatitis B virus and Human Papilloma Virus (HPV). We have discovered VLP can also act as vehicles for heterologous vaccines, dramatically increasing the immunogenicity of a non-viral protein attached to the surface. We are currently investigating the use of VLP as vaccines against TB, Chlamydia and several tumours. The method we use to attach the relevant proteins to VLP is rapid and simple. Our preliminary work has shown that immunising mice with either a TB or Chlamydia protein attached to VLP from *Rabbit hemorrhagic disease virus*, can enhance immune responses against infectious diseases. In addition we have shown that VLP linked to tumour antigens (proteins) delay the growth of tumours much more effectively than protein alone.

10:20 am - 10:40 am
Dr. Sarah Hook, University of Otago

Designing Sustained Release, Particulate, Sub-unit Vaccines

Sarah Hook, Julia Myschik[1,2], Sarah Gordon[1], Shakila Rizwan[1], Warren McBurney[1,3], Thomas Rades[1]

[1]School of Pharmacy, University of Otago, Dunedin, New Zealand
[2]Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics, Ludwig-Maximilians-University Munich, Germany
[3]Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand

A number of different approaches have been utilized in the development of vaccines to protect against the development of infectious diseases. The polar extremes of these are live attenuated bacterial or viral vaccines and purified peptide or protein based sub-unit vaccines. While sub-unit vaccines have the advantage of being safe, well defined and easily up-scaled, they have poor immunogenicity and low in vivo stability. Therefore much emphasis must be placed on vaccine formulation and delivery.

The aim of this work is to design and test formulations whereby the vaccine antigen is released over a period of several days, encapsulated into a highly immunogenic particles, stimulating CD8 as well as CD4 immune responses. Stimulation of cellular CD8 responses is vital as the diseases for which vaccines are still desperately needed require some aspect of CD8 immunity.

We have developed and evaluated a number of sustained release systems. These include implants which releases self-assembling lipidic particles, sustained release cubosomes and thermogelling hydrogels. Thermogelling systems are liquid at room temperature but gel upon exposure to physiological temperatures, providing a sustained release biodegradable depot. These systems have been characterised physically, chemically and immunologically.

10:40 am - 11:00 am
Professor Philip Hill, University of Otago

Introduction of Prevenar into Africa: The Gambian pneumococcal surveillance project

Philip C Hill, Grant Mckenzie, Martin Antonio and Richard A Adegbola

MRC Laboratories, The Gambia

Pneumonia is the biggest killer of children world-wide and *Streptococcus pneumoniae* is its biggest cause. In The Gambia, nasopharyngeal carriage of *S. pneumoniae* is universal in Gambian children by the end of infancy and a randomised trial of a 9-valent pneumococcal conjugate vaccine in The Gambia showed high efficacy against pneumococcal disease, including near 80% efficacy against invasive disease caused by vaccine serotypes. Most surprisingly it showed 12% efficacy against overall mortality. In The Gambia there are at least 65 different serotypes circulating, creating a huge pool of organisms available for serotype replacement with the introduction of the 7-valent vaccine Prevenar. In order to monitor the introduction of Prevenar into the Gambian population routine vaccination system, a formal surveillance system has been established within a population of 130,000 people under demographic surveillance.

Symposium Oral Presentations: Abstracts

Symposium Keynote Address

11:00 am - 12:00 noon

C. Jo White
Chief Medical Officer
VGX Pharmaceuticals

How to get Prophylactic or Cancer Vaccines Licensed in 10 (Not So) Easy Steps

2:00 pm - 2:20 pm
Professor John Tagg, University of Otago

Something old and something new - the amazing repertoir of bacteriocins produced by Streptococcus salivarius

John Tagg[1] and Philip Westcombe[2]

[1]University of Otago, Department of Microbiology and Immunology
[2]BLIS Technologies Ltd, Dunedin

Streptococcus salivarius is a numerically predominant member of the human oral microflora. *S. salivarius* K12, the first strain of this species to be distributed commercially as an oral probiotic, is a producer of several bacteriocin-like inhibitory substances (BLIS) including the well-characterised lantibiotics salivaricin A and salivaricin B.

A feature of the BLIS activities of *S. salivarius* is that their production appears typically to be mega-plasmid encoded. The 180 kb mega-plasmid in strain K12 appears to be transmissible to other *S. salivarius* both in vitro and in vivo. Other *S. salivarius* produce homologs of streptin and SA-FF22, two lantibiotics originally characterised in *Streptococcus pyogenes*. However, the *S. salivarius* lantibiotic, salivaricin 9, does not appear to have any equivalent produced by *S. pyogenes*. *S. salivarius* Mia has attracted recent interest because of its production of non-lantibiotic peptides active against the dental pathogen *Streptococcus mutans*. Other *S. salivarius* such as strains MPS and SN are distinctive in their production of heat labile BLIS activity principally directed against the significant oral pathogen, *Streptococcus pyogenes*.

Minimal pathogenicity for heathy individuals, an ability to achieve large populations on oral mucosa and the production of a varied and extensive repertoire of BLIS activities, directed against potentially pathogenic oral bacteria, are characteristics of *S. salivarius* that equip this species perfectly for a role as the prototype of oral probiotics for use in humans.

2:20 pm - 2:40 pm
Dr. Margi Butler, University of Otago

Chloramphenicol cures chytridiomycosis in frogs

Margi Butler, Phil Bishop, Rick Speare, Russell Poulter

University of Otago

Chytridiomycosis is a disease caused by the presence of a pathogenic fungus *Batrachochytrium dendrobatidis* on the skin of frogs and other amphibians. *Batrachochytrium* has been shown to cause sudden and severe population declines in numerous amphibian species on different continents. The chytrid fungus is probably the most serious acute threat to almost a third of amphibian species.

For the 500 species that cannot be conserved in the wild, the only solution is to take them out of their habitat and attempt to breed them in captivity. However, these efforts remain vulnerable to the introduction of *Batrachochytrium*.

We have developed a protocol to cure frogs that have been experimentally infected with *Batrachochytrium*. This protocol involves bathing the frogs in solutions of chloramphenicol or thiamphenicol. We assessed the effect of the treatment on the presence of the fungus by using quantitative, real-time PCR of skin swabs taken from the frogs. The PCR assay was able to detect very low levels of *Batrachochytrium*. We demonstrated that chloramphenicol at concentrations of 20 mg/L had no apparent adverse effects on adult frogs or tadpoles. Amphenicols are normally used as antibacterial antibiotics and the discovery that chloramphenicol and thiamphenicol are effective against a fungus is unexpected.

2:40 pm - 3:00 pm
Muriel Dufour, ESR NCBID

Surveillance of enteric pathogens in New Zealand

Muriel Dufour

ESR, NCBID

NCBID is the National Centre for Biosecurity and Infectious Disease based in Wallaceville (Upper Hutt). It was opened in April 2008 with the mission to increase New Zealand's capability to safeguard human and animal health. It provides centralised coordination and emergency response for disease outbreaks, biosecurity investigations and chemical and biological threats.

ESR has two laboratories at NCBID: Bacteriology and Virology. The Bacteriology laboratory includes the Enteric Reference Laboratory (ERL) and the Leptospira Reference Laboratory. The role of ERL is to provide national reference and surveillance laboratory services for human, animal, food and environmental enteric bacterial pathogens. The surveillance role allows ERL to detect any outbreak clusters as well as the introduction of new species/serotypes (exotic) into New Zealand. This is achieved using a combination of serology, phage typing and molecular methods. Examples of recent outbreaks include the *Salmonella typhimurium* Phage Type 42 (South Island), *Salmonella typhimurium* Phage Type 1 (Auckland and Gisborne) and cluster of *Escherichia coli* O157:H7 (North Island).

3:00 pm - 3:20 pm
Associate Professor Michael Baker, University of Otago at Wellington

The Epidemiology of Tuberculosis in New Zealand: Re-inforcing the need to address national and global health inequalities

Michael Baker, Dilip Das, Kamalesh Venugopal, Philippa Howden-Chapman

University of Otago, Wellington

Background: TB incidence in NZ declined steadily from the mid 1940s until the mid 1980s. Since then rates have remained static at 7-10 cases per 100,000 (290-446 cases per annum). This presentation will describe the epidemiology of TB in NZ and review evidence about the factors that are sustaining the current disease burden.

Methods: TB notification and laboratory data from 1995 to 2004 and population data from the 1996 and 2001 census were used to calculate incidence rates of TB by age, ethnicity and country of birth. A negative binomial regression model was used to estimate the association between TB incidence and household crowding, household income and migrants from high-TB-incidence countries at the neighbourhood (census area unit) level. Anonymous HIV surveillance data were matched with TB surveillance data to measure the extent of HIV/AIDS co-infection.

Results: Migration of people from high-TB incidence countries is the main source of TB in NZ. Rates of local TB transmission and reactivation of old disease are declining steadily for NZ born populations, except for New Zealand born Māori and Pacific people under 40. TB incidence at the neighbourhood level was associated with household crowding and household income. The association with migrants from high-TB-incidence countries disappeared when the analysis was restricted to the subgroup of people likely to have been infected more recently (NZ-born people aged <40 years). HIV/AIDS, multi-drug-resistance, and zoonotic transmission of M. bovis are not significant contributors to TB incidence in NZ.

Conclusion: TB incidence in NZ is mainly sustained by migration of TB infected people from high incidence countries and subsequent development of disease in NZ. Migrants are not spreading TB to the NZ born population to a significant extent. Transmission within NZ is associated with household crowding and relative poverty. These findings: (1) Support the current direction of TB control in NZ including a focus on immigrant health screening & treatment; (2) Reinforce efforts to reduce socioeconomic deprivation, ethnic inequalities, and household crowding in NZ; (3) Emphasise the importance of regional and global TB control initiatives.

3:20 pm - 3:40 pm

Clifton E. Barry, III
Chief, TB Research Section
NIAID/NIH

TBA

Energy metabolism of slow-replicating *Mycobacterium smegmatis*

Michael Berney and Gregory M. Cook

Department of Microbiology, University of Otago

The energy metabolism of slow-replicating mycobacteria is not well understood and is a potential source for identifying new drug targets. We have established a continuous culture system for the fast-growing *Mycobacterium smegmatis* to study metabolism at low growth rate under different nutritional and environmental conditions. A global gene expression study of slow-growing (doubling time of 70 h) versus fast-growing (4 h) *M. smegmatis* under aerobic and hypoxic conditions revealed that most enzymes of the respiratory chain are tightly coupled to growth rate and not to dissolved oxygen concentration. In contrast, *M. smegmatis* genes homologous to the DosR regulon of *M. tuberculosis* did not exhibit growth rate-dependent expression. Our data demonstrates that *M. smegmatis* and *M. tuberculosis* share a similar expression pattern of genes involved in energy metabolism when the bacteria experience a downshift in growth rate and under hypoxia. Alternative pathways for the recycling of reducing equivalents under these conditions will be discussed.

A homogeneous cell-based bicistronic fluorescence assay for high-throughput sceening

Tony S. Cardno, Elizabeth S. Poole, Suneeth F. Mathew, Ryan Graves and Warren P. Tate

University of Otago

Recoding mechanisms are programmed protein synthesis events used commonly by viruses but only very rarely in cells for cellular gene expression. For example, HIV-1 has an absolute reliance on frameshifting to produce the correct ratio of key proteins critical for infectivity. To exploit such recoding sites as therapeutic targets, a simple homogeneous assay capable of detecting small perturbations in these low-frequency (< 5%) events is required. Current assays based on dual luciferase reporters use expensive substrates and are labor-intensive, both impediments for high-throughput screening. We have developed a cell-based bi-fluorophore assay able to measure accurately small recoding changes (< 0.1%) with a high Z!-factor in 24- or 96- well formats that could be extended to 384-wells. In cases of nonsense mutations arising within coding regions of genes, the assay is suitable for assessing the potential of screened compounds to increase readthrough at these non-programmed stop signals of variable termination efficiency.

Investigation into the feasibility of disrupting the quaternary structure of DHDPS from M. tuberculosis as an alternative approach to drug design

G. L. Evans[1], S. R. A. Devenish[1], F. G. Pearce[1], L. Schuldt[2], G. Kefala[2], M. A. Perugini[3], M. S. Weiss[2], J. A. Gerrard[1]

[1]School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand
[2]EMBL Hamburg Outstation, c/o DESY, Notkestr. 85, D-22603 Hamburg, Germany
[3]Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, 30 Flemington Road, University of Melbourne, Melbourne, Australia

Dihydrodipicolinate synthase (DHDPS) is an important antibiotic target, yet no potent inhibitor based on substrates, intermediates or product has been found. A thorough understanding of the mechanism of DHDPS has lead to questions about its homotetrameric quaternary structure, often described as a dimer of dimers. The active site residues are compartmentalized in the dimeric subunits, with no obvious need for their association into the tetrameric structure. The importance of the tetrameric structure in *E. coli* DHDPS has been demonstrated by the 100-fold decrease in activity observed in a dimeric variant created by mutagenesis. This suggested a new approach for inhibitor design; that is targeting the dimer-dimer interface, and disrupts tetramer formation.

Our aim was to engineer a dimeric *M. tuberculosis* DHDPS through rational designed mutation, and analyse the effect of disrupting quaternary structure on enzyme function. A single point mutation resulted in a dimeric *M. tuberculosis* DHDPS, as determined by analytical centrifugation and gel filtration chromatography. X-ray crystallography showed that the active site was conserved. Surprisingly, the dimeric variant had similar activity to the wild type, thus suggests that disrupting the tetramer formation will not provide an alternative direction for antibiotic design for DHDPS from *M. tuberculosis*.

* Genevieve Evans is eligible for the Student Poster Presentation Award

TrpD, MbtI & HRP1: from structure towards therapy

J. Shaun Lott, Esther Bulloch, Chen Gao, Clare Lee, Fran Short, Caroline Charlier, Miriam Sharpe, Hannah Kelly[1], Joanna Kirman[1] and Edward N. Baker.

School of Biological Sciences, University of Auckland, Auckland 1142, New Zealand.
[1]Malaghan Institute of Medical Research, Victoria University, Wellington 6012, New Zealand.

We have solved the structures of two enzymes from *M. tuberculosis* known to be essential for pathogenesis: anthranilate phosphoribosyl transferase (AnPRT; TrpD), the enzyme which catalyses the second committed step in tryptophan biosynthesis, and salicylate synthase (MbtI), which catalyses the first step in the biosynthesis of the siderophore mycobactin, essential for iron uptake into the bacterium (Lee et al., J. Mol Biol. 355: 784 (2006); Harrison et al., J. Bacteriol. 188: 6081 (2006)). Through a combination of in silico modelling and in vitro assays, we have identified sets of AnPRT and MbtI compounds which will form the basis for the structure-guided synthesis of more potent inhibitors of both enzymes. Additionally, we have solved the structure of Hypoxic Response Protein 1 (HRP1), the most strongly upregulated protein in the dosR regulon. HRP1 is a CBS-domain containing protein, but unlike other characterised CBS domains, it is a secreted protein which does not bind AMP (Sharpe et al., J. Mol Biol. 383: 822–836(2008)). It appears to be an important antigen in persistent infection. We are testing HRP1 as a vaccine candidate in mice with the aim of producing protective immunity against persistent infection.

The Role of VapBC Toxin-Antitoxin Proteins in Mycobacteria

Joanna McKenzie[1], Jennifer Robson[2], Gregory Cook[2], Ray Cursons[1], Ali Ruthe[1], Vic Arcus[1]

[1]Department of Biological Sciences, University of Waikato
[2]Department of Microbiology, University of Otago

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB) in humans, is a devastating infectious organism. The current suite of antibiotics used to treat TB face two main difficulties: (1) the emergence of multidrug resistant (MDR) strains of *M. tuberculosis* and (2) the persistent state of the bacterium that is less susceptible to antibiotics. *M. tuberculosis* has a surprisingly large repertoire of 46 VapBC proteins and are thought to form an array of auto-toxins which arrest the growth of the organism in response to external stress. VapC toxin proteins from the thermophilic bacterium *Pyrobaculum aerophilum* which are homologues of those from mycobacteria display Mg2+/Mn2+ dependent RNase activity against a variety of substrates which is inhibited when the toxin (VapC) is bound to its cognate antitoxin (VapB). Thus VapBC proteins act as metabolic managers by virtue of their ability to cleave mRNA and inhibit translation thereby inhibiting protein synthesis. The VapBC proteins from *Mycobacterium smegmatis* have been shown to form a tight complex in a 1:1 toxin to antitoxin ratio, interactions between the Vap proteins result in a tetramer of VapBC heterodimers. *M. smegmatis* is a model organism for *M. tuberculosis* and contains only one VapBC toxin-antitoxin operon, thereby making it an ideal for system for uncovering the role of toxin-antitoxins in the mycobacteria.

* Joanna McKenzie is eligible for the Student Poster Presentation Award

An optimised real-time PCR approach for mycobacteria

Ali Ruthe, Ray Cursons and Vic Arcus

Department of Biological Sciences, University of Waikato, New Zealand

Mycobacterium smegmatis is a non-pathogenic bacterium used as a model organism for mycobacterial studies as it shares many features with *Mycobacterium tuberculosis*, which kills approximately two million people each year and latently infects one third of the world's population.

Our particular focus involves the regulation and expression of chromosomal toxin-antitoxin systems in mycobacteria which are thought to act as metabolic regulators in the stress response. We have developed optimised protocols for the isolation and quantification of mRNA levels using real-time PCR analysis so we can more efficiently extract, preserve and detect the low abundance target genes.

* Ali Ruthe is eligible for the Student Poster Presentation Award

Lsr2 from *M. tuberculosis* forms DNA-protein bundles

Emma Summers[1], Alok Mitra[2], Roberto Colangeli[3] and Vickery Arcus[1]

[1]Department of Biological Sciences, The University of Waikato, Hamilton, New Zealand. Email: esummers@waikato.ac.nz
[2]Laboratory of Structural Biology, School of Biological Sciences, University of Auckland, Auckland, New Zealand
[3]Department of Medicine, University of Medicine and Dentistry of New Jersey, New Jersey, USA.

Lsr2 is a small (12 kDa) basic protein of unknown function that is highly conserved among Mycobacteria. Recent work from Colangeli et al (1) showed that Lsr2 regulates the iniBAC operon, which is upregulated when *M. tuberculosis* is treated with the front-line antibiotic isoniazid. The overexpression of Lsr2 represses this isoniazid-mediated induction. Results from Colangeli and colleagues show that Lsr2 is capable of binding to DNA and forming large oligomers and it has been suggested that Lsr2 is novel histone-like protein. Our recent results, using transmission electron microscopy, show that Lsr2 may be more similar in its mode of DNA binding to RecA, a protein responsible for homologous recombination and DNA repair (2) than to a histone-like protein. Lsr2 appears to bind dsDNA non-specifically and in large quantities, requiring conditions such as high salt buffer or DNase digestion, for DNA removal. The dimensions of the Lsr2-DNA bundle is consistent with double stranded DNA coated by a single layer of folded protein. Our Lsr2-DNA bundle dimensions are similar to that of the RecA-DNA supercoils. We hypothesize that Lsr2 has an essential role in the regulation of gene expression in Mycobacteria by controlling DNA topology, but that it is not histone-like in it's orientation on DNA.

(1) Colangeli, R, et al (2007). PLoS Pathogens, 3 (6), e87-1-14.
(2) Shi, W., and Larson, R.G. (2005). Nano Letters, 5 (12), 2476-2481.

* Emma Summers is eligible for the Student Poster Presentation Award

Dr. Robert E. Webster

The Webster Centre for Infectious Diseases is named after the noted virologist and international expert in influenza, Robert Webster, an Otago Graduate.

New Zealander Professor Robert Webster, based in the Department of Infectious Diseases at St. Jude Children's Research Hospital in the USA, is a world authority on avian influenza. He is an expert in the structure and function of influenza virus proteins and the development of new vaccines and antivirals and was the first to acknowledge a link between human and avian flu. His team isolated and identified the avain-adapted strain of H5N1, the causative agent of the H5N1 flu, commonly known as "avian influenza" or "bird flu".

Born in Balclutha New Zealand, Webster grew up on a farm, studied at Otago University Department of Micobiology where he completed a BSc and MSc. During his distinguished career, he has held many research posts starting out as a virologist with the New Zealand Department of Agriculture, then Research Fellow at ANU's John Curtin Medical School. After receiving his PhD, he moved to USA where he became a member of both the Departments of Microbiology and Immunology at the St. Jude Children's Research Hospital in Memphis, Tennessee.

Webster Centre Steering Committee, University of Otago

Professor Greg Cook

Professor Greg Cook (Department of Microbiology & Immunology, University of Otago) researches key issues in antibiotic resistance and microbial physiology with a focus on Mycobacteria, Enterococcus and thermophiles. His group aims to understand how bacteria adapt to different environments, particularly at the level of the cytoplasmic membrane.

Professor Frank Griffin

Professor Frank Griffin (Head of Department of Microbiology & Immunology, University of Otago) heads the Disease Research Labooratory. The DRL research targets immunity to mycobacterial infections in domestic livestock (sheep and deer) and wildlife (Cape buffalo). The DRL studies all facets of immunity to infection including improved diagnosis, vaccination and selection for heritable resistance.

Professor Philip Hill

Professor Philip Hill (Department of Preventative & Social Medicine, University of Otago) is the Director for the Centre for International Health. His research interests include studies of *Mycobacterium tuberculosis* infection and disease and *Streptococcus pneumoniae* carriage, disease and vaccination, particularly in developing countries such as The Gambia.

Professor Andy Mercer

Professor Andrew Mercer (Department of Microbiology & Immunology, University of Otago) is Director of the Virus Research Unit. This group has broad interests in viral pathogenesis, vaccine development and the generation of virus-derived therapeutics. His research has a particular focus on poxviruses and their interactions with infected hosts.

Associate Professor Russell Poulter

Associate Professor Russell Poulter (Department of Biochemistry, University of Otago) researches the molecular genetics of eukaryote microorganisms, especially pathogens including fungi and chytrids. His laboratory is especuially interested in the origin and evolution of these pathogens.

Professor Warren Tate

Professor Warren Tate (Department of Biochemistry, University of Otago) and his group approach infectious disease research from a molecular standpoint and they have a long-term interest in understanding protein synthesis. Due to their 1980s discovery of translational frameshifting they developed a key interest in the frameshift mechanism of HIV-1 as a potential site of vulnerability in the virus.

Webster Centre for Infectious Diseases

UNIVERSITY of OTAGO
Te Whare Wānanga o Otāgo
NEW ZEALAND

Newsletter: Issue 5, March 2009 — University of Otago, Dunedin, NZ

A Message from the Director:

Professor Kurt Krause
Webster Centre Director

Hello and welcome to the March edition of the Webster Centre newsletter. This month the newsletter features an update on our upcoming symposia including a description of the exciting venue for Dr. Clifton Barry's keynote address at Glenfalloch Woodland Gardens on the evening of 23 April. The entire event is complementary, and transportation will be provided, so be sure not to miss this key element of the program. On page 2, please have a look at what drives Otago's newest Professor, Gregory Cook, from the Department of Microbiology. Finally there is still room to register to attend next months symposia, but space is getting tight, so please click on our link now at http://www.otago.ac.nz/webstercentre and pop your rego to us in an e-mail. We look forward to seeing new next month in Dunedin. Cheers!

Webster Centre for Infectious Diseases Symposium: Updates

Glenfalloch Woodland Gardens chosen as dinner venue

Glenfalloch Chalet

Glenfalloch Woodland Gardens, a picturesque site on the Otago peninsula has been chosen to host the evening keynote address and conference dinner at next month's upcoming Symposium.

Located about a 15 minute drive from the University of Otago, Glenfalloch Gardens and Restaurants was once an estate, established on the peninsula in 1871. It subsequently fell into disrepair and now is owned by the Otago Peninsula trust. It now serves as a multifunctional event centre that, in addition to the restaurant and bistro, contains thirty acres of woodland gardens with walking tracks throughout.

In addition to providing the dinner service, Woodland Gardens/Restaurants will serve as the venue for the evening keynote speaker, Dr. Clifton E. Barry, III.

Transportation will be provided to the venue from campus by bus for those who desire it. Individuals with special dietary requirements are still encouraged to attend the dinner. We have spoken to the restaurant staff and they will accommodate your needs. Please inform Rebecca Psutka (Webster Centre Coordinator) by email in advance. Email: rebecca.psutka@otago.ac.nz

Symposium Registration Deadline: 3 April 2009

This could be you!

We still have limited openings for speakers wishing to present their research at the 2009 Webster Centre for Infectious Diseases Symposium. Our categories for talks are as follows:

- Tuberculosis and Mycobacterial Diseases
- Vaccine Discovery and Development
- Current Topics in Infectious Diseases

These topics are designed to be broad and inclusive as the Webster Centre is seeking to feature infectious disease research from many disciplines

Scientists researching infectious diseases in disciplines as far ranging as chemistry, biochemistry, microbiology, molecular genetics, immunology, pharmacology, pharmacy and public health are members of the Webster Centre for Infectious Diseases and are attending the 2009 Webster Centre Symposium.

The Webster Centre has sent out invitation emails to the Webster Centre email list, and to the health science departments in each university and research institute in New Zealand. Additionally there are 300+ posters disseminated at the Otago campus and through our network of researchers.

All interested researchers academics and students are encouraged to register for this free Symposium and hear the talks from our excellent keynote speakers. Please encourage individuals you know who may be interested to register for the 2009 Webster Centre Symposium if they have not already done so.

Webster Centre profiled researcher: Professor Greg Cook

Professor Greg Cook
University of Otago

Steering Committee Member, WCID

Webster Centre Coordinator Rebecca Psutka met up recently with Professor Gregory Cook, of Department of Microbiology & Immunology at the University of Otago for a Q & A session. Professor Cook's research targets key issues in antibiotic resistance and microbial physiology with a focus on Mycobacteria, Enterococcus and thermophiles.

RP: Can you briefly describe for me: your research interests?

GC: Broadly our research is aimed at understanding how bacteria adapt to different environments, particular at the level of the cytoplasmic membrane. The second goal is then to conduct structure-function studies on key players such as membrane proteins in this adaptation to understand how proteins have been designed to function under different environments.

RP: Why your research is important?

GC: One of our projects focuses on how *Mycobacterium tuberculosis* adapts to changing conditions in the host macrophage and the switch to slow growth/ metabolism. This is a metabolic adaptation and therefore the metabolic enzymes involved in this process may represent new targets for drug discovery. By understanding how *Mycobacterium tuberculosis* metabolizes and generates energy may also provide us with new ways to combat latency and disease.

RP: To you, what is the most frustrating aspect of ID research?

GC:. At the moment, we have no PC3 facility at Otago so we can't actually do research on pathogenic species of interest: specifically on *Mycobacterium tuberculosis.* There is also a lack of membrane protein crystallographers at the University, which hinders our ability to advance membrane protein structural work at Otago.

RP: You have quite a few post-grad students (seven) and post-docs (five) working under your supervision currently. What general advice do you have for somebody who is considering post-graduate study?

GC: The number 1 factor should always be the research project. If you are interested and passionate about a project you will make it your own and work day and night to see it succeed. As long you have enough to live, money will not be important. The best supervisor in the world will help, but really it comes down to the motivation of the researcher and this will be high if they like the project.

Another crucial point is to have a good game plan - be focused when you come into the lab and have a good plan of attack about what you will achieve that day. Don't plan around a 5 day week otherwise you will accomplish very little. Work in blocks and plan rest at the end of a series of experiments - this may last longer than 5 days, but plan to have a break when you are finished. Finding a balance between work and recreation is essential - so often it is the time spent out of the lab where good ideas will come to you. If you get it right, it should be fun and you won't be able to sleep because you will be anxious to get into the lab to get that next result.

I will never forget my first day at Cornell University as a postdoc, my boss told me "I expect you to be here at 8 am and I don't care what you do after 5 pm or the weekends - if you can't get your act together during this time you will be of no use to anybody". Strong words, but I will never forget how true they really are. I worked harder as a PhD student, but I was far more productive as a postdoc working less hours.

General Announcements and Upcoming Meetings

Rebecca Psutka
Coordinator,
Webster Centre for Infectious Diseases

Please email the Webster Centre at: webstercentre@otago.ac.nz

- if there is a **scientific meeting** you would like highlighted
- if you are not on the Webster Centre **email list**
- if your research relates to infectious diseases and would like to **join** the Webster Centre for Infectious Diseases
- if you have any comments or suggestions about this newsletter or any other Webster Centre related activity

Upcoming Meetings

2009 Webster Centre Symposium

23-24 April, 2009
St. Margaret's College
University of Otago, Dunedin, New Zealand

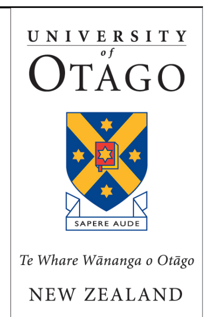
An advertisement and a registration form are attached with this newsletter.

Please submit registration forms via emailing the word document ASAP.

More information can be found on our website: www.webstercentre.otago.ac.nz

The **Webster Centre for Infectious Diseases** can be contacted at:

107 Biochemistry Bldg
University of Otago,
Dunedin, New Zealand
Email: webstercentre@otago.ac.nz
Phone: (03) 479-5148
Website: http://www.otago.ac.nz/webstercentre



Webster Centre is also online at: www.webstercentre.otago.ac.nz

UNIVERSITY of OTAGO

Te Whare Wānanga o Otāgo NEW ZEALAND

Webster Centre for Infectious Diseases

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Symposium Program Now Available: 23-24 April 2009

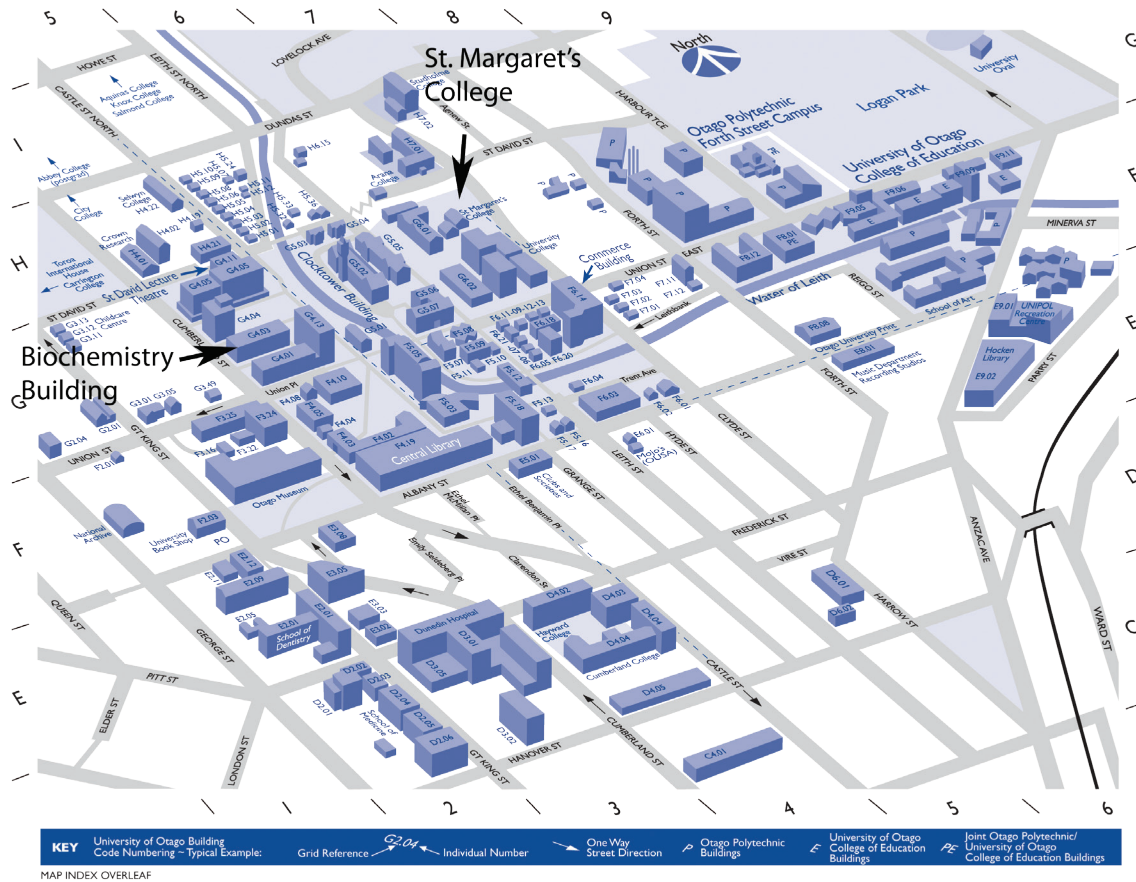
The Webster Centre for Infectious Diseases is based at the University of Otago.

Check here for information on:

Webster Centre Symposium	Undergraduate Studentships	Postgraduate Travel Scholarship	Sponsored Seminars

University of Otago Campus, located in north Dunedin.

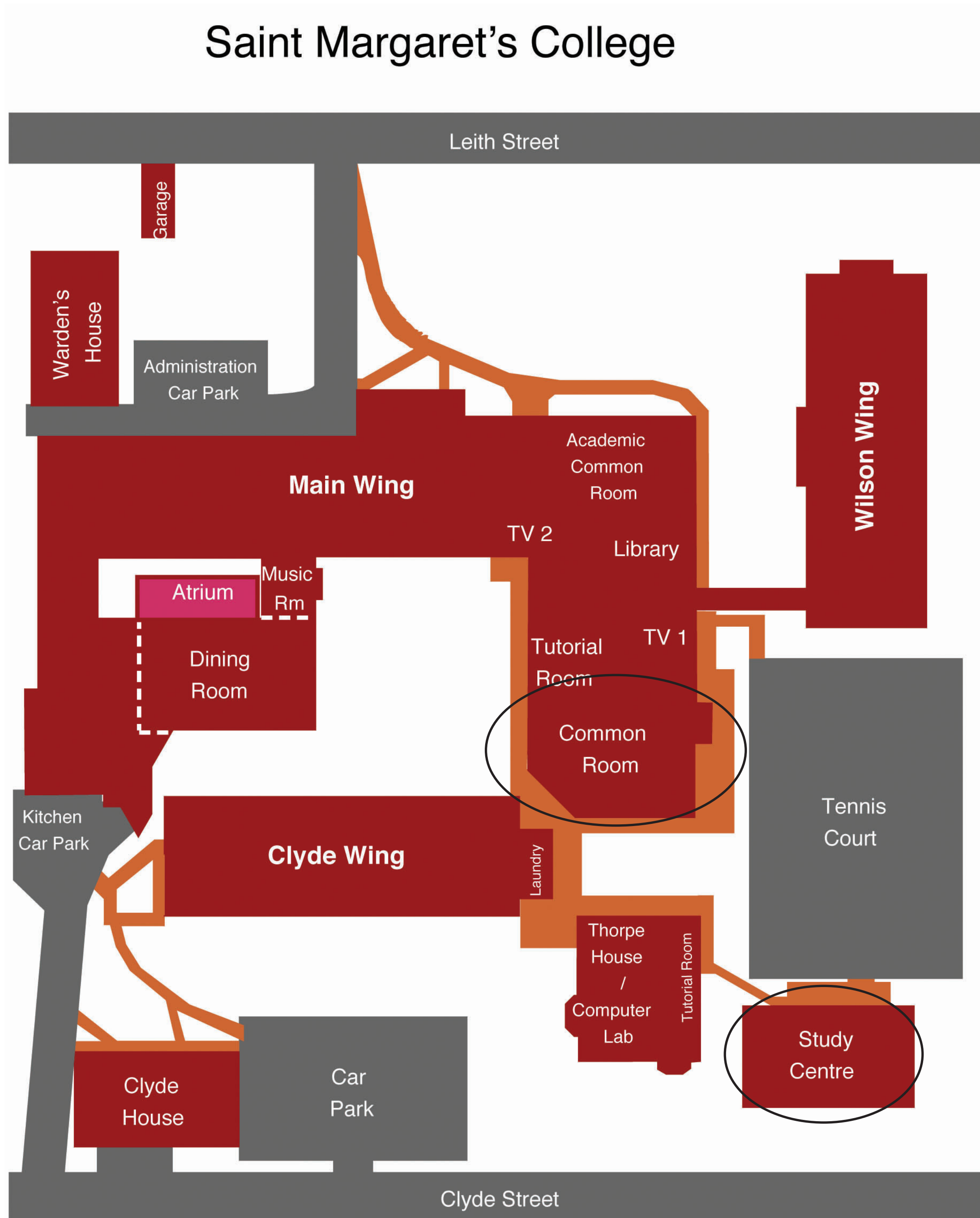
Webster Centre Symposium takes place at **St. Margaret's College,** indicated below.



Maps

Common Room: oral presentations, location of pre-symposium tea/coffee (8:30am)

Study Centre: poster session, location of Morning/Afternoon Tea and Lunch breaks



University of Otago campus - Glenfalloch Restaurants

Ⓐ 710 Cumberland St N, Dunedin North, 9016, New Zealar

Ⓑ 430 Portobello Road, Colinswood, Otago 9077 (Glenfall(

Add Destination - Show options

By car | Get Directions

Driving directions to Glenfalloch Restaurants

10.7 km – about **19 mins**

Ⓐ 710 Cumberland St N
Dunedin North, 9016

1. Head **south** on **Cumberland St Nth** toward **Union St West** — 0.3 km
2. Continue on **Gowland St** — 0.3 km
3. Continue on **Castle St** — 0.4 km
4. Turn **left** at **St Andrew St** — 0.4 km
5. At the roundabout, take the **3rd** exit onto **Thomas Burns St** — 0.6 km
6. Continue on **Wharf St** — 0.9 km
7. Continue on **Portsmouth Dr** — 1.2 km
8. Continue straight onto **Portobello Rd** — 25 m
9. Slight **left** to stay on **Portobello Rd** — 6.5 km

Ⓑ Glenfalloch Restaurants
430 Portobello Road
Colinswood, Otago 9077
Edited - Show original

These directions are for planning purposes only. You may find that construction projects, traffic, weather, or other events may cause conditions to differ from the map results, and you should plan your route accordingly. You must obey all signs or notices regarding your route.

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