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Introduction

Overview
The ARC Centre of Excellence in Structural and Functional Microbial Genomics brings together a team of internationally-recognised researchers from Monash University with complementary expertise in microbiology, molecular biology, biochemistry, structural biology, and vaccinology. The Centre conducts integrated research that elucidates key aspects of microbial pathogens and the hosts they infect, focussing on diseases of importance to Australian primary industry. At the core of the Centre’s applied research program is a genomics-based development process, which utilises high throughput, robotics-facilitated protein production and analysis to identify and characterise lead candidates for novel vaccines or drug targets. Major projects include the development of vaccines against leptospirosis, fowl cholera, ovine footrot, avian necrotic enteritis and gastrointestinal nematode infection in sheep. In 2013, fundamental research within the Centre into microbial genomics, pathogenesis and immunity continued to be published in high quality scientific journals.

The Centre also works in partnership with scientists within Australia at The University of Sydney, The University of Queensland, CSIRO Livestock Industries, and the Victorian Bioinformatics Consortium, as well as with numerous collaborators in Europe, Asia and North America, as detailed in this report.
Financial Support

The Centre’s main sources of funding are the ARC Centre grant through the Centre of Excellence program and Monash University. The ARC provides approximately $1.5 million per annum, with Monash University contributing $699,000 per annum. In-kind contributions from Monash University, Centre partners, associates and collaborators amounted to approximately $5 million in 2013. Additionally, the Centre obtained funding from collaborating partners, including The Australian Poultry CRC, The Australian Sheep CRC, Meat and Livestock Australia, and the Norwegian Veterinary Institute.

Income sources for 2013

* Denotes total funds allocated for the project over the next 3 years (2013–2016)
It is again with pleasure that I present this report which outlines the activities of the ARC Centre of Excellence in Structural and Functional Microbial Genomics for 2013. My first task is an especially pleasant one, namely to report that Scientific Advisory Board member John Egerton was made a Member (AM) in the General Order of Australia for “significant service to the livestock industry, particularly the eradication of infectious disease, and to education” in the 2014 Australia Day Honours list. John has been a member of the Board since the inception of the Centre and on behalf of all Centre personnel, I extend to him our heartiest congratulations.

It is a tribute to all Centre staff and students that the quality of research output in 2013 continued at a very high level and was accordingly published in the most highly respected international scientific journals. I was likewise delighted with the level of science and the quality of presentations at the Centre’s Annual Scientific Meeting, held in December at the Yarra Valley Lodge. A most pleasing aspect was the standard of presentations by our younger students. I express my appreciation to Advisory Board members for their participation, especially to our two international members for making the long journey to be present.

It is also extremely gratifying to note the substantial advancement of applied projects within the Centre. Vaccine projects on necrotic enteritis in poultry and on gastrointestinal nematodes in ruminants are now with commercial partners, while preliminary discussions are also in progress with industry on leptospirosis vaccines. The ovine footrot vaccine work is now funded by Meat and Livestock Australia. Work on the identification of antimicrobial drug targets has likewise made significant progress, while a PCR-based scheme for identifying Pasteurella multocida (the cause of fowl cholera) is now used internationally.

So, yet again I express my heartfelt appreciation to all Centre staff and students for their enthusiasm and dedication, to our administrative staff for their support and assistance and to all members of the Scientific Advisory Board for their ongoing sagacity, advice and commitment to the success of the Centre.

Prof Ben Adler

Director, ARC Centre of Excellence in Structural and Functional Microbial Genomics
Organisation and Governance

Centre Governance and Management Structure

The Centre was formed in 2006 as a partnership of the participating institutions under a formal Centre agreement, with Monash University as the administering institution.

Organisational chart
Scientific Advisory Board

The Scientific Advisory Board provides advice on research programs of the Centre, commercialisation opportunities and matters of strategic direction in research and other areas as may be relevant.

The Board meets twice a year or more frequently if necessary.

The Centre’s Board members are:

**Professor Jim Pittard, AM, FAA**, (Chair) is a microbial geneticist with a major interest in the regulation of gene expression, transport of small molecules across membranes and the molecular genetics of plasmids and their role in evolution. He is currently an Emeritus Professor at the University of Melbourne where for a number of years he alternated with Professor David White as Head of the Department of Microbiology (now Microbiology and Immunology).

**Professor John Egerton, AM**, is an Emeritus Professor of Animal Health at the University of Sydney, New South Wales. His research interests include lameness in sheep goats and cattle, treatment and vaccination against footrot in sheep, role of conventional and rDNA vaccines in eradication of footrot, and heritability of resistance to footrot in Merinos. His other research interests include anthrax in pigs, and necrotic enteritis (**Clostridium perfringens** Type C infection) in New Guinea highlanders.

**Dr Emanuela Handman** is a parasitologist with an interest in intracellular pathogens and their interaction with the host at the molecular, cellular and organisinal levels. Over a career spanning 30 years as Head of the **Leishmania** Laboratory at the Walter and Eliza Hall Institute of Medical Research she has focused on two main themes. On the parasite side, the elucidation of the structure, function and biochemistry of surface molecules involved in invasion of host cells and establishment of intracellular infection. On the host side, understanding the genetics of host responses to infection and their role in susceptibility or resistance to disease. More recently, using **Leishmania** functional genomics her group has made significant progress in the identification of novel targets for anti parasite drugs. She is a Fellow of the Australian Society for Parasitology, an Associate Research Fellow at the Walter and Eliza Hall Institute of Medical Research and an Adjunct Associate Professor in the Department of Microbiology at Monash University.
Professor Joachim Frey is the Director of the Institute of Veterinary Bacteriology at the University of Bern, Switzerland since 2000. His research interests are the molecular mechanisms of pathogenic Mycoplasma species. He is Chairman of the Board of the International Organization for Mycoplasmology (IOM) and is member of the international committee on systematics of prokaryotes, subcommittee on the taxonomy of Mollicutes.

Professor John F. Prescott is based in the Department of Pathobiology, Ontario Veterinary College, University of Guelph, Ontario, Canada. He is best known for work in the area of Rhodococcus equi pneumonia in foals, an area on which he has organized four international Workshops. He has been Co-Editor-in-Chief of Veterinary Microbiology (Elsevier Science), a member of the Canadian Veterinary Medical Association Council, and a Director of the Canadian Committee on Antibiotic Resistance. His current active research interests are in immunity and virulence in clostridial infections in animals.

International Adjunct Board Members

Professor Marshall Lightowlers is a Professor in the Department of Veterinary Science at The University of Melbourne and an NHMRC Principal Research Fellow. His research career has focused on the immunology and molecular biology of taeniid cestode parasites in developing highly effective, recombinant vaccines against cysticercosis caused by Taenia saginata and Taenia solium as well as recombinant vaccines against the tapeworm parasite Echinococcus granulosus which causes hydatid disease. Professor Lightowlers has served as President of the Australian Society for Parasitology, and was awarded that society’s Bancroft-Mackerras Medal in recognition of an outstanding contribution to the Science of Parasitology. He was recently awarded an appointment as Melbourne Laureate Professor at the University of Melbourne.

Professor Nick Samaras is founder of consulting firm Australis Biosciences. He has an interest in translational research at the academic-industry interface. He runs his own corporate advisory practice, which specialises in providing strategic advice to a range of Australian and international companies in the health care and biotechnology fields. Prof. Samaras has over 25 years experience in the global life sciences industry and holds a PhD from the Walter and Eliza Hall Institute, University of Melbourne. He is also the Chairman of Genetic Signatures, NHMRC Research Committee member 2006–12, NHMR Assigners Academy 2012–14, Adjunct Professor La Trobe University, and Director of the AGRF and MuriGen Therapeutics.

Professor Ben Adler, Centre Research Director (ex officio)
Centre Management and Administration

Director

Professor Ben Adler, as Centre Research Director, is responsible for decisions affecting Centre scientific, financial, human and infrastructure resources. In addition, his role oversees the overall direction of the Centre Scientific projects. He also manages a joint research group co-headed by Centre Associate Dr John Boyce.

Centre Operations Officer

Desmond Gul provides support to the Centre Director and Scientific Committee, by management of a wide range of Centre operational activities, particularly those associated with Centre funding bodies’ reporting requirements, marketing and communications initiatives, and the identification of Centre business development opportunities.

Personal Assistant

Sherrie Young provides administrative and secretarial support to the Centre Director. She is the minutes secretary for the Scientific Committee and Advisory Board meetings. Sherrie is also PA to Professor Christian Doerig (Head of Department, Microbiology), and assists with postgraduate and undergraduate student administration matters for Centre and Microbiology Department students.
Scientific Committee

The Scientific Committee, which comprises the Centre Director and Chief Investigators, is responsible for the overall scientific direction of the Centre’s fundamental and applied research programs. The Scientific Committee meets monthly, with meetings open to all Centre staff, students and associates.

The Centre's Scientific Committee members are:

Centre Director

Professor Ben Adler holds a personal chair in the Department of Microbiology at Monash University. In addition to his managerial role as Centre Director he is recognised internationally for his work on bacterial pathogens, especially Leptospira and other spirochaetes, Pasteurella and Shigella. His area of scientific expertise is in the application of genomics to elucidate molecular mechanisms of bacterial pathogenesis and in immunity to bacterial infection and vaccine development. He is, since 1986, a member of the Subcommittee on the Taxonomy of Leptospira of the International Union of Microbiological Societies (IUMS) and an executive member of the International Leptospirosis Society. He served on the IUMS Scientific Program Committee in 2011 and 2014. Together with Centre CI Prof Paul Hertzog, he established the Monash Infection and Immunity Network, which has now expanded to the Victorian Infection and Immunity Network (www.viin.org.au).

Deputy Director

Professor Rod Devenish is Professor in the Department of Biochemistry and Molecular Biology at Monash University. He is also the Academic Director of the university’s Research Graduate School. His major research interest is in the area of autophagy and he has served for a number of years on the Editorial Board of the specialist journal, Autophagy. In collaboration with Centre colleagues, he is investigating the interaction of bacterial pathogens with mammalian host cell autophagy. The particular focus of this research is how Burkholderia pseudomallei, the aetiological agent of melioidosis, avoid destruction by host cell autophagy after it escapes from phagosomes. Other aspects of his work on autophagy concern the turnover of organelles, particularly mitochondria and the nucleus, in yeast and on autophagy and autophagic cell death in cortical neurons.

Professor Ross Coppel is the Deputy Dean and Director of Research of the Faculty of Medicine, Nursing and Health Sciences at Monash University and a former Howard Hughes Medical Institute Infectious Diseases Fellow. He is an internationally recognised authority in molecular biology and genetic engineering as applied to infectious diseases and primary biliary cirrhosis. His work in bioinformatics led to the establishment of the Victorian Bioinformatics Consortium (VBC).

Professor Paul Hertzog is the Director of the Centre for Innate Immunity and Infectious Disease at the Monash Institute of Medical Research (MIMR), Clayton, Deputy Director of the MIMR, and a Senior Principal Research Fellow of the National Health and Medical Research Council of Australia (NHMRC). Professor Hertzog is the Co-convenor of the Victorian Infection and Immunity Network (VII) and the Lorne Infection and Immunity conference, as well as the Monash Health Translation Precinct’s Inflammation and Infection Theme and its Medical Genomics facility. His research interests cover an integrated approach to understanding the molecular mechanisms of the innate immune response to infections. His group specialises in gene targeted mouse models of disease integrated with cellular, genomics and bioinformatics approaches to identify new immunoregulatory approaches to preventing and treating infections.
Professor Els Meeusen heads the Biotechnology Research Laboratories (BRL) within the School of Biomedical Sciences at Monash University. She is internationally recognised for her expertise in large animal immunology, parasite biology and vaccine development and has a special interest in translating basic research findings into practical applications for animal and human health. Within the ARC Centre, her group is conducting research into parasite vaccines and developing diagnostics for parasite infection and parasite resistance.

Professor Julian Rood has an international reputation for his extensive research on the genetics, regulation and pathogenesis of anaerobic bacteria, especially Clostridium and Dichelobacter species. His current Centre research, in the Department of Microbiology at Monash University is focused on the genomics and pathogenesis of ovine footrot and avian necrotic enteritis and understanding how bacteria transfer their virulence and antibiotic resistance genes.

Professor Jamie Rossjohn is an NHMRC Australia Fellow and a Professor of the Department of Biochemistry and Molecular Biology at Monash University. His group investigates the structural basis for defined events central to infection and cellular immunity. Specifically he has provided an understanding of receptor-recognition events at the immunological synapse as well as an understanding of processes central to bacterial physiology and host-pathogen interactions.

Professor Ian Smith is Pro-Vice Chancellor (Research & Research Infrastructure) and as such he has responsibility for the oversight and management of the university’s research infrastructure as well as developing and implementing strategies to meet future university infrastructure needs. Ian is also the Director of the Victorian node of the NCRIS funded Proteomics Australia Consortium. As a protein biochemist Ian brings to the Centre internationally recognised expertise in protein purification, characterisation as well as providing access to high throughput, high sensitivity, quantitative proteomic analysis of complex protein mixtures.

Professor James Whisstock is a biochemist in the Department of Biochemistry and Molecular Biology at Monash University. He has particular expertise in structural biology and bioinformatics. His research focus includes proteases and their inhibitors as well as bacterial virulence factors. He was the recipient of the 2006 Science Minister’s prize for Life Scientist of the year and the 2008 Commonwealth Health Minister's Award for Excellence in Health and Medical Research. In 2008 he was awarded an ARC Federation Fellowship, in 2010 the Australian Academy of Science Gottschalk Medal and in 2012 the ASBMB Merck Millipore Medal.
Centre Associates

**Associate Professor Travis Beddoe** is a Pfizer Australia Senior Research Fellow in the Department of Biochemistry and Molecular Biology at Monash University. He works on various proteins from a number of bacterial pathogens using X-ray crystallography and biophysical methods to determine their function.

**Professor Stephen Bottomley** is an NHMRC Senior Research Fellow in the Department of Biochemistry and Molecular Biology at Monash University. He is internationally recognised for his work on understanding protein misfolding and its links with disease. In collaboration with the ARC Centre he has established the Protein Production Unit (PPU), which is an automated protein production facility that enables researchers to express and purify their proteins in a high throughput manner.

**Dr John Boyce** has extensive experience in the identification and characterisation of virulence factors of bacterial pathogens, especially *Pasteurella multocida*. His work has focused on using whole-genome approaches such as DNA microarrays, signature-tagged mutagenesis, in vivo expression technology and proteomics to identify factors critical for the bacteria during the infectious process. This work has identified capsule and LPS as critical *P. multocida* virulence factors. He has recently used new generation sequencing technologies for the analysis of bacterial genomes and for the identification of the regulator of capsule expression. Dr Boyce also has extensive experience in targeted mutagenesis procedures in *P. multocida*. As a former senior Centre Research Fellow he is project manager for the Centre vaccine pipeline projects.

**Professor Brian Cooke** is a NHMRC Senior Research Fellow and Professor in the Department of Microbiology at Monash University. Over more than two decades, Brian’s work has focused on understanding the cellular and molecular basis by which parasites of red blood cells (particularly malaria and *Babesia*) cause disease and death in humans and animals. His research group is internationally recognized as playing a vital role in a worldwide consortium toward the functional analysis of novel genes identified in the recently sequenced genomes of malaria and *Babesia* parasites. Brian is an elected member of the International Advisory Editorial Boards for *Trends in Parasitology* and *Blood* and Editor of the *International Journal for Parasitology*.

**Associate Professor Stuart Cordwell** is a graduate of the University of Sydney. He was an author on the original manuscript that defined the term ‘proteome’ in 1995 and has been involved in proteomics research throughout his career. He was Senior Research Fellow at the Australian Proteome Analysis Facility from 1999–2003, and Director of Research and Development from 2003–2004. He returned to the University of Sydney in 2004 as Sesqui Senior Lecturer in Proteomics in the School of Molecular and Microbial Biosciences and the Department of Pathology. He is a member of the Bosch Institute and a Director of the University of Sydney Proteome Research Unit. He was awarded the Selby Research Award in 2006 and is a member of the Editorial Boards for the field-leading journals *Proteomics* and *Proteomics (Clinical Applications)*. His biological research interests lie in two major areas – bacterial pathogens and myocardial ischemia / reperfusion injury.
Professor John Davies is former Head of the Department of Microbiology and Deputy Head of the School of Biomedical Sciences at Monash University. He is internationally recognised for his work on a variety of bacterial pathogens, especially Neisseria species. He has extensive experience in bacterial genomics and regulation of gene expression. Professor Davies retired as Chief Investigator in June 2010, but remains linked with the Centre of Excellence as a Research Associate.

Associate Professor Dena Lyras is based in the Department of Microbiology at Monash University and has played a major role in the development of methods of genetic analysis in Clostridium species. Her current research is focussed on the pathogenesis of infections caused by the emerging pathogens Clostridium difficile and Clostridium sordelli, particularly with respect to the role of toxins, adhesion factors and spores in disease. Her work has also investigated mobile DNA and antibiotic resistance mechanisms employed by Clostridium species, especially involving the analysis of mobilisable and conjugative transposons.

Professor Bruce McClane is a Professor in the Microbiology and Molecular Genetics Department of the University of Pittsburgh, Pennsylvania, USA. He is internationally known for his work on Clostridium perfringens, particularly the toxins and toxin-encoding plasmids of this bacterium.

Dr Ashley Mansell heads the Toll-Like Receptor (TLR) Group at the Monash Institute of Medical Research (MIMR), Monash Medical Centre. He is internationally recognized for his work in Pattern Recognition Receptor signal transduction and negative regulation of these pathways. He initiated the formation and heads the Australian TLR research network and chairs the Australasian Society of Immunology Infection and Immunity special interest group.

Dr Rob Moore is the leader of the Modulation of Host Responses group at the CSIRO Livestock Industries’ Australian Animal Health Laboratories in Geelong, Victoria. He works on a number of bacterial pathogens including Clostridium perfringens, Campylobacter jejuni, and Corynebacterium pseudotuberculosis, studies the host response to pathogens, and is investigating the role of gut microbiota in the health and productivity of animals. He has co-supervised several PhD students with Centre CIs.
Professor Phillip Nagley is an Emeritus Professor of the Department of Biochemistry and Molecular Biology at Monash University. His research field is biochemistry and molecular biology, and his interests extend to cell biology, genetics, infectious disease and neuroscience. The broad goal of his research is to understand the response of cells to stresses that may lead to death. Professor Nagley retired as Chief Investigator in June 2010, but remains linked with the Centre of Excellence as a Research Associate.

Associate Professor David Piedrafita works in the School of Applied Sciences and Engineering at Monash University. He works on worm parasites such as the liver flukes, blood flukes and gastrointestinal nematodes that infect grazing ruminants, particularly in Asia and Africa. These parasites result in enormous economic loss to the agricultural sector or subsistence farmers and negatively impacting on efficient food production in developing countries. Research is centred in understanding how parasites and hosts interact to provide information we need to understand disease susceptibility and design better vaccines or drugs to prevent and treat parasitic disease.

Professor Richard Whittington is a veterinary pathobiologist at the University of Sydney, Camden, New South Wales. He leads research on *Mycobacterium avium* subspecies Paratuberculosis, the causative agent of Johne’s disease in ruminants, ovine footrot and infectious diseases of fish and wildlife. His major studies involve functional analysis and molecular studies of viruses and bacteria, immune responses, pathology and epidemiology in individual animals and animal populations.
Centre Personnel and Collaborators

Research Fellows
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Monash University, Australia

Dr Julian Vivian,
Monash University, Australia

Dr Ilia Voskoboinik,
Peter MacCallum Cancer Centre, Australia
Mission

To conduct integrated research that will elucidate key aspects of microbial pathogens and the hosts they infect. The research will encompass genomic analyses, development of modern veterinary vaccines, identification of antimicrobial targets and development of antimicrobial agents.

Objectives

• To develop vaccines against microbial pathogens of importance to Australian primary industry
• To identify and validate genes essential for microbial survival and thus facilitate the development of novel antimicrobial agents
• To characterise key host-pathogen interactions at the molecular and cellular levels in order to elucidate or control the processes whereby microbes evolve and cause disease
• To train a new generation of multi-skilled researchers based on the Centre’s broad range of advanced technologies
Research Infrastructure

To aid in the Centre’s high throughput microbial pipeline, the Centre has priority access to state-of-the-art instrumentation and technology. These facilities (listed below) are housed within the Centre’s host institution, Monash University, at the School of Biomedical Sciences.

Biomedical Proteomics Facility (BPF)

Mass spectrometry is one of the leading tools for identifying proteins, quantifying levels of expression and sequencing. A proteomics approach helps to focus on low expressing proteins that could serve as potential targets for therapy.

Services offered by BPF include:

- Protein characterisation and identification
- De novo and confirmatory protein sequencing
- Post-translational modifications
- Protein quantitation
- Membrane protein identification

For more information: www.med.monash.edu.au/biochem/facilities/proteomics

Dr David Steer (BPF Manager): david.steer@monash.edu (Tel: 9902 9323)

Protein Production Unit (PPU)

The PPU has the capability to purify a large number of recombinant proteins in a high throughput manner. It offers expertise in the optimisation of the protein expression systems and once established, is capable of rapidly optimising the purification protocol for the protein of interest.

Services offered by PPU include:

- Automated high-throughput protein purification
- Purification protocol development
- Protein expression optimisation
- Protein refolding screens and protein stability analysis

For more information: proteinproductionunit@med.monash.edu.au

Dr Noelene Quinsey (PPU Manager): noelene.quinsey@monash.edu (Tel: 9902 0020)
Macromolecular Crystallisation Facility (MCF)

Macromolecular crystallography provides unparalleled details of 3D structure of biological macromolecules and provides the basis for the rational design of therapeutics. The facility houses Australia’s largest fully-automated platform for the high-throughput crystallisation of biological macromolecules (CrystalMation).

Services offered by MCF:

- Crystallisation of macromolecules
- Macromolecular structure determination through X-ray diffraction


Prof Matthew Wilce (MCF Director): matthew.wilce@monash.edu (Tel: 9902 9244)

Micromon

Micromon provides genomics services such as Sanger DNA sequencing and also the more current and innovative Next-Generation sequencing. This latest technology can also be used for genome sequencing, transcriptomics and expression profiling.

For more information: www.micromon.monash.org

Mr Mark Cauchi (Micromon Manager): mark.cauchi@monash.edu (Tel: 9905 4830)
The High-throughput Microbial Pipeline

Leveraged as an entire system, the previously described infrastructure forms the Centre’s High-throughput Microbial Pipeline.

The High-throughput Microbial Pipeline allows the Centre to adopt the reverse vaccinology or genomic approach to vaccine development and a rational drug design approach to antimicrobial drug target identification.

Independently each component can add value to specific projects as stand alone capability.

Key Features – Specialised Capability

- Propriety sequence information
- ID of novel genes and gene products
- Specialised infrastructure
- Access to public and proprietary databases
- High throughput and output
- A variety of expression systems
- Rapid
- Synchrotron
- X-ray crystallography
- Mass Spectrometry
- Vaccines
- Drug targets
- Diagnostics
- Knowledge
Centre Research Projects

Identification and characterisation of antimicrobial drug targets

Structural determination of novel drug targets in *Mycobacterium* spp.

(Prof Ross Coppel, Prof Jamie Rossjohn, A/Prof Travis Beddoe, Dr Paul Crellin, Ms Rajini Brammananth, Mr Adam Shahine)

Mycobacteria are the causative agents of serious diseases in animals and humans. To address the increasing problem of resistance to existing antimicrobial agents, we are identifying and characterising essential mycobacterial enzymes involved in cell wall synthesis and survival in macrophages as potential targets for the design of specific antimicrobial drugs.

These studies have two basic components:

a) functional characterisation of essential mycobacterial enzymes using model species *Corynebacterium glutamicum* and *Mycobacterium smegmatis*

b) structural characterisation of essential mycobacterial enzymes.

In 2013, we have completed a structure/function study on a protein essential for survival of *M. smegmatis* in J774 macrophages. Mutants of *Rv0807*, a small gene of unknown function, show a reduced capacity to survive in the first 8 hours following phagocytosis, relative to wild-type *M. smegmatis*. Recent work on these mutants has revealed a possible defect in phosphate metabolism or uptake, since they grow poorly in phosphate-free medium while phosphate supplementation rescues growth. Interestingly, phagosomes are known to be low-phosphate environments and a reduced capacity to scavenge this vital nutrient may explain the macrophage survival defect. Inhibition of Rv0807 could be a novel strategy for clearing intracellular mycobacteria.

To better understand the role of Rv0807, we have cloned, expressed and purified the *M. smegmatis* (MSMEG_5817) and *M. tuberculosis* versions of the protein using *E. coli* and *M. smegmatis* expression systems. High yields of soluble protein were obtained and diffraction-quality crystals of the *M. smegmatis* protein were produced. To gain insights into function, the crystal structure of MSMEG_5817 has been solved to 2.4Å resolution (Figure 1). The structure reveals a high level of structural homology to the Sterol Carrier Protein (SCP) family, suggesting a potential role of MSMEG_5817 in the binding and transport of biologically relevant lipids required for bacterial survival. The lipid binding capacity of MSMEG_5817 was confirmed by ELISA, revealing binding to a number of phospholipids with varying binding specificities compared to the *Homo sapiens* SCP. A potential lipid-binding site was probed by alanine-scanning mutagenesis, revealing structurally relevant residues and a potentially different binding mechanism to the SCPs.

Research Achievements and Outcomes:

- Structural and functional characterisation of MSMEG_5817, a lipid binding protein implicated in mycobacterial survival in macrophages.
Characterisation of mycobacterial cell wall biosynthesis pathways
(Prof Ross Coppel, Dr Paul Crellin, Ms Rajini Brammananth, Mr Arek Rainczuk, Ms Tamaryn Cashmore)

In 2013, we continued our characterisation of a genetic locus in C. glutamicum, conserved in mycobacteria and previously shown to have a role in late stages of the mycolic acid biosynthesis pathway. Previously, mutants with deletions in methytransferase, glycosyltransferase and membrane acyltransferase had been shown to have slow rate of flux of cell wall trehalose monocorynomycolate (TMCM) to trehalose dicorynomycolate (TDCM). Recently, the remaining two genes of this locus were also mutated. The first mutant shared the phenotype of the existing mutants while studies on the second mutant have revealed that his gene (NCgl2760) plays a key role in the production of lipoarabinomannan (LAM), the major cell wall lipoglycan. This is an unexpected finding, and characterisation of this mutant is continuing.

In 2013 we completed our studies on one gene of this locus, NCgl2759, encoding a putative membrane acyltransferase previously implicated in mycolic acid biosynthesis. Mycolic acids are synthesized on the inner leaflet of the cell membrane and subsequently transported to the periplasmic space after conjugation to the disaccharide, trehalose. The exported TMCM functions as the donor for multiple cell wall mycolation reactions and the synthesis of TDCM. Recent studies suggest that mycobacterial membrane protein MmpL3, and the related Corynebacterium glutamicum proteins NCgl0228 and NCgl2769, mediate TMCM export, although it is not known how this process is regulated. Our work has provided evidence that transient acetylation of the mycolyl moiety of TMCM is required for periplasmic export. Disruption of the NCgl2759 gene caused accumulation of TMCM and a block in the surface transport of this glycolipid, as well as reduced synthesis of TDCM.
A low level of TMCM export continued in the mutant, since mycolation of cell wall arabinogalactan was unaffected. The NCgl2759 mutant accumulated a novel glycolipid species that was transiently labelled when wild type bacteria were pulse-chase labelled with 14C-acetate. We identified this glycolipid as acetyl-TMCM (AcTMCM) by mass spectrometry of the native species and a chemically synthesized authentic standard. Further analysis supported the conclusion that NCgl2759 corresponds a to novel TMCM mycolyl acetyltransferase (TmaT). Finally, the biochemical and TMCM/TDCM transport phenotype observed in the ∆NCgl2759 mutant was replicated by simultaneous chemical inhibition and genetic disruption of the two C. glutamicum MmpL3 homologues, NCgl0228 and NCgl2769, respectively. These studies show that acetylation regulates trehalose mycolate transport and indicate that TmaT is a potential drug target in pathogenic species. We have incorporated our findings into a new model for mycolic acid biosynthesis and transasport (Figure 2).

**Research Achievements and Outcomes:**
- Functional characterisation of TmaT as an acyltransferase that modifies trehalose mycolates and thereby acts as a regulator of mycolic acid transport in corynebacteria and mycobacteria.

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**Figure 2:** Proposed mycolic acid transport pathways in Corynebacteria and Mycobacteria. Our studies suggest that acetylation of TMCM by the acyltransferase TmaT (NCgl2759) is essential for TMCM transport to the periplasm and subsequent conversion to TDCM or attachment of mycolic acids to the arabinogalactan (AG) layer of the cell wall.
Mechanisms of pathogenesis in fowl cholera

(Prof Ben Adler, Dr John Boyce, Dr Marina Harper, Dr Andrew Cox, Dr David Powell, Dr Paul Harrison, Dr Torsten Seemann, Ms Marianne Mégroz, Ms Marietta John, Ms Amy Wright, Mr Mark Edmunds)

Pasteurella multocida lipopolysaccharide (LPS): its role in pathogenesis and immunity

Genetic analysis of *P. multocida* strains representing the sixteen LPS serovars has revealed that in this species there are eight different loci encoding the LPS outer core biosynthesis genes. However, LPS structural and genetic analysis of the type strains as well as more than 50 field isolates revealed that many more LPS structures are possible as a result of mutations within each locus, that lead to inactivation of genes involved in LPS assembly. In 2013, we completed the characterisation of the L6 LPS outer core biosynthesis locus and related LPS structures. Genetic analysis of the L6 locus revealed that it has significant synteny with the previously characterized L3 locus but as a result of nucleotide divergence, gene duplication, and gene redundancy, the LPS outer core produced by the L6 locus is structurally quite different from the L3 LPS outer core (Figure 3). The serovar type strains 10, 11, 12 and 15 all contain the L6 LPS outer core biosynthesis locus. Bioinformatic analysis along with LPS structural differences, have allowed us to define a role for each of the L6 glycosyltransferases in LPS assembly. Importantly, we identified two glycosyltransferases, GctD and GatB, which differed by only a single amino acid but had different donor sugar specificity (UDP-Glc and UDP-Gal respectively). The terminal region of the L6 outer core oligosaccharide produced by the Heddleston serovar 12 type strain consisted of β-Gal-(1,4)-β-GlcNAc-(1,3)-β-Gal-(1,4)-β-Glc which is identical in structure to paragloboside, the precursor for P1 antigen and the type 2 oligosaccharide that, in humans, carry the ABH blood group antigens. Interestingly molecular mimicry of host glycosphingolipids has been observed previously in *P. multocida* with the L3 strains expressing LPS similar to the Forssman, P and P' antigens present on vertebrate red blood cells. The expression of a paragloboside-like oligosaccharide suggests that *P. multocida* strains belonging to LPS genotype L6 may also engage in molecular mimicry.

Figure 3: Schematic representation comparing the LPS outer core produced by *P. multocida* L3 and L6 strains and the outer core biosynthesis loci. Percentage similarity at nucleotide level between the L6 glycosyltransferase genes and the L3 glycosyltransferase genes is shown. The *P. multocida* glycosyltransferase genes known or predicted to be required for the assembly of the outer core are shown below structures expressed by the serovar 3 (P1059) and 12 (P1573) type strains. Important mutations in the L6 locus and the corresponding LPS outer core structures are also indicated for each type strain. Residues are Gal, galactose; GalNAc, N-acetyl-galactosamine; Glc, glucose; GlcNAc, N-acetyl-glucosamine; Hep, heptose.
Analysis of the global regulator Hfq

It is becoming increasingly clear that small RNA (sRNA) molecules play critical roles in bacterial gene expression. The protein Hfq is an RNA-binding protein that facilitates the interaction of sRNAs with their target mRNAs, altering either the rate of mRNA translation or the stability of the mRNA. In this way, Hfq and sRNAs act together to regulate gene expression. Nothing is currently known about the importance of Hfq or sRNAs in the regulation of P. multocida gene expression. To determine whether sRNAs play an important role in gene regulation in P. multocida, we constructed an hfq mutant in the highly virulent P. multocida strain VP161. The mutant displayed normal growth in liquid BHI broth but displayed reduced colony size and a less mucoid appearance on solid BHI medium. Hyaluronic acid assays showed that the production of capsule was significantly reduced in the mutant; complementation with an intact hfq restored capsule production. The hfq mutant showed reduced in vivo survival in mice and reduced virulence in chickens when inoculated via the intratracheal route. We compared the transcriptomes of the wild-type and hfq mutant strains by RNA-sequencing; 129 genes were differentially expressed in the hfq mutant with 111 genes up-regulated and 18 genes down-regulated (expression ratio > 2-fold and false discovery rate \( p < 0.05 \)). The majority of the genes in the capsule biosynthesis locus were significantly down-regulated in the hfq mutant, consistent with the reduced capsule production. A number of genes involved in bacterial stress responses (including the genes encoding the sigma factors RpoE and RpoH) and amino acid metabolism were also differentially expressed in the hfq mutant. Taken together, these data show that the action of Hfq is critical for full P. multocida virulence and capsule production. Furthermore, they strongly suggest that sRNAs are important regulators of virulence genes in P. multocida.

The P. multocida LPS phosphoethanolamine (PEtn) transferases

P. multocida expresses two lipopolysaccharide (LPS) glycoforms simultaneously (glycoform A and B) that differ only in their inner core structure. Glycoform A inner core contains a single phosphorylated 3-deoxy-D-manno-octulosonic acid (Kdo) residue (Figure 4), whereas the glycoform B inner core contains two un-phosphorylated Kdo residues. In other bacteria the LPS inner core is decorated with PEtn at a number of positions, reducing the net negative charge of the LPS molecule and increasing resistance to host antimicrobial peptides. Analyses of the LPS structures expressed by P. multocida serovar 1 strains VP161 and X73 identified multiple positions that may be substituted with PEtn, including lipid A, the phosphorylated Kdo molecule specific to glycoform A and the second heptose on the tri-heptose side chain. Unusually, X73 also decorates the both terminal galactose residues in the outer core.

![Figure 4: Serovar 1 LPS glycoform A inner core structure showing relative positions that PEtn may be added to the LPS structure. Shown in red are the PEtn residues. The predicted transferases responsible for PEtn addition are also shown (red italics). Residues are: PEtn, phosphoethanolamine; PCho, phosphocholine; Gal, galactose; Hep, heptose; Glc, glucose; KDO, 3-deoxy-D-manno-octulosonate; P, phosphate.](image-url)
Genome sequence analysis of the serovar 1 strains VP161 and X73 allowed us to identify the full cohort of LPS-specific PEtn transferase genes. Two of the identified PEtn transferases, PetL (required for addition of PEtn to lipid A) and Lpt3 (required for the addition of PEtn to the second heptose) share a high degree of identity with PEtn transferases identified and characterized in other bacterial species. However, the terminal galactose-specific PEtn transferase, PetG, and the PEtn transferase specific for the phosphorylated Kdo residue, PetP, have no close homologues.

Antimicrobial susceptibility assays on VP161 petL and petP mutants revealed that the loss of the PEtn residue from the lipid A or the phosphate group (attached to the Kdo residue in glycoform A) reduced the ability of P. multocida to survive in the presence of the chicken cathelicidin antimicrobial peptide, fowlcidin 1. However, inactivation of petG or lpt_3 did not alter the sensitivity to fowlcidin 1. To assess the ability of the petP, petL and petG mutants to cause disease, virulence assays in chickens were performed. Wild type or mutant bacteria were introduced into birds via the intramuscular route or intra-tracheal route (to mimic the natural route of infection) and monitored for signs of fowl cholera. However, no difference was observed between wild-type and the PEtn mutants in the ability to cause disease indicating that these PEtn mutants retain virulence in chickens.

Research Achievements and Outcomes:
- Elucidation of the P. multocida L6 lipopolysaccharide (LPS) outer core biosynthesis locus and related LPS structures.
- Determination of the role that the RNA chaperone, Hfq, plays in gene regulation.
- Characterization of the LPS phosphoethanolamine genes in P. multocida and their role in antimicrobial resistance and virulence.

Genomics and pathogenesis of the ovine footrot pathogen, *Dichelobacter nodosus*
(Prof Julian Rood, Dr Ruth Kennan, Dr Dieter Bulach, Ms Marianne Gilhuus, Dr Hannah Jorgensen, Dr Torsten Seemann, Dr John Boyce)

The aims of this project have been to develop a detailed understanding of how *Dichelobacter nodosus* is able to infect the sheep hoof and cause clinical footrot and to examine genomic variation in diverse isolates of *D. nodosus*. Over the course of the project we have shown that virulence in *D. nodosus* is dependent upon the twitching motility imparted by its type IV fimbriae and upon the production of extracellular serine proteases. We have determined the genome sequences of 103 *D. nodosus* isolates, primarily from Australia and Norway and analysed them in depth (Figure 5). The results have shown that *D. nodosus* isolates can be divided into two distinct clades that correlate with the virulent or benign designation of those isolates and with their ability to produce either the AprV2 or AprB2 proteases, which are identical except for a single amino acid substitution.

These studies have made a very major contribution to the field. They have led to a detailed understanding of both the disease process and the genetic relationship between different isolates of *D. nodosus*.

Research Achievements and Outcomes:
- Determination of the key virulence factors that enable the footrot pathogen *Dichelobacter nodosus* to cause footrot in sheep.
- Elucidation and comparative analysis of the genome sequences of 103 isolates of *D. nodosus*.
- Determination that *D. nodosus* isolates can be divided into two distinct clades that correlate with virulence and the ability to produce a specific extracellular protease.
Figure 5: Comparative genomic analysis of 103 isolates of Dichelobacter nodosus. The colours indicate the geographic origin of the isolates: green and gold rings (Australian isolates) red and pink (Norwegian isolates) light blue and dark blue (Swedish isolates) grey (Danish isolates) the four remaining rings are individual isolates from Nepal, UK, Bhutan and India
Cellular and molecular basis of host pathogen interactions

Effect of autophagic induction on host-pathogen interactions

(Prof Rod Devenish, Dr Xuelei Li, Dr Mark Prescott, Ms Shu-Il Lai, Prof Ben Adler, Dr John Boyce)

The process of LC3-associated phagocytosis (LAP) of Burkholderia pseudomallei (the cause of melioidosis in animals and humans) by murine macrophage (RAW 264.7) cells is an intracellular innate defense mechanism. We have previously reported that both rapamycin and starvation treatment enhance LAP of B. pseudomallei. Beclin-1, a protein with several roles in autophagic processes, is required for LAP of B. pseudomallei in RAW 264.7 cells. We have reported that both rapamycin and starvation treatment enhanced LAP of B. pseudomallei in wild type cells, but that the rapamycin response is Beclin-1 independent whereas the starvation response is Beclin-1 dependent. To further investigate the role of Beclin-1 in LAP, we evaluated Beclin-1 phosphorylation in B. pseudomallei-infected cells by Western blotting. Our preliminary results show that a significant band representing Beclin-1 protein phosphorylated at serine14 can be detected in B. pseudomallei-infected cells in comparison to non-infected cells or cells exposed to dead bacteria. We have also addressed whether other autophagic proteins (in addition to Beclin-1) are involved in the regulation of LAP of B. pseudomallei in wild type cells, but that the rapamycin response is Beclin-1 independent whereas the starvation response is Beclin-1 dependent.

To further investigate the role of Beclin-1 in LAP, we evaluated Beclin-1 phosphorylation in B. pseudomallei-infected cells by Western blotting. Our preliminary results show that a significant band representing Beclin-1 protein phosphorylated at serine14 can be detected in B. pseudomallei-infected cells in comparison to non-infected cells or cells exposed to dead bacteria. We have also addressed whether other autophagic proteins (in addition to Beclin-1) are involved in the regulation of LAP of B. pseudomallei in wild type cells, but that the rapamycin response is Beclin-1 independent whereas the starvation response is Beclin-1 dependent. To further investigate the role of Beclin-1 in LAP, we evaluated Beclin-1 phosphorylation in B. pseudomallei-infected cells by Western blotting. Our preliminary results show that a significant band representing Beclin-1 protein phosphorylated at serine14 can be detected in B. pseudomallei-infected cells in comparison to non-infected cells or cells exposed to dead bacteria. We have also addressed whether other autophagic proteins (in addition to Beclin-1) are involved in the regulation of LAP of B. pseudomallei in wild type cells, but that the rapamycin response is Beclin-1 independent whereas the starvation response is Beclin-1 dependent. To further investigate the role of Beclin-1 in LAP, we evaluated Beclin-1 phosphorylation in B. pseudomallei-infected cells by Western blotting. Our preliminary results show that a significant band representing Beclin-1 protein phosphorylated at serine14 can be detected in B. pseudomallei-infected cells in comparison to non-infected cells or cells exposed to dead bacteria. We have also addressed whether other autophagic proteins (in addition to Beclin-1) are involved in the regulation of LAP of B. pseudomallei in wild type cells, but that the rapamycin response is Beclin-1 independent whereas the starvation response is Beclin-1 dependent.

While some phagocytosed bacteria are killed as a result of LAP, the majority escape from phagosomes to the cytosol, but are not targeted by canonical autophagy. The mechanism by which B. pseudomallei can avoid targeting by autophagy remains to be elucidated. A recently recognized signal for autophagic targeting of different bacteria is conjugation to ubiquitin (Ub). Ub conjugation can be reversed by the action of deubiquitinating enzymes (DUBs). We are investigating whether modulation of host ubiquitin responses following B. pseudomallei infection, might lead to evasion of autophagy. BPSS1512, encoded by a type VI secretion system (T6SS5) gene, has almost 100% identity with a B. mallei protein, recently shown to display DUB activity in vitro. bpss1513 encodes a small protein that may interact with the Ub C-terminal hydrolase (UCH) domain of BPSS1512. We have constructed mutants disrupted in BPSS1512 or BPSS1513 and a double mutant. Testing of mutant bacteria for virulence using a BALB/c mouse infection model shows that they are not attenuated. Furthermore, the BPSS1512 mutant exhibited no difference in its co-localisation with GFP-LC3 (Figure 6), indicating the bacteria can still escape from LAP. While mutant bacteria did show a higher co-localization with polyubiquitinated protein at later time points after infection, the overall percentage was low. Immunoblotting of polyubiquitinated proteins in whole cell lysates of infected cells showed no gross difference between infection with mutant or wild-type bacteria, suggesting that any change is subtle and confined to a small number of specific proteins.

In collaboration with Dr Patrick Tan (Genomics Institute of Singapore) we have shown that B. pseudomallei bpss0180, another T6SS cluster-associated gene, may be a novel inducer of host cell autophagy that contributes to production of nutrient resources for utilization in intracellular replication of bacteria. Three putative homologues of bpss0180 have been identified cloned for expression in yeast and mammalian cells to test if they also act as inducers of host autophagy.
**Burkholderia pseudomallei**
two-component signal transduction systems

(Dr John Boyce, Prof Rod Devenish, Prof Ben Adler, Dr Mark Prescott, Ms Priya Alwis, Ms Alexandra Dimitropoulos)

We have continued our characterization of some *B. pseudomallei* two-component signal transduction systems (TCSTSs).

The *bvrRS* (*Burkholderia virulence regulator Response/Sensor*) locus comprises the genes *bpss0688* (*bvrR*), encoding a putative response regulator and *bpss0687* (*bvrS*), its putative sensor histidine kinase. Single *bvrR* and *bvrS* mutants and a double *bvrR/bvrS* mutant were constructed by double-crossover insertional mutagenesis. Both single gene mutants were attenuated for virulence in BALB/c mice.

High-throughput RNA sequencing was used to compare the transcriptomes of the *bvrS* mutant and the wild-type strain K96243. These analyses identified 126 differentially expressed genes in the mutant, of which 104 demonstrated increased expression and 22 showed decreased expression. Interestingly, expression of the genes *motA* and *motB* (encoding components of the flagellar motor) and *fliD* (encoding the flagellar hook) was increased in the *bvrS* mutant. Consequently, we assayed the mutant and wild-type strains for motility. Both a *bvrS::Tn5* and the directed *bvrS* mutant were significantly reduced in swarming motility. Complementation with an intact copy of the *bvrSR* gene pair restored motility. Transmission and scanning electron microscopy indicated significantly reduced expression of flagella on the surface of the *bvrS* mutant and a *bvrR* mutant. We therefore conclude that this novel virulence-associated TCSTS in *B. pseudomallei* plays a critical role in regulating expression of flagellin and in controlling motility.

Other work has focussed on three other *B. pseudomallei* TCSTSs, namely the BPSSL1036-BPSSL1037 OmpR-EnvZ–like TCSTS, the BPSS2246 hybrid histidine kinase, and the BPSSL1669 OmpR-like response regulator. Allelic-exchange mutagenesis was used to delete either the bpss1512-bpss1513 operon (*Δ*1036–1037), *bpss1037* (*Δ*1037), *bpss1036* (*Δ*1036), *bpss1669* (*Δ*1669) or *bpss2246* (*Δ*2246). Subsequently, the virulence for mice of each TCSTS mutant was assayed. All five mutants caused disease at a similar rate to the wildtype strain.

The OmpR-EnvZ system is essential for osmoregulation in *Escherichia coli*; however, all five *B. pseudomallei* TCSTS mutant strains displayed similar survival to the wildtype on high-osmolarity medium. The *Δ*1036–1037, *Δ*1037 and *Δ*1036 strains also displayed similar swarming motility to that shown by the wildtype. A notably distinct, mucoid morphology was observed when *Δ*1037, *Δ*1036, *Δ*2246 and *Δ*1669 colonies were cultured on solid medium containing sucrose or glycerol for 5–20 days. This morphology was absent in similarly-cultured *Δ*1036–1037 and wildtype colonies. Mucoidy can be caused by alterations to bacterial capsule; however, analysis of crude mucus preparations indicated that type I capsule levels were unaltered in mucus-producing strains. Interestingly, the preparations from mucoid strains were found to contain significant quantities of extracellular DNA. Increased cell lysis was not observed in the mucoid strains, suggesting that they actively release DNA. In static liquid media two mutant strains, *Δ*1037 and *Δ*1036, developed significantly altered pellicle architecture compared to the wildtype. We conclude that while neither BPSSL1036–BPSSL1037, BPSS2246 or BPSSL1669 are virulence-associated, all three TCSTSs influence the release of extracellular DNA. The BPSSL1036–BPSSL1037 system also functions in pellicle biofilm formation.

Research Achievements and Outcomes:

- Strains deleted for expression of the type VI cluster 1-associated genes *bpss1512* and *bpss1513*, either singly or in combination, are not attenuated for virulence in mice.
- The *BvrRS* TCSTS is virulence-associated and plays a critical role in regulating expression of flagellin and in controlling motility.
- None of the three TCSTS, BPSSL1036–BPSSL1037, BPSS2246 or BPSSL1669, are virulence-associated; however all influence the release of extracellular DNA. The BPSSL1036–BPSSL1037 system also functions in pellicle biofilm formation.
Figure 6: Confocal images of RAW264.7 cells expressing GFP-LC3 (green) and infected with Burkholderia pseudomallei BPSS1512 mutant. Cells were fixed 6 h post infection, permeabilised, and stained: bacteria (blue), ubiquitin (Ub; red). Examples of bacteria co-localized with Ub only (pink arrow, indicated bacteria shown in the bottom insert); with UB and GFP-LC3 (white arrowhead, indicated bacteria shown in the top insert). Images by Ms Alicia Lai.
Mechanisms of innate immunity

Structural aspects of innate immunity

(Prof Jamie Rossjohn, Dr Peter Vella)

The innate immunity project centres on innate-like T-cells and innate cytokine receptors. In collaboration with Prof James McCluskey, Prof Dale Godfrey (University of Melbourne) and Assoc Prof Laurent Gapin (University of Colorado at Denver, USA) we have focussed on natural killer T (NKT) cells and mucosal associated invariant T (MAIT) cells. NKT cells recognise lipid based antigens presented by CD1d, whereas the identity of the MAIT cell ligands, which are restricted to the MHC-I like molecule, MR1, is unknown.

In 2013, we provided an understanding of the fine specificity of human NKT cells towards lipid antigens and published two prominent reviews on NKT recognition. We also showed how CD1d-lipid complex can be recognized by γδ T-cell receptors (TCRs) (Figure 7). In relation to MAIT cells, we described how the innate-like MAIT TCR recognised microbial vitamin B-based metabolites and described a generic method for the tracking of MAIT cells.

Research Achievements and Outcomes:

- Understanding and structural characterisation of CD1d-lipid antigen recognition by γδ TCR.
- Understanding MAIT TCR recognition of vitamin B metabolites.

Figure 7: γδTCR recognition of CD1d-lipid.
Systems biology of innate immune signal transduction pathways

(Prof Paul Hertzog, Ms Irina Rusinova, Mr Sam Forster, Ms Jodee Gould, Mr Kim Linton, Mr Simon Yu, Mr Phillip Chan)

We have previously established a database and allied set of bioinformatics tools to search all available gene expression microarray data for predicted signal transduction pathways, functional clusters or signatures that might identify a disease, vaccine or therapeutic response, etc. (Interferome.org). We have now implemented and published an improved workflow for data queries on the website, improving and validating prediction pathways for Transcription Factor Binding Sites. Together with the Australian National Data Service program based at Monash e-Research, we have compiled an improved data capture workflow, improved storage and uploading to our database, and have catalogued the associated metadata. An optimized database structure and supporting infrastructure have now been developed and 59 published datasets have been subjected to our pipeline of statistical analyses, annotation and uploading to the database. Pipelines for processing different formats of microarray experiments were established and we now include Agilent, Affimetrix and others. The database went live in December 2012 (Interferome v 2.0; http://interferome.its.monash.edu.au/interferome). Interferome v2.0 was used to identify an interferon-innate immune response important to host defence against cancer metastases, surprisingly similar to the signalling pathways usually associated with response to infections.

The second aspect of this project was to extend our current analyses of innate immune responses based on measurement of changes in gene expression determined by microarrays, to a more global assessment of transcriptional regulation. We have thus undertaken a next generation sequencing profile of whole transcriptome changes in macrophage transcription in response to interferon. In the analysis of the mRNA encoding proteins, 800 showed altered expression, 50% elevated and 50% suppressed; in addition, many altered transcripts showed evidence of alternative splicing. We also identified long and short non-coding RNAs (ncRNA) that will be important to characterise for regulatory function. We have validated examples of many of these changes, including many novel non-coding RNAs and are in the process of building interaction networks of the ncRNA and target genes. In order to accurately determine new features in the sequencing data, we have devised a novel feature extraction program called RNA eXpress. This has been used to identify new ncRNA and forms an integral part of a new ncRNA identification pipeline. During this year we undertook HITS CLIP experiments to validate the ncRNA – target predictions in collaboration with colleagues at Harvard University and Rockerfeller University in the USA. This work has led to the identification and validation of 84 IFN-regulated microRNAs actively associated with 3874 regulatory interactions for further investigation.

Research Achievements and Outcomes:

• Interferome v2.0 going live and its applications.
• Analysis of next generation sequencing experiment on the innate immune response of macrophages to IFN.
Structure-function studies of interferon-receptor signaling

(Prof Paul Hertzog, Dr Nicole de Weerd, Dr Thao Nguyen, Ms Jodee Gould, Dr Julian Vivian, Dr Hugh Reid, Prof Jamie Rossjohn)

The type I interferons (IFNs) form an integral component of the host response to bacterial and viral pathogens, both modulating the early innate immune response and sculpting ensuing adaptive immunity. They have practical applications as directly administered anti-infectives, as targets for blockade in some cases to avoid excessive inflammatory disease, or can be induced or administered as integral components of vaccine responses. This project aimed to elucidate the structural details of how members of the IFN family, particularly IFN\(\beta\), which is commonly induced by bacteria, interact with their receptor components, Ifnar1 and Ifnar2 to orchestrate complex cellular responses.

The structure of the IFN\(\beta\)-Ifnar1 complex was solved to a resolution of 2.9 Å and its specificity characterised to be different from other type I IFN/Ifnar interactions (Figure 8). We have made significant inroads into characterising the nature of signals transmitted and to demonstrating physiological relevance to disease. Microarray studies and quantitative real-time PCR have demonstrated and confirmed the repertoire of genes regulated by this signalling axis, revealing genes encoding known inflammatory molecules. An animal model has demonstrated the importance of the signalling axis in contributing to the lethal effects of endotoxic shock.

Together, our studies have revealed a new IFN/ receptor signalling complex and indicate that the resultant signalling axis is of primary importance in endotoxic shock, regulating the expression of several candidate effector molecules previously implicated in this syndrome, and other inflammatory conditions.

Research Achievements and Outcomes:

- Characterisation of the crystal structure of the IFN\(\beta\)-Ifnar1 complex and signaling from the IFN\(\beta\)-Ifnar1 axis.
- Filing of provisional patent application of the method used in identifying an interferon receptor modulator.

Systems biology of innate immune responses to clostridial infection

(Prof. Paul Hertzog, Mr Sam Forster, Ms Jodee Gould, Ms Lee-Yean Low, Dr Paul Harrison, Dr Jackie Cheung, Prof. Julian Rood)

In collaboration with Prof Julian Rood’s group, we have undertaken an integrated whole transcriptome analysis of bacteria and host of an in vivo infection of muscle by Clostridium perfringens. Using RNASeq, we have assessed global changes in gene expression in bacteria isolated from an infected murine muscle, as well as from the mammalian muscle tissue. This study identified new gene changes in both pathogen and host which will give us new insights into the mechanisms of disease pathogenesis, as well as candidate targets for disease-modifying drugs.

Figure 8: Structure of the IFNAR-IFN complex. Orthogonal views of the IFNAR1–IFN-\(\beta\) complex with IFNAR1 SD1 in yellow, SD2 in green, SD3 in blue and SD4 in magenta. Helices of IFN-\(\beta\) (orange; labeled A–E) are also indicated. Image by Dr Julian Vivian.
Structural and functional biology

Structural biology and drug target characterisation
(Prof Jamie Rossjohn, A/Prof Travis Beddoe, Dr Dene Littler, Dr Jerome Le Nours, Dr Natasha Ng)

We have continued the collaboration with Dr Adrienne Paton and Prof James Paton (University of Adelaide) that investigates AB5 toxins from pathogenic bacteria. We have addressed the basis of SubAB toxin assembly. Further, we have determined the structures of a number of AB5 members such as EcxAB in *E. coli*, which is providing insight into the evolution of AB5 specificity towards glycan substrates.

Structural biology and bioinformatics
(Prof James Whisstock, Dr Siew Siew Pang, Mr Gordon Lloyd, Dr Douda Traore, Dr Cyril Reboul, Prof Julian Rood)

We have crystallised several portions of the clostridial conjugation machinery; most notably we have determined the structure of the ATPase (motor) that drives conjugation, and shown that this region interacts with DNA.

Using the *Drosophila* model system, we have shown that the sole perforin-like protein in the fly (Tsl) plays an important role in immune defence. To our surprise, we find that Tsl is key for defence against Gram positive organisms (but not Gram negative pathogens), and likely functions via the Toll pathway.

We have determined the structure of a key adhesin from the *Helicobacter pylori*, the organism that causes *H. pylori*, ulcers and gastric cancer. This major finding reveals how this important gastric pathogen interacts with host cells (Figure 9).

Research Achievements and Outcomes:
- Crystallization and determination of structure of novel AB5 toxin, EcxAB, from *E. coli*.
- Determination of the structure of SabA.
- Determination of the structure of the conjugation ATPase.
- Understanding the immune function of ancestral perforin-like proteins.

Figure 9: Cartoon illustrating the structure of SabA. We determined the structure (crystal top left) of the N-terminal ligand binding ectodomain comprising the head and handle. The position of the two hypervariable loops (magenta) and the ligand binding residue Q159 (pink sphere) are shown. A 60 amino region (dashed line) links into a C-terminal outer membrane barrel (model only).
Functional genomics of large clostridial plasmids

(Prof Julian Rood, Dr Vicki Adams, Dr Xiaoyan Han, Prof James Whisstock, Ms Jessica Wisniewski, Ms Lakmini Weeramantri, Ms Xu-Xia Yan, Mr Thomas Watts, Dr Trudi Bannam, Dr Daouda Traore, Dr Corrine Porter, Dr Rob Moore, Dr Jackie Cheung, Prof Bruce McClane, Dr Francisco Uzal)

The overall objectives of this project have been to understand how large clostridial plasmids are transferred between strains of *Clostridium perfringens* and to determine the role of toxin plasmids in *C. perfringens*-mediated disease. Research on the tcp conjugation locus from the paradigm conjugative tetracycline resistance plasmid, pCW3 has shown that eight of the 11 genes in the tcp locus, plus the tcpK gene are required for optimal conjugative transfer. These genes are common to all conjugative *C. perfringens* toxin and antibiotic resistance plasmids. We have identified the origin of transfer, oriT, and a novel relaxase protein IntP and shown that IntP binds to the oriT site. This binding is enhanced in the presence of the TcpK protein. Genetic studies have revealed that the ε-toxin, β-toxin, β2-toxin and NetB toxin genes are encoded on conjugative plasmids in *C. perfringens*, respectively, and have led to the identification of a bacitracin resistance locus that is located on a Tn916-like transposon, within a large conjugative plasmid. We have determined the structure of the NetB toxin and the TcpC conjugation protein and have carried out structure-function studies on these proteins and their derivatives. Finally, we have developed a hypothesis that explains how *C. perfringens* can maintain four closely related conjugative plasmids in the same cell.

Research Achievements and Outcomes:

- Demonstration that the conjugation proteins TcpD and TcpE localise to the cell membrane in *C. perfringens*, independently of the other Tcp proteins.
- Identification of residues that are essential for IntP function.
- Demonstration that β-toxin and β2-toxin are encoded on conjugative plasmids in type C and type D strains of *C. perfringens* respectively.
- Identification of a novel bacitracin resistance transposon that is located on a conjugative plasmid.

![Figure 10: A model for the Tcp.](image-url)
Development of veterinary vaccines

Vaccine development against ovine footrot

(Prof Julian Rood, Prof Richard Whittington, Dr Om Dungyel, Prof Steve Bottomley, Dr Noelene Quinsey)

*Dichelobacter nodosus* is the causative agents of ovine footrot. We utilised a range of bioinformatics analyses of the annotated *D. nodosus* genome sequences and previously published experimental data to select genes encoding proteins likely to have vaccine potential. The central premise of this work is that protective antigens are likely to be surface exposed or secreted by the bacteria and therefore accessible to the host immune response.

There is a need to identify suitable antigens that can form the basis of a multivalent vaccine against ovine footrot. The ability of 63 recombinant antigens to protect against ovine footrot has been examined in five separate pen-based virulence trials. In these trials sheep were immunized with recombinant proteins and then challenged with highly virulent *D. nodosus*. Analysis of quantitative data obtained from these trials has led to the identification of potential vaccine candidates. Several of these candidates were retested both individually and in combination in a further vaccine trial. The results provided evidence that one combination of antigens may provide protection against disease. Current studies are aimed at testing additional vaccine candidates in pen trials and testing antigen combinations in field trials.

Research Achievements and Outcomes:
- Use of bioinformatics to identify potential vaccine candidates from the ovine footrot pathogen *Dichelobacter nodosus*.
- Use of vaccine trials in sheep to identify a potentially protective combination of antigens against ovine footrot.

Vaccine development in necrotic enteritis in chickens

(Dr Robert Moore, Prof Julian Rood, Dr Anthony Keyburn, Dr John Boyce, Dr Trudi Bannam, Dr Xiaoyan Han, Prof Ben Adler, Dr Noelene Quinsey, Ms Xu-Xia Yan).

The objective of this project is to develop new methods for the control of necrotic enteritis in chickens, which is caused by specific avian strains of *Clostridium perfringens*. A reverse vaccinology approach was used to identify novel vaccine candidates, including NetB toxin, which was discovered by this research team. Vaccine studies using recombinant antigens, especially the NetB toxin, are on-going. Recent results provide evidence that in combination with either bacterins or toxoids derived from *C. perfringens*, recombinant NetB protein can protect chickens against challenge with necrotic enteritis-causing strains of *C. perfringens*. In addition, we have shown that the immunization of boiler breeder hens with NetB-enhanced toxoid has significant potential for the control of necrotic enteritis in young broiler chickens.

Research Achievements and Outcomes:
- Demonstration that recombinant NetB, in combination with other antigens, can protect chickens against necrotic enteritis.
- Demonstration that maternal immunization with NetB-based vaccines can protect progeny from necrotic enteritis.
Identification of markers for resistance against gastrointestinal nematode infection in sheep
(Prof Els Meeusen, Assoc Prof David Piedrafita, Ms Amanda Peers-Adams, Ms Sarah Preston, Prof Steve Walkden-Brown)

Gastrointestinal nematode (GIN) parasites present a major problem for the sheep industry in Australia and worldwide. One strategy to control parasite infection, is to selectively breed sheep that show natural resistance to GIN. Measuring faecal egg counts after infection is the standard technique for assessing levels of resistance in individual sheep but is very labour intensive. Using a flock of sheep assessed for parasite resistance by the Australian Sheep CRC, we have measured a wide range of innate and adaptive immune parameters and correlated these with resistance status. Two immune readouts showed significant correlation with parasite resistance and are being further developed for in the field.

Research Achievements and Outcomes:
• Identification of two novel immune parameters associated with parasite resistance.

Identification of candidate vaccine antigens against blood fluke
(Assoc Prof David Piedrafita, Prof Els Meeusen, Mr Hamish McWilliam, Mr Chris Hosking, Dr Mike de Veer, Dr Yusheng Lee, Prof Don McManus)

Infection with the blood fluke, Schistosoma japonicum, causes major health problems for animals and humans in many Asian countries. Water buffalos in particular pose a continuous reservoir for human infections and vaccination of these animals has been proposed as a valid goal to both reduce human infections and increase animal productivity. Based on our studies of infection in rat models and in the natural water buffalo host, we have identified several candidate vaccine antigens. One of these antigens has been fully characterised and cloned and is currently the subject of vaccine trials. In addition, a single chain antibody fragment (scFv) phage display library has been generated from infected lymph nodes of water buffalo and is being used to further identify and characterise larval specific antigens.

Research Achievements and Outcomes:
• Identification of candidate vaccine antigens to protect against blood fluke infections.
• Production of one of the antigens in recombinant form for ongoing vaccination trials.
• Generation of a scFv phage display library from infected water buffalo, specific for larval surface binding.

Figure 11: Inspection of water buffalo lung for blood flukes
Characterising the mechanism of action of different adjuvant systems

(Prof Ben Adler, Dr Gerald Murray, Dr Thanatchaporn Bartpho, Prof Rasana Sermswan)

Leptospirosis is a global zoonosis and a major cause of economic loss in production animals. Identification of antigens able to elicit cross protective immunity against unrelated serovars is a major goal in leptospiral vaccine research. We have generated a mutant in serovar Manilae with altered lipopolysaccharide (LPS), which is attenuated for virulence in both the hamster model of acute infection and in the mouse renal persistence and excretion model. A single immunisation with the live mutant (M1352), but not with killed mutant (bacterin), was able to stimulate protective immunity against heterologous Pomona and Autumnalis serovars (Figure 12). Immunity was unrelated to LPS, indicating that cross-protective immunity can be mediated by protein antigens that are expressed only in vivo, but whose identity remains unknown.

Immunity and vaccine development in leptospirosis

(Prof Ben Adler, Dr Gerald Murray, Dr Thanatchaporn Bartpho, Prof Rasana Sermswan)

Leptospirosis is a global zoonosis and a major cause of economic loss in production animals. Identification of antigens able to elicit cross protective immunity against unrelated serovars is a major goal in leptospiral vaccine research. We have generated a mutant in serovar Manilae with altered lipopolysaccharide (LPS), which is attenuated for virulence in both the hamster model of acute infection and in the mouse renal persistence and excretion model. A single immunisation with the live mutant (M1352), but not with killed mutant (bacterin), was able to stimulate protective immunity against heterologous Pomona and Autumnalis serovars (Figure 12). Immunity was unrelated to LPS, indicating that cross-protective immunity can be mediated by protein antigens that are expressed only in vivo, but whose identity remains unknown.

Research Achievements and Outcomes:
• Demonstration of cross protective immunity elicited by a genetically defined strain of Leptospira

Research Achievements and Outcomes:
• Application of a systems biology approach to characterise the initial response to vaccination with a commercial adjuvant.
• Elucidation of the mechanisms of different delivery systems in boosting an immune response.

Figure 12: Heterologous protection of hamsters by live, but not by killed, serovar Manilae vaccine.
Key Performance Indicators

Research Findings

Quality of publications
Centre researchers continued to perform well in terms of their outputs of peer-reviewed publications, with a total of 51 papers and reviews published in scholarly refereed journals in 2013. Of these papers, 71% appeared in journals with an impact factor of 3.75 and above. Centre researchers also published 6 invited book chapters. Details of publications are shown in Appendix 1: Publications.

Patents

Centre hosted conferences and scientific meetings

Annual Scientific Meeting, Yarra Valley Lodge

For 2013, the Centre held its Annual Scientific Meeting from 8–10 December at Yarra Valley Lodge. About 55 Centre staff and students, including Board members and Centre Associates attended the meeting. Prof Christian Doerig Head of Microbiology Department at Monash and Prof Mingan Choct, Director of the Poultry CRC, with whom the Centre has a collaborative relationship were present as guests.

The program was intense and stimulating, with representative talks from each research group, as well as excellent and thought-provoking presentations from each of our two international advisory board members. It was an excellent interactive meeting and provided opportunities for Centre staff and students to discuss together and exchange ideas. Indeed some of the scientific discussion even continued to midnight over drinks at the Lodge Bar.

Participants at the Centre’s Annual Scientific Meeting at the Yarra Valley Lodge during one of the presentations.
Invitations to address and participate in international conferences

Professor Ben Adler
- Première Journée Scientifique de l’Institut Pasteur de Nouvelle Calédonie
- Eighth International Leptospirosis Society Scientific Conference, Fukuoka, Japan
- Chair, Gordon Research Conference on the Biology of Spirochetes, Ventura, USA

Professor Rod Devenish
- Hunter Cell Biology Meeting, Pokolbin, Australia
- 26th International Conference on Yeast Genetics and Molecular Biology, Frankfurt, Germany

Professor Paul Hertzog
- Bill and Melinda Gates Foundation’s Collaboration for AIDS Vaccine Discovery Annual Meeting, Seattle, USA
- Bill and Melinda Gates Foundation’s Grand Challenges Meeting, Rio de Janeiro, Brazil
- International Cytokine Society/International Society for Interferon and Cytokine Research, San Francisco, USA
- International Congress in Immunology, Milan, Italy

Professor Julian Rood
- Chair, Organising Committee, Seventh International Conference on the Molecular Genetics and Pathogenesis of the Clostridia, Palm Cove, Australia

Professor Jamie Rossjohn
- 38th Lorne Conference on Protein Structure and Function, Lorne, Australia
- Seventh International Symposium on CD1 and NKT Cells, Tours, France
- Cardiff Institute of Infection and Immunity Annual Meeting, Cardiff, UK

Professor James Whisstock
- Eighth Aso International Meeting, Kumamoto, Japan
- 30th Winter School on Proteases and their Inhibitors, Salzburg, Australia
- 38th Lorne Conference on Protein Structure and Function, Lorne, Australia
Invitations to visit leading international laboratories

Professor Ben Adler
- Institut Pasteur de Nouvelle Calédonie, Nouméa
- Universidad Nacional de Colombia, Bogota, Colombia
- Universidad de la Salle, Bogota, Colombia
- Pontificia Universidad Javeriana, Bogota, Colombia
- Instituto de Investigaciones Biológicas del Trópico de la Universidad de Córdoba, Monteria, Colombia
- Universidad del Norte, Baranquilla, Colombia
- Universidad del Valle, Cali, Colombia

Professor Paul Hertzog
- Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China
- Laboratory of Lymphocyte Biology, Rockefeller University, New York City, USA
- Epigenetic Control of Innate Immunity Laboratory, Massachusetts General Hospital, Boston, USA
- Janssen Cilag, Beerse, Belgium
- Trinity College, Dublin, Ireland
- Imperial College, London, UK

Professor Julian Rood
- Norwegian Veterinary Institute, Oslo, Norway
- University of Helsinki, Helsinki, Finland

Professor Jamie Rossjohn
- Leiden University Medical Centre, Leiden, The Netherlands
- Cardiff Institute of Infection and Immunity, Cardiff University, Cardiff, UK
Commentaries about the Centre’s achievements

Publications


‘Horse a little punky? It might be lepto’, Modern Equine Vet, Issue 6 2013

‘A first in front line immunity research’, Monash Memo, 24 July 2013


‘Footrot research steps up’, MLA Feedback Magazine, Oct 2013

‘Footrot vaccine hope’, Weekly Times Now, 27 November 2013

Research training and professional education

Post graduate student education continued to be an important component of the Centre’s activities in 2013. A primary focus is the training of students in advanced technologies. In 2013, the Centre recruited 8 new PhD students. In addition to project-based training, Centre postgraduate students have access to professional development provided by the Monash Institute for Graduate Research (MIGR), the Monash Postgraduate Association (MPA) and the MBio Graduate School platform at the School of Biomedical Sciences. Students are encouraged to engage in teaching activity (demonstrating to undergraduate classes) for which training is provided.

In particular, Centre PhD students are encouraged to participate in an annual young investigator symposium as part of the Victorian Infection and Immunity Network. Centre Chief Investigator, Professor Paul Hertzog is also the co-covenor of VIIN.

Centre post graduate recruitments

Abdullah Al-Mamun (PhD)
Selecting superior production animals through microbiome characterization: Interactions between *Haemonchus contortus* and the gut microbiome of sheep

Shoeib Moradi (PhD)
An investigation into natural killer cell receptor recognition

Rebecca Piganis (PhD)
Characterisation of suppressor of cytokine signalling 1 regulation of Type I interferon signalling

Dhanasekaran Sakthivel (PhD)
The role of parasite proteases of *Haemonchus contortus* in infection and disease

Thomas Stent (PhD)
Plasmid-determined virulence genes in necrotic enteritis strains of *Clostridium perfringens*

Srinivasan Sundararaj (PhD)
Elucidating the role of NKT cells in asthma

Praveena Thirunavukkarasu (PhD)
Natural killer T cell recognition of lipid-based antigens

Shakif Ul-Azam (PhD)
Gene analysis of mediators upregulated in parasite resistant sheep
Honours Students

Gautham Balaji (Hons)
Structural and functional characterisation of the natural killer cell receptor KIR2DS4

Marianne Mégroz (Hons)
The role of Hfq in the virulence of Pasteurella multocida

Eenu Monga (Hons)
The role of the Chp system in Dichelobacter nodosus

Thomas Watts (Hons)
The analysis of Clostridium perfringens plasmid partitioning and incompatibility

Centre post graduate course completions

Andrew Clarke (PhD)
Structural insights into the recognition of CD1d-restricted self antigens by the natural killer T cell antigen receptor

Deanna Deveson Lucas (PhD)
The bovine immune response to Leptospira borgpetersenii serovar Hardjo

Amy King (PhD)
The characterisation of putative virulence determinants of Leptospira interrogans

Hamish McWilliam (PhD)
Schistosome vaccine development using the local immune response

Putayalai Treerat (PhD)
Characterisation of Burkholderia pseudomallei type III secretion system III components

Wilson Wong (PhD)
The structural and biochemical basis for host tissue destruction mediated by subtilisin-like proteases from the ovine footrot pathogen, Dichelobacter nodosus
Centre Student Achievements

Abdullah Al-Mamun
Recipient of one of only 60 positions nationwide in the exclusive European Molecular Biology Laboratory (EMBL) Australia PhD course scholarship

Priyangi Alwis
Recipient of Poster Prize at the Seventh World Melioidosis Conference, Bangkok, Thailand

Sam Forster
Recipient of Monash Institute of Medical Research Travel Award of $1,500

Dhanasekaran Sakthivel
Recipient of Victorian India Doctoral Scholarship

Thomas Stent
Recipient of Poultry CRC Postgraduate Scholarship

Jessica Wisniewski
Recipient of Outstanding Student Speaker Award at BacPath12 meeting

Shakif Ul-Azam
Recipient of 3rd Prize in the recent Postgraduate Research Poster Competition at Monash University

PhD Student Priya Alwis with her winning poster "Characterisation of an OmpR-EnvZ-like two-component signal transduction system in Burkholderia pseudomallei" at the 7th World Melioidosis Congress in Bangkok.
Undergraduate teaching

In 2013, Centre Chief Investigators delivered undergraduate lectures at first to fourth year levels in microbiology, biochemistry, bioinformatics, molecular biology, proteomics, pathogenesis, and structural biology at Monash University.

Professor Ben Adler
MIC 3990 – Microbiology in action research project (convener)

Professor Ross Coppel
MED2031 – Medical Studies in Microbiology and Infectious Diseases
MED2042 – Medical Studies in Microbiology and Infectious Diseases
MIC3041 – Medical Microbiology

Professor Rod Devenish
MED1011 – Medicine 1
BCH3031 – Advanced Molecular Biology: Modern concepts and applications
MIC3032 – Pathogenesis of bacterial infectious diseases

Professor Els Meeusen
BTH3012 – Biotechnology

Professor Julian Rood
MED1022 – Medicine 1
GMA1011 – Medicine 1 (Gippsland)
MIC3011 – Molecular Microbiology
MIC3032 – Pathogenesis of Bacterial Infectious Diseases
MIC4100 – Microbiology research project (Honours – convener)
MIC4200 – Advanced Studies in Microbiology (Honours – convener)

Other research training and professional education

Bioinformatics Workshop

Centre Director, Prof Ben Adler convened a Bioinformatics workshop on 11 Oct in Fukuoka, Japan. This workshop was conducted as part of the Eighth International Leptospirosis Society scientific meeting. Dr Dieter Bulach from the Victorian Bioinformatic Consortium, who has collaborated on a number of Centre research projects, presented the workshop. Using the Centre’s Leptospira genome project, the workshop provided participants with some simple approaches to accessing genome data and also experience in working with these data using specialised software available freely to users. Centre staff and student, Dr Gerald Murray and Ms Amy King were also present to assist the workshop participants.
International, national and regional links and networks

The centre has an extensive international network. Its researchers collaborate and publish together with researchers and institutes across the world in over 55 cities, as shown on the map below.
International Engagement

Through its extensive international network, the Centre had the privilege to engage in a number of international activities and host a number of international scientists and students who chose to spend time at the Centre doing research and interacting with Centre staff and students.

A team of three academic Malaysian researchers (Prof Raja Noor Zalih, Dr Adam Chor and Dr Mohd Shukuri) from the faculty of Biotechnology and Biomolecular Sciences at the University Putra Malaysia (UPM) visited the Centre on 9 April. Their main aim was to learn how the Centre operates so that they can have a model to set up and run a similar centre in their own university. During their visit, they spoke with some of the Chief Investigators about their research and also learnt more about the equipment and research technology platforms made available for the Centre’s research projects. The UPM delegates had chosen to visit the Centre because of its reputation in microbial genomics and structural biology as well as the ongoing collaboration that the Centre already has with other institutions in Malaysia.

Centre Director Prof Ben Adler was invited on a lecture tour of Colombia in June. He visited six universities in four cities and presented seminars on Centre research on the fowl cholera and leptospirosis projects. He was also able to promote other Centre research activities and generate interest in future research and educational collaborations.

CI Prof Jamie Rossjohn continued his collaborative program of research with Prof. Nor Mahadi of the Malaysian Genome Institute and Prof. Rosli Illias of Universiti Teknologi Malaysia. This program initiated since 2011, allows two Malaysian students to conduct research at Monash for 6 months (5 students in total to date), as well as a Malaysian post-doctoral researcher to locate at Monash for 18 months. The research from this program takes a structural genomics approach on proteins isolated from extremophiles and makes use of the Centre microbial genomics pipeline.
Other International and National Visitors

Throughout the year, the Centre received a number of visitors who were interested in Centre research activities and wanted to discuss scientific matters with Centre researchers or have a closer look at our activities and facilities. Some also had the opportunity to present their work at a Centre seminar.

The visitors in 2013 included:

Dr Walid Arafa, Beni-Suef University, Egypt
Dr Motti Gerlach, Walter and Eliza Hall Institute, Australia
Dr Marianne Gilhuus, Norwegian Veterinary Institute, Norway
Prof Lars Juhl Jensen, Panum Institute, Denmark
Dr Candice Lee, Australian National University, Australia
Dr Natacha Lorsuwannarat, Mahidol University, Thailand
Prof Robert Miller, University of New Mexico, New Mexico, USA
Prof D Branch Moody, Harvard Medical School, Massachusetts, USA
Dr Nor Muhammad Mahadi, Malaysia Genome Institute, Malaysia
Dr Rosli Md Illias, Malaysia Genome Institute, Malaysia
Dr Abdul Munir Abdul Murad, Malaysia Genome Institute, Malaysia
Dr Dominic de Nardo, University of Bonn, Germany

Centre Associate Professor Davie Piedrafita and Centre PhD student Hamish McWilliam with Chinese students and researchers from the Hunan Institute of Parasitic Diseases on their fieldtrip experiment in Hunan, China for the Schistosoma vaccine project.
Income derived from other sources

This list includes all active grants received by Centre Chief Investigators in 2013. Monetary figures denote the amount allocated for 2013 and principal collaborators are indicated in parenthesis.

**Professor Ben Adler**
Australian Poultry CRC – Rapid multiplex PCR assay for differentiating *Pasteurella multocida* serovars. $94,782 (Boyce, Harper)

**Professor Ross Coppel**
National Institutes of Health (NIH), USA – Malaria and the red blood cell. US$130,000 (Mohandas)

NHMRC Project Grant – Investigating the mechanisms of regulation of mycobacterial cell wall biosynthesis. $192,244 (Crellin, McConville)

2014 NHMRC Project Grant – Regulation from the outside: control of transport and assembly. $210,186 (Crellin, McConville)

**Professor Rod Devenish**
ARC SuperScience Grant – Design and fabrication of molecular machines: the nanomachines of the future. $278,400 (Lithgow, Rossjohn, Martin, Strugnell)

NHMRC Project Grant – How *Burkholderia pseudomallei* subverts host ubiquitination and autophagy pathways. $165,098 (Kleifeld, Boyce, Prescott)

**Professor Paul Hertzog**
NHMRC Development Grant – Development of novel antivirals. $211,209 (Sullivan)

NHMRC Hendra Virus Urgent Call – Understanding pathogenicity and immunity in an encephalitic mouse model of Hendra virus infection. $187,450 (Stambas, Ninuesa, Middleton, Wang, Marsh)

NHMRC Project Grant – Protection of the reproductive tract against *Chlamydia* infection by a new interferon epsilon. $174,000 (Hansboro)

NHMRC Project Grant – The role of BAFF, its receptor TACI and Toll-like receptors in autoimmunity and tolerance. $156,674 (Mackay)

NHMRC Project Grant – Structural characterization function analyses of type 1 interferon-receptor. $165,500 (Reid)

NHMRC Project Grant – The role of interferon signalling in the regulation of stroke. $199,500 (Crack)

Cancer Council Victoria – Silencing of Irf7 expression in breast cancer cells as a mechanism of immune escape during metastasis. $197,016 (Parker)

NHMRC Project Grant – Tumour induced innate immune responses that control breast cancer metastases. $177,240 (Parker)

NHMRC Project Grant – Characterising the novel signalling mechanism for a new interferon. $169,115

Victorian Infection and Immunity Network Industry Alliance, Collaborative Networks Pilot Program, Department of Business and Innovation, State Government of Victoria. $150,000

**Professor Els Meeuwen**
ARC Linkage Grant – Exploiting the lymphatic system for next generation vaccine development. $180,000

ARC Discovery Grant – Designing new generation adjuvants for allergy and parasite vaccines. $110,000 (O’Hehir)

Meat and Livestock Australia – New approaches to innate immunity in livestock and the potential for manipulation. $20,000

Novartis – Testing of anti-fluke compounds $30,000 (Piedrafita)

Spanish Ministry of Science and Education National Grant – In vivo depletion of γδ T cells and eosinophils and its effects on the resistance of the Canaria Hair Breed sheep against *Haemonchus contortus*. $50,000 (Gonzalez, Piedrafita, Molina, Rodriguez, Hernandez)

**Professor Julian Rood**
Australian Poultry CRC – Vaccine against *Clostridium perfringens* to protect birds from necrotic enteritis. $61,438 Monash component only (Moore, Keyburn)

NHMRC Project Grant – Host-pathogen interactions in clostridial myonecrosis. $138,205 (Lyra, Awad)

NHMRC Project Grant – Novel therapeutic and preventive strategies for *Clostridium difficile* infections. $163,775 (Lyra, Carter, Sonenshein)

ARC Linkage Grant – The development and evaluation of new therapy for the prevention and treatment of bacterial infections in hospitals. $165,000 (Lyra, Rawlin)
NIH/NIAID – *Clostridium perfringens* type B-D virulence plasmids USD $90,481, Rood lab allocation (McClane, Uzal)

NHMRC Project Grant – The role of *Clostridium difficile* virulence factors in mediating the host-pathogen interactions that lead to gastrointestinal disease. $143,000 (Lyras, Carter)

NHMRC Project Grant – The role of *Clostridium difficile* spore surface structures in initiating gastrointestinal infection and disease. $150,470 (Lyras, Awad)

NHMRC Project Grant – The mechanism of conjugative transfer of antibiotic resistance genes in Gram positive pathogens. $202,262 (Whisstock)

Meat & Livestock Australia – Development of a cross-protective footrot vaccine using reverse vaccinology $107,464 (Kennan, Whittington)

### Professor Jamie Rossjohn

NHMRC Hendra Virus Urgent Call – Understanding the host pathogen relationships of Hendra virus in bats, horses and humans. $212,340

Association for International Cancer Research – Investigating the role of immune system cells in cancer. $105,075

NHMRC Australia Fellowship – $800,000

Roche Organ Transplantation Research Fund – To monitor the mechanism and tissue specificity of cross-reactive allogenic T-cells. $98,000 (McCluskey, Bharadwaj)

NIH/NIAID Grant – iNKT cell recognition of endogenous lipid antigens. $127,450 (Gapin)

ARC Superscience Grant – Design and fabrication of molecular machines: the nanomachines of the future. $278,400 (Lithgow, Devenish, Martin, Strugnell)

### Professor James Whisstock

ARC Federation Fellowship – Structural and functional studies on Membrane Attack Complex / Perforin-like proteins $163,873

ARC Discovery Grant – Membrane attack complex/perforin-like proteins in developmental and neurobiology $120,000 (Warr, Freeman)

ARC Superscience Grant – Engineering pore forming proteins as machines for the delivery of proteins and nanoparticles into cells. $163,873 (Porter, Friend, Hourigan, Boyd)

NHMRC Project Grant – Understanding how perforin forms pores: the role of calcium and lipids. $256,759 (Norton)

NHMRC Project Grant – The structural basis for plasminogen activation. $178,866
Awards and recognition

**Professor Jamie Rossjohn**

- 2013 Leiden University Medical Centre Boerhaave Medal.
- 2013 Australian Museum – University of New South Wales Eureka Prize for Scientific Research (jointly with Dr. Lars Kjer-Nielsen & Prof. James McCluskey).

**Professor Paul Hertzog**

- Recipient of the 2013 International Cytokine and Interferon Society Milstein Award. This award recognizes individuals who have made exceptional contributions to interferon and cytokine research, either in a basic or applied field. Many of these achievements have led to the advancement of human health.
End-user links

Commercialization and Technology Transfer

• Our extensive studies have shown that we can protect sheep against *Haemonchus contortus* infection by vaccinating in the field with a single native, larval-specific antigen. This antigen can be easily prepared from the abundant L3 larval stage and the vaccine may be ideal for small farm holders of sheep and goats. Through our collaborators in the Canary Islands and the local Pfizer representatives, field trials are under way to validate this vaccine as a commercially viable product for use in the Canary Islands, where small farm holdings are the mainstay for animal production. The culture and preparation of the vaccine has been set up at the University of Las Palmas on Gran Canarias and suitable farms have been identified where field trials are due to commence shortly. Based on the result of these trials, further distribution of this vaccination strategy will be implemented worldwide.

• The *Schistosoma japonicum* vaccine antigen is currently being evaluated for a patent application. In addition, 2 patent applications have already been filed in 2013:
  1. MR1 ligand and its uses thereof
  2. Method for identifying an interferon receptor (IFNAR) modulator and its uses thereof

• Validation of a diagnostic PCR for identification of fowl cholera-causing isolates of *Pasteurella multocida*, in collaboration with the Australian Poultry CRC and University of Queensland.

• The footrot vaccine project is now supported by Meat and Livestock Australia.

• The commercialization of an avian necrotic enteritis vaccine is now proceeding under the auspices of the Australian Poultry CRC.

Government, Industry and Business Briefings

Prof Paul Hertzog continued activities under the $300,000 grant of the Collaborative Networks Pilot Program from the Department of State Development, Business and Innovation, State Government of Victoria. This grant funds the Victorian Infection and Immunity Network Industry Alliance (VIIN-IA), which aims to build relationships between industry and VIIN of which the Centre is a component. In 2013, the VIIN-IA held a series of meetings, including its Roadshow and Research Showcases in Clayton, Parkville and Geelong during May. Together with BioMelbourne Network, it also co-hosted the BioBriefing Breakfast in November on “Emerging Superbugs: why we should be concerned and why we need a new approach.” The VIIN-IA also funded a series of collaborative projects involving a scientist from VIIN, working with a Victorian-based biotech or pharmaceutical business to translate basic research outcomes or to address a specific business problem. Over 40 companies have been engaged through the VIIN-IA’s activities.
Public Awareness Activities

National Science Week

This year for National Science Week, the Centre organised a public lecture on 15 Aug. Given the rising interest in superbug infection and antibiotic resistance, the lecture was aptly entitled “Will the superbugs inherit the earth?” It was delivered by one of the Centre’s Associates, Associate Professor Dena Lyras, who gave an interesting account of how the threat of antibiotic-resistant bacteria is rising and measures that could be taken to control and overcome this problem.

Also as part of National Science Week, the Centre ran a program called “Parasites in Focus”, in conjunction with the Gene Technology Access Centre (GTAC) and the Australasian Society of Parasitology. This workshop took place on 16 August and was attended by 80 secondary school students of Years 10–11 from 10 schools across Victoria. Centre Associate, Assoc Prof David Piedrafita, was invited to present the opening address and he gave a fun and engaging introduction to the world of parasitology and the life of a field parasitologist. The students then participated in three rotating laboratory workshops. In one of the workshops, David and his graduate students from the Centre (Amanda Peers-Adams, Sarah Preston, Md Abdullah Al-Mamun and Md Shakif Ul-Azam) guided students using microscopy to explore adaptations of parasites.

Centre Associate Assoc Prof David Piedrafita also represented the Centre to take part in the ConocoPhillips Science experience in 2013. This is a program organised by the Science Schools Foundation Inc. to provide Year 9 and 10 students with an interest in science to engage further by given them opportunities to participate in a wide range of science activities under the guidance of scientists. For this program which ran from 21–23 January at the Monash Gippsland campus, Assoc Prof Piedrafita gave lectures to the students and also ran a practical on parasites. About 40 students attended this program.
Other Chief Investigator professional activities

Professor Ben Adler
- Joint Editor-in-Chief, Veterinary Microbiology
- Academic Editor, PLoS One
- Scientific Advisory Board, Institut Pasteur de Nouvelle Calédonie
- Scientific Advisory Committee, Eighth International Leptospirosis Society Scientific Conference, Fukuoka, Japan
- Scientific Advisory Committee, International Union of Microbiological Societies (IUMS)
- Chair, Gordon Research Conference on The Biology of Spirochetes
- Chair, Third Prato Conference on the Pathogenesis of Bacterial Infections of Animals
- Committee Member, International Pasteurellaceae Conference

Professor Ross Coppel
- Member of the NHMRC Academy

Professor Rod Devenish
- Editorial Board, Autophagy

Professor Paul Hertzog
- Initiated the International Innate Immunity Consortium linking CiiiD, University of Cambridge UK, University of Massachusetts USA, Trinity College Dublin, University of Bonn, Germany.
- Convenor of the Victorian Infection and Immunity Network
- Convenor of the Lorne Infection and Immunity Conference
- Chair of the Monash Medical Centre Animal Facility Coordinating Committee
- Chair of the Monash Health Translation Precinct Translational Research Facility Platform Technologies Committee
- Editor of the 2013 Australia Issue of the Cytokine & Growth Factor Research journal

Professor Els Meeusen
- Editorial board member, Parasite Immunology

Professor Julian Rood
- Editor-in-Chief, Plasmid
- Editorial Board, BMC Microbiology, Anaerobe, Veterinary Research
- Ambassador for Australia, American Society for Microbiology

Professor Jamie Rossjohn
- Reviewed applications for University of Massachusetts Medical School and Wellcome Trust Investigator Award

Professor Ian Smith
- Editorial Board Member, Journal of Molecular and Cellular Proteomics, 2002–
- Editorial Board Member, Protein & Peptide Letters, 2005–
- Editorial Board Member, International Journal of Peptide Research and Therapeutics, 2005–
- Editorial Board Member, Current Proteomics, 2007–
- Editorial Board Member, The Open Proteomics Journal, 2007–
- Editorial Board Member, Clinical Proteomics, 2007–
- Board member NCRIS funded National Imaging Facility (NIF)
- Board member VLSCI (Victorian Life Science Computing Facility)
- Director, Monash Biomedical Imaging (MBI)
- Chair, NHMRC’s RGMS User Reference Group (RURG) 2010–
- Member The BioSciences Victoria Collaborative 2010–
- Chair, Victorian node Proteomics Australia
- Chair, Victorian BioMedical Imaging Consortium 2010–
- Member, BioImaging External Advisory Board, ESFRI Roadmap for Research Infrastructures, Euro-Bioimaging Project, 2010–
- Member, Victorian Biomedical Imaging Consortium 2010–
- Member, Victorian BioMedical Imaging Consortium 2010–

Professor James Whisstock
- Organising Committee, 38th Lorne Conference on Protein Structure and Function
Producing solutions for vaccine and drug development

Appendix 1: Publications

Journal articles

This list includes all publications by Centre affiliated researchers accepted in 2013. These publications have varying levels of input from Centre staff and students. Centre byline publications are indicated with an asterisk.


Producing solutions for vaccine and drug development


Producing solutions for vaccine and drug development


**Conference Abstracts**

This list includes all conference presentations in 2013 by Centre affiliated researchers. These presentations have varying levels of input from Centre staff and students.


Book Chapters


Rossjohn, J. (2013) T-cell receptor recognition of a monomorphic Ag-presenting molecule. 7th International Symposium on CD1 and NKT Cells, Tours, France.


## Appendix 2: Financial statement

Financial statement for year ended 31 December 2013.

### Income

<table>
<thead>
<tr>
<th>Source</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARC</td>
<td></td>
</tr>
<tr>
<td>CoE Program</td>
<td>1,711,181</td>
</tr>
<tr>
<td><strong>Industry/Private Funds</strong></td>
<td></td>
</tr>
<tr>
<td>Meat &amp; Livestock Australia</td>
<td>940,000</td>
</tr>
<tr>
<td>Sheep CRC</td>
<td>7,500</td>
</tr>
<tr>
<td>Poultry CRC</td>
<td>144,782</td>
</tr>
<tr>
<td>Australian Society for Parasitology</td>
<td>7,064</td>
</tr>
<tr>
<td><strong>Host Institution Support</strong></td>
<td>699,000</td>
</tr>
<tr>
<td><strong>Carried forward from 2012</strong></td>
<td></td>
</tr>
<tr>
<td>ARC funds</td>
<td>105,151</td>
</tr>
<tr>
<td>Host Institution</td>
<td>950,285</td>
</tr>
<tr>
<td><strong>Total Income</strong></td>
<td>4,564,963</td>
</tr>
</tbody>
</table>

### Expenditure

<table>
<thead>
<tr>
<th>Category</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salaries</td>
<td>2,193,609</td>
</tr>
<tr>
<td>Scholarships, Prizes &amp; Grants</td>
<td>81,872</td>
</tr>
<tr>
<td>Equipment &amp; IT</td>
<td>8,498</td>
</tr>
<tr>
<td>Books, Print &amp; Media</td>
<td>8,434</td>
</tr>
<tr>
<td>Maintenance/Consumables</td>
<td>744,240</td>
</tr>
<tr>
<td>Travel and Related Expenses</td>
<td>12,696</td>
</tr>
<tr>
<td><strong>Other Expenditure</strong></td>
<td></td>
</tr>
<tr>
<td>Meat &amp; Livestock Project</td>
<td>940,000</td>
</tr>
<tr>
<td>Poultry CRC Project</td>
<td>26,243</td>
</tr>
<tr>
<td>Centre Annual Scientific Meeting</td>
<td>38,057</td>
</tr>
<tr>
<td>Administrative Charges</td>
<td>3,386</td>
</tr>
<tr>
<td><strong>Total expenditure</strong></td>
<td>4,057,035</td>
</tr>
</tbody>
</table>
### Appendix 3: Key result areas and performance measures 2013

<table>
<thead>
<tr>
<th>Key Result Area</th>
<th>Performance Measure</th>
<th>Target</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Research findings</strong></td>
<td>Quality of publications</td>
<td>At least 75% on journals with an impact factor (IF) of ≥3.75</td>
<td>71% of papers were published in journals with IF ≥3.75.</td>
</tr>
<tr>
<td></td>
<td>Number of publications</td>
<td>22 refereed publications in international journals, 2 invited book chapters or reviews</td>
<td>51 refereed publications, including 1 review. 6 invited book chapters.</td>
</tr>
<tr>
<td></td>
<td>Number of patents</td>
<td>1 per year</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Invitations to address and participate in international conferences</td>
<td>7 per year</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Invitations to visit leading international laboratories</td>
<td>7 per year</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Number and nature of commentaries about the Centre’s achievements</td>
<td>1 per year</td>
<td>8 published commentaries</td>
</tr>
<tr>
<td><strong>Research training and professional education</strong></td>
<td>Number of postgraduates recruited</td>
<td>4 per year</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Number of postgraduate completions</td>
<td>3 per year</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Number of Honours students</td>
<td>4 per year</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Participation in professional courses</td>
<td>1 per year</td>
<td>Centre convened the Bioinformatics Workshop as part of the International Leptospirosis Society scientific meeting in Fukuoka, Japan</td>
</tr>
<tr>
<td></td>
<td>Number and level of undergraduate and high school courses in the priority area(s)</td>
<td>2 at 2nd year level</td>
<td>MED2031 and MED 2042 – Medical Studies in Microbiology and Infectious Diseases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 at 3rd year level with substantial microbial genomics components</td>
<td>BCH3031 – Advanced Molecular Biology</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BTH3012 – Biotechnology Science, Industry and Commercialisation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MIC 3011 – Molecular Microbiology</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MIC3032 – Pathogenesis of Bacterial Infectious Diseases</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MIC3990 – Microbiology in Action</td>
</tr>
<tr>
<td>Key Result Area</td>
<td>Performance Measure</td>
<td>Target</td>
<td>Outcome</td>
</tr>
<tr>
<td>-----------------------------------------------------</td>
<td>-----------------------------------------------------------</td>
<td>--------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>International, national and regional links and networks</td>
<td>Number of international visitors</td>
<td>4 per year</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Number of national and international workshops</td>
<td>1 per year</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Number of visits to overseas laboratories</td>
<td>7 per year</td>
<td>17</td>
</tr>
</tbody>
</table>
| End-user links                                      | Nature and number of commercialisation activities         | 2 partnerships with industry or industry bodies         | Australian Poultry CRC  
                          Australian Sheep CRC  
                          Meat & Livestock Australia  
                          Zoetis Australia           |
|                                                     | Number of government, industry and business briefings     | 1 per year   | 1                                                                       |
|                                                     | Number of Centre associates trained/ing in technology transfer and commercialisation | 1 per year   | 1                                                                       |
|                                                     | Number and nature of public awareness campaigns           | 1 per year   | 3                                                                       |
| Organisational support                              | Annual in-kind contributions from Collaborating Institutions | $300,000 per year | ~$460,000 salary in-kind contributions                                  |
|                                                     | Number of new organisations recruited to or involved in the Centre | 1 per year   | Hunan Institute of Parasitic Diseases, China University of Copenhagen, Denmark Universitat Autònoma de Barcelona, Spain |
|                                                     | Level and quality of infrastructure provided to the Centre | $800,000 per year in equipment infrastructure, and $700,000 per year cash | ~$699,000 cash contribution from Monash University.  
                          ~$2,6M in equipment infrastructure and  
                          ~$2,35M in Chief Investigator and other staff salaries.       |
|                                                     | Annual cash contributions from other Organisations         | $200,000     | ~$152,000 from Poultry CRC and Sheep CRC  
                          ~$22,000 from Norwegian Veterinary Institute  
                          ~$940,000 from Meat and Livestock Australia             |
<p>|                                                     | Annual in-kind contributions from other Organisations     | $150,000     | $240,000 in-kind contributions                                           |</p>
<table>
<thead>
<tr>
<th>Key Result Area</th>
<th>Performance Measure</th>
<th>Target</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Governance</strong></td>
<td>Breadth and experience of members of Advisory Board</td>
<td>6 Members specified</td>
<td>Existing board of 5 members and 2 international Adjunct Board Members.</td>
</tr>
<tr>
<td></td>
<td>Frequency and effectiveness of Advisory Board meetings</td>
<td>Twice per year</td>
<td>Met once on Feb 5. Second meeting was held during the Centre’s annual scientific meeting 8–10 Dec.</td>
</tr>
<tr>
<td></td>
<td>Quality of Centre Strategic Plan</td>
<td>As outlined in submitted research plan</td>
<td>Post-review changes incorporated into plan.</td>
</tr>
<tr>
<td></td>
<td>Effectiveness of arrangements to manage Centre nodes</td>
<td>Publication of cross-node papers</td>
<td>Centre is single node</td>
</tr>
<tr>
<td></td>
<td>Adequacy of the Centre’s key Performance Measures</td>
<td>Annual review by the Scientific Advisory Board and other reviewers as determined by the Board</td>
<td>Review conducted by Scientific Advisory Board and the Board is pleased with the progress of the Centre.</td>
</tr>
<tr>
<td><strong>National benefit</strong></td>
<td>Measure of expansion of Australia’s capability in the priority area(s)</td>
<td>Substantial increase in knowledge base. Progress in vaccine development and drug target identification. Production of quality PhD graduates</td>
<td>See list of publications. Vaccine development and drug target pipeline projects progressed satisfactorily in 2013. A number of vaccine targets are being supported by various industry partners. See progress reports in Annual Report.</td>
</tr>
<tr>
<td></td>
<td>Case studies of economic, social, cultural or environmental benefits</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>