Department of Microbiology

2019 Honours Programs in Microbiology

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The Honours programs for both Bachelor of Biomedical Science (BBimedSci) and Bachelor of Science (BSc) contain coursework and an independent research project. The objectives of these courses are to develop the laboratory skills required for research in microbiology and the ability to critically evaluate microbiological research. Students also achieve a detailed understanding of specialised topics in microbiology and enhance their communication skills in written and oral presentations.

The Department looks forward to welcoming you in 2019. We feel that our friendly, constructive and highly productive working environment provide an excellent opportunity for honours students to develop an understanding of the research process and to achieve their full research potential.

Formal application process

Application for Microbiology Honours entry involves a two part application process.

1. Formal application to the relevant faculty by
   B. Sc (Hons): November 16, 2018
   [link]
   B. Biomed. Sci (Hons): November 16, 2018
   [link]

2. Submission of project preferences to Associate Professor John Boyce (no later than November 16, 2018).

Research projects

The research project is the major component of both programs. All efforts are made to accommodate students in the laboratory of their choice, and to develop research projects that take into account the student’s, as well as the supervisor’s, interests. Brief outlines of the available projects for 2019 are in the following section.

Supervisor interviews

Applicants are encouraged to discuss research projects with potential supervisors at any suitable time, by appointment. Following these discussions, students will need to give Associate Professor John Boyce their Microbiology application forms (see last page) indicating their project preferences, and any additional documentation required. You do not need to wait until November 16th to hand in your preference forms, the earlier the better.

Projects outside the department

It is possible for students to complete their coursework within the Department of Microbiology at Clayton, and their research project off-campus. Under these circumstances, students must travel between locations when required. The thesis examination takes place at the same time for all students enrolled through Microbiology.

Microbiology coursework

The coursework conducted within the Department of Microbiology consists of short courses termed colloquia, a statistics course and a seminar series. BSc students need to complete two colloquia, BBimedSci students complete one colloquium. Each colloquium is held during a one month period in the first half of the year, so that the coursework is usually completed, and students receive some feedback on their progress, by mid-year. The format of the colloquia will vary. Most involve reading recent research papers, an oral or poster presentation, and a written assignment.

BBimedSci common core coursework

In addition to one colloquium, all BBimedSci Honours students must complete a centrally assessed common coursework component consisting of:

- A statistics module, an accompanying workshop and test
- A written critique of a scientific paper, in a three-hour examination format
Literature survey

During first semester the students must submit a literature survey on their research project. The literature survey (which can be used as the basis for the introduction in the final report) allows the identification early in the year of those students who have problems with English expression so this can be addressed by directed English writing instruction. It also, of course, compels the students to become thoroughly conversant with their area of research.

Additional requirements

The programs will commence on February 25, 2019 (mid year begins July 15) with a series of introductory lectures, before the students start work on their research projects. These lectures contain information on the course, departmental facilities and laboratory safety. In the second half of the year students may be given specific training in the presentation of written reports, and in oral presentation of their work. It is compulsory for students to attend the introductory lecture course, all departmental seminars, and any short courses on written and oral presentations.

Assessment

Final assessment of the BSc Honours program follows the format:

<table>
<thead>
<tr>
<th>Component</th>
<th>Weight</th>
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<tbody>
<tr>
<td>Literature survey</td>
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<tr>
<td>Research report/report review</td>
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<tr>
<td>Seminar</td>
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<tr>
<td>Microbiology coursework</td>
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Final assessment of the BBiomedSci Honours program follows the format:

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<tr>
<td>Seminar</td>
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<tr>
<td>Microbiology coursework</td>
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<tr>
<td>Statistics Module</td>
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<tr>
<td>Common written component</td>
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Eligibility

Monash BSc Students

Entry to the course is restricted to those students who have qualified for the award of the pass degree of BSc (all subjects completed before enrolment), and have an average of at least 70% in 24 points of relevant level-three science units. This generally includes at least 18 points of Microbiology units. Students studying combined Science degrees must be eligible for the award of BSc.

BSc Graduates of Other Universities

As for Monash students, applicants are required to have a BSc and distinction grades in Microbiology or closely related subjects. A certified copy of the applicant’s academic record and a statement to the effect that they have qualified for a pass degree are required as soon as they are available.

Monash BBiomedSci students

Students must have completed all requirements for the award of the pass degree of Bachelor of Biomedical Science offered at the Monash University. They must also have an average of 70% or higher in at least 24 points at third year level, with 12 points from third year core units.

BBiomedSci graduates from other universities

Students applying for admission based on a qualification other than the pass degree of Bachelor of Biomedical Science offered at Monash University will need to demonstrate that they have achieved an appropriate standard in studies comparable to 24 points of BBiomedSci subjects as stipulated above.

Part-time study and mid-year entry

The department prefers students to study on a full-time basis. However, it may be possible under special circumstance to complete the Honours degree in two consecutive years by doing the coursework and research project in separate years. It may also be possible to start the course mid-year. In both of these circumstances, the arrangements are made on an individual basis between applicants and supervisors.
Research Projects
2019
Characterisation of the *Acinetobacter baumannii* type VI secretion system

A/Professor John Boyce, Dr Marina Harper and Dr Deanna Deveson Lucas

*Acinetobacter baumannii* has been identified as one of the top three dangerous Gram-negative hospital pathogens as it can cause a range of life-threatening infections and many strains are now resistant to almost all current antibiotics.

Mechanisms of virulence and gene regulation are still poorly understood in this organism. The identification of novel virulence genes will identify new targets for therapeutic intervention. In this project we will further characterise the type VI secretion system of *A. baumannii*, which we have shown is involved in interbacterial killing of both other *A. baumannii* strains and other bacterial species. We have shown that the system secretes at least 9 proteins including three toxins, two immunity proteins and four proteins of unknown function. This project will define the function of uncharacterized type VI secretion system structural proteins, antibacterial toxins and secreted effectors using complementation and directed mutagenesis approaches. These novel toxins are promising antimicrobial agents.

Defining the mechanisms of *Pasteurella multocida* pathogenesis and identifying novel virulence regulators

Dr Marina Harper and A/Professor John Boyce

*Pasteurella multocida* is a Gram-negative bacterial pathogen that causes a number of different diseases in cattle, pigs and poultry, resulting in serious economic losses worldwide in food production industries. We are interested in understanding the molecular mechanisms of pathogenesis in this bacterium with an aim to developing new, more effective and widely applicable vaccines or antimicrobial drugs.

The mechanisms by which *P. multocida* controls the expression of virulence genes is poorly understood. Indeed, we are the only group worldwide to have characterized virulence regulators in this important pathogen. In this project we will mutate predicted virulence regulators (alternative sigma factors and/or two component signal transduction system components) and assess their effect on *P. multocida* gene expression and virulence. We will analyse the changes in virulence gene/protein expression using both whole transcriptomic and proteomic techniques and assess the phenotype of the mutants both *in vitro* and *in vivo* using established assays.
Understanding antibiotic tolerance and persistence mechanisms in *Pseudomonas aeruginosa* hypermutator strains

A/Professor John Boyce, Dr Cornelia Landersdorfer (Department of Pharmacy Practice) and Dr Deanna Deveson Lucas

*Pseudomonas aeruginosa* is a Gram-negative bacterium that is one of the most common pathogens of people with cystic fibrosis. This organism causes chronic lung infections that are very difficult to eradicate. During long-term, chronic infections, mutations often occur within the *P. aeruginosa* DNA repair genes, leading to a hypermutator phenotype; these mutant bacteria are more persistent and more antibiotic resistant than their wild-type counterparts. We are currently aiming to understand how hypermutation affects the response to clinically relevant antibiotics (both in mono and combination therapy) in order to optimize treatment regimens. In this project we will identify novel putative antibiotic resistance and/or persistence genes in clinically relevant hypermutator strains and characterize the function of these genes using, directed mutagenesis, heterologous expression, complementation and qRT-PCR, together with a range of phenotypic assays including ability to form biofilms and susceptibility to various antibiotics and their combinations. These experiments will identify novel targets of direct relevance to treatment of chronic lung infections in cystic fibrosis patients.
Research in our laboratory focuses on understanding the ways in which parasites of red blood cells cause disease and death in humans or animals. We aim to provide a friendly and helpful environment in which to gain knowledge and expertise in the process of modern biomedical research. Honours students will have the opportunity to design an original research project in one of our two major areas of interest in close consultation with their supervisors. Initially, students will be closely supervised and work side-by-side with experienced researchers in the laboratory. Importantly, you will acquire a wide range of skills including bioinformatics, proteomics, molecular techniques (cloning, PCR, Southern blotting, etc.), immunoblotting, immunofluorescence, cell culture, biophysical assays and sub-cellular fractionation. Graduates will be well prepared to either enter the work force or begin a higher research degree.

Studies on malaria

Malaria causes severe morbidity, mortality and socio-economic hardship particularly in Africa, South America and Asia. The disease is caused by protozoan parasites of the genus Plasmodium, with at least five species known to infect humans. Symptoms, including fever, chills, headaches and anaemia, are attributable to replication of parasites within red blood cells (RBCs) and vary in severity depending on the parasite species and the immune status of the host. In the case of falciparum malaria, serious complications can arise due to sequestration of parasitised RBCs (pRBCs) in the microvasculature of the brain or the placenta resulting in cerebral malaria and pregnancy-associated malaria respectively.

Characterisation of malaria PHIST-domain proteins

Professor Brian M. Cooke and Dr Nicholas I. Proellocks

Proteins containing a PHIST (Plasmodium helical interspersed sub-telomeric) domain constitute a multi-member family that are present in the most important species of human malaria parasites. Although the family is largely uncharacterised, preliminary information on the function of a few characterised members suggest that they play major roles in pathogenesis. This project will use a combination of molecular, cellular, proteomic and biophysical approaches to characterise defined members of PHIST proteins to gain a better understanding of the mechanisms by which malaria parasites cause human disease. This will involve both *in vitro* studies of protein interaction and *ex vivo* studies of protein fate and action in transgenic parasites. Specifically, your studies will contribute to an overall analysis of the expression and cellular localisation of previously uncharacterised PHISTs, determine their function in PRBCs and identify the host and/or parasite proteins with which they interact.

Understanding the function of unique P. falciparum FIKK kinases.

Professor Brian M. Cooke and Professor Christian Doerig

FIKK kinases are unique among apicomplexan parasites. Interestingly the genome of *P. falciparum* encodes 20 FIKK kinases, but very little is known about their localisation or biological function. This project will focus on a sub-group of
FIKK kinases, which our preliminary work strongly suggests are essential for parasite survival. The divergence of these enzymes from mammalian eukaryotic protein kinases, together with their essentiality to parasite proliferation, makes them highly attractive potential targets for the development of next-generation anti-malarial drugs. The overall objective is to characterise one or more essential FIKK kinases in order to understand the mechanisms by which they regulate essential biological processes in the parasite. This will be achieved in part by confirming their essentiality, localisation and trafficking in blood stage *P. falciparum* parasites and identification of their target proteins by biochemical and proteomic approaches.

Discovering the functions of essential exported malaria proteins

**Professor Brian M. Cooke and Dr Paul Gilson**

The functions of at least half of the proteins encoded in the genome of *Plasmodium falciparum* malaria parasites remain unknown. Understanding protein function is important because many are critical for pathogenesis and/or could become future drug targets. Proteins exported by the malaria parasite into its host red blood cell are particularly intriguing as they aid parasite survival and immune avoidance. Disrupting expression of a protein in malaria parasites and then studying the resultant phenotypic changes has traditionally been used to understand function. However, about 25% of genes for exported proteins cannot be disrupted and are considered to be essential. This makes the study of these proteins and their functions difficult. To overcome this we have been developing genetic knockdown systems where a switch is inserted into the gene of interest so it can rapidly be turned down at will and the resultant phenotypes examined. The aim of this project is to genetically knockdown essential genes for novel exported proteins in malaria parasites and determine their function using a suite of molecular and cellular biological assays.

Studies on babesia

*Babesia* spp. are widespread and economically-important protozoan parasites that cause severe, often fatal diseases in animals and humans. *Babesia bovis* (that shows striking similarities with human malaria parasites) and *B. bigemina* are the causative agents of babesiosis in cattle. *Babesiosis*, is of major national and international importance and imposes huge economic burdens on the beef and dairy industries. A better understanding of the basic biology of these parasites and the relationship between parasites and their host is urgently required for the development of anti-parasitic vaccines, drugs and new therapeutic regimens for this important disease. The new therapeutic regimens arising from our research to control bovine babesiosis have potential for research translation in many countries worldwide. New control measures for bovine babesiosis will play a crucial role in improving animal welfare and the livelihood of livestock farmers globally. We are also interested in learning more about the basic biology of this parasite since it offers a unique opportunity to answer important questions about malaria infection that are not currently possible to perform in humans.

Characterisation of novel *Babesia* parasite proteins and the discovery of new vaccines against tick fever

**Professor Brian M. Cooke, Dr Vignesh Rathinasamy and Dr Carlos Suarez, (United States Department of Agriculture)**

The mechanisms by which *Babesia bovis* causes severe disease in susceptible cattle are not well understood; however, it is clear that alterations to the structure and function of infected RBCs, secondary to the export of currently uncharacterised parasite-encoded proteins, play a critical role. Using a rational bioinformatic analysis of the genome sequence of *B. bovis*, we have identified a subset of parasite proteins that we predict will be exported from the parasite into the host RBC and play a major role in host cell modification. In addition, a subset of *B. bovis* exported proteins share an orthologue in *B. bigemina* and evaluation of these proteins provides an opportunity to use the same vaccine candidates to control both *B. bovis* and *B. bigemina*. The overall aim of this project is to characterise these novel exported proteins by determining their localisation within PRBCs and ultimately elucidating their function and evaluation as vaccine targets in cattle. A combination of bioinformatic, molecular, cellular and proteomic approaches will be employed in order to shed some light on the mechanisms by which these parasites induce dramatic changes to the infected RBC. A complete analysis of the parasites’ ‘exportome’ will result in a better understanding of the pathogenesis of babesiosis and facilitate the identification of new vaccine targets to combat bovine babesiosis.

Identification of the *Babesia bovis* ridge protein

**Professor Brian M. Cooke, Dr Vignesh Rathinasamy and William Poole**

Shortly following invasion, *Babesia* induces the formation of unique structures on the RBC surface, which we have termed ‘ridges’. Importantly, the appearance of ridges appears to correlate with the level of parasite virulence and are believed to be the structures responsible for the binding of infected RBC to endothelial cells therefore mediating the ‘vasculature-blocking’ phenotype of the parasite. The protein responsible for the formation of these ridges on the cell surface is currently unknown. In this project we will use a combination of molecular, biochemical and proteomic approaches as well as transgenic parasites to identify the ‘ridge’ protein. Elucidation of this protein, and ultimately its function in the infected RBC, will give us a better understanding of the pathogenesis of babesiosis.
Malaria is one of the most devastating infectious diseases worldwide, causing major public health, social and economic problems globally. The lack of an efficient vaccine and the alarming emergence of resistant parasites to currently available drugs urgently call for the identification of new treatment strategies.

Protein kinases are prominent drug targets in cancer and represent highly interesting targets for malaria drug development as well. Our laboratory is a world-leader on the study of functional kinomics of the human malaria parasite *Plasmodium falciparum*. Unravelling the biological functions of protein kinases of the host cell and of the parasite during infection will inform on specific potential targets for antimalarial drug discovery.

In the context of our work on the kinomics of erythrocytes infected with *P. falciparum*, we made the surprising observation that infection with *P. falciparum* activates a signalling pathway involving p21-activated kinase (PAK) and MAP/ERK kinase (MEK1) of the host erythrocyte, and that this is required for parasite survival. This prompted us to implement a system-wide approach to assess the modulation of signalling pathways of the host cell by intracellular pathogens. A comprehensive antibody microarray detected dynamic variations in the expression levels and phosphorylation status of host cell signalling proteins during the *P. falciparum* asexual cycle in erythrocytes.

We provided proof of concept that selective small molecule-mediated inhibition of host cell kinases that are among the targets identified by the antibody microarray approach does indeed impair parasite proliferation. This opens the way for host-directed therapy (HTD), a strategy that would limit the emergence of resistance (since the target is not encoded by the parasite).

The first proposed project relates to this strategy, while the second one addresses the function of an intriguing *Plasmodium*-encoded secreted kinase.

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**The kinomics of malaria erythrocytic infection**

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PROJECT 1: Hijacking of red blood cell kinases by *P. falciparum*

The infection of erythrocytes by *P. falciparum* causes major changes in the host cell. We have recently discovered that a range of host erythrocyte signalling proteins are activated and hijacked by the parasite, and that chemical inhibition of the human MEK and PAK kinases kills parasites. This discovery opens a new approach for antimalarial drug development, namely targeting human proteins. Given that MEK and PAK inhibitors are currently being developed as anticancer drugs, we propose that such compounds could be repositioned as potential antimalarials.

Little is known about why red blood cell kinases are essential for the parasite, or how *Plasmodium* activates kinases in the red blood cell. This project will combine the aforementioned antibody microarray approach with molecular (mutagenesis, cloning), biochemical (Western Blots, recombinant protein assays), cellular (fluorescence microscopy, tissue culture, flow cytometry) and proteomic (pull-downs, mass spectrometry) techniques to identify how red blood cell kinases are activated by *Plasmodium* infection, and will test a range of potential downstream effectors within the parasite.

You will acquire a wide range of techniques and your studies will contribute to a better understanding of the role human kinases play in *Plasmodium*.

PROJECT 2: Secreted IRAK-like kinase of *P. falciparum* – a modulator of immune response?

Exacerbated immune responses play a major role in malaria pathogenesis. Many other pathogens are known to produce molecules that modulate the signalling events regulating response by cells of the immune system. We have recently discovered that red blood cells infected with the malaria parasite secrete parasite-encoded protein kinases into the extracellular medium. Intriguingly, one of the secreted enzymes, called PfTKL2, belongs to the IRAK family, a group of kinases that regulate the innate immune response to pathogens. Our preliminary data suggest that secreted PfTKL2 associates with microparticles, produced by infected erythrocytes; microparticles are known to deliver proteins to bystander immune and endothelial cells.

The hypothesis that the project aims to test is that secreted PfTKL2 modulates the human innate response to infection by interfering with signalling in bystander immune and endothelial cells.

The aims are:

1. to determine whether microparticles deliver PfTKL2
2. to determine whether PfTKL2 affects signalling in human immune and endothelial cells

The approaches will include tissue culture techniques (transwell experiments), molecular cloning (genetic manipulation of *P. falciparum* to implement reverse genetics for PfTKL2), and immunological techniques to phenotype the immune response. If the central hypothesis is confirmed, this will be a major development in malariology and will give opportunities for high impact publications and translational research into innovative malaria control strategies.
The research projects described here will be conducted using the facilities at the Centre of Biospectroscopy (CfB) directed by me and Associate Professor Bayden Wood from the School of Chemistry. The CfB, housed on the third floor of the new Green Chemical Futures building, is the best equipped laboratory of its kind in the Southern Hemisphere. The CfB is a world recognized centre of excellence in this area of research and collaborates on interdisciplinary research projects with the Doherty Institute, the Burnet Institute, the Walter and Eliza Hall Institute, the Alfred Hospital and Monash Health. It has developed a number of medical diagnostic devices for detecting bloodstream infections and is currently testing these in a number of clinical pilot trials in conjunction with commercial backers. We are most concerned with diagnostics that target diseases of greatest burden in the developing world such as malaria, HIV and hepatitis.

Biospectroscopy studies the interaction of light with biological tissues, cells and body fluids. Vibrational spectra can be obtained that provides a spectral “fingerprint” of all the major macromolecules (proteins, nucleic acids, lipids, carbohydrates) in the biological material in a snapshot and hence defines the compositional phenotype of the sample. Multivariate data analysis and artificial intelligence systems are used to model and recognize the molecular phenotypes. For example: biospectroscopy methods can reliably identify bacteria down to the strain level and is routinely used in a number of European hospitals; malaria infected blood samples can be distinguished from uninfected ones with very high sensitivity and specificity; the presence of hepatitis viruses in blood serum can be readily detected. What is being developed here are diagnostic approaches akin to ‘Dr McCoy’s tricorder’ in the science fiction series ‘Star Trek’, where light is passed through a diseased sample and the disease is diagnosed immediately and directly without any change to the sample. The approach is truly revolutionary for the medical sciences!

Please do not feel intimidated if you do not have a chemistry and mathematical science background and would like to share in the research conducted by the CfB. Many of our students come from a purely biomedical background! You do not need to be a chemist to understand the spectroscopic signatures that we examine and because we employ developed software packages to analyse and model the data you don’t need to be a mathematician either!

**PROJECT 1**

**Dr Philip Heraud, A/Professor Bayden Wood and Professor Anton Peleg**

Resistance to antimicrobial drugs is a key issue in medicine with the rise of so-called ‘superbugs’ that are resistant to most or all antibiotics and are commonly observed in hospital settings threatening the lives of sick people. Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most common organisms found in hospital and community infections, resistant to methicillin and all β-lactam antimicrobials. Vancomycin is a drug of choice for the treatment of serious MRSA infections; however, recently a number of vancomycin non-susceptible *S. aureus* strains have been reported because of the overuse of this drug.
Currently, point-of-care detection and characterization of methicillin and vancomycin resistant *S. aureus* strains is not available. The state of the art detection methods involve characterization by mass spectroscopy (MALDI-TOF); however, this relies on amplification of bacterial numbers from a serum sample using blood culturing methods taking at least 12 h to achieve. The delay in detection means that appropriate treatment is delayed, which can be critical in terms of patient outcomes.

Infrared spectroscopy (IRS) and Raman spectroscopy (RS) present as possible solutions in this area. Preliminary studies indicate that IRS and RS can provide a molecular fingerprint that is very sensitive to changes in phenotype related to antimicrobial resistance in *S. aureus*.

This project would explore whether different methicillin and vancomycin resistance strains can be detected and classified using IRS and RS.

**PROJECT 2**

*Dr Philip Heraud and Professor Christian Doerig*

Malaria was responsible for almost half a million deaths in 2015, the majority of which were children under 5 years old. The disease feeds into the cycle of poverty by reducing the growth of GDP by up to 1.3% per annum in some African countries. Increasing resistance to front line antimicrobials used to treat malaria is posing a real threat that could worsen this situation dramatically. Many potential anti-malarial drugs are known, such as the “malaria box” of 500 drugs with known anti-malarial action being offered up by Glaxo Smith Cline company for research investigations. Unfortunately, the pace of development of new anti-malarials is slowed by the lack of knowledge of the precise mechanisms of action of even commonly employed agents such as chloroquine.

This project would use infrared spectroscopy to study the phenotypic response of the malaria *Plasmodium* organism to a number of antimalarial drugs with known and different modes of action. The aim would be to discover phenotypic signatures related to the different modes of action of the various drugs. If this is possible a drug discovery platform could be developed for rapid testing of potential new drugs that can classify them in terms of their modes of action thus speeding the development of new anti-malarial drugs.
The molecular mechanisms by which *Helicobacter pylori* causes stomach cancer

*Helicobacter pylori* is a Gram-negative gastric bacterium that has co-evolved with humans for more than 50,000 years. It colonises the stomach of over 50% of the world’s population, making it one of the most prevalent human pathogens. It is a causative agent of severe gastric diseases including chronic gastritis, peptic ulcer and stomach cancer. *H. pylori* has been classified as a Group I (high-risk) carcinogen.

Highly virulent strains of *H. pylori* harbour a type IV secretion system (T4SS), a secretion machinery that functions as a “syringe” for injecting virulence proteins and peptidoglycan into the host cell. We discovered that CagL, a specialised adhesin present on the surface of the *H. pylori* T4SS, binds to the human integrin α5β1 receptor on stomach lining cells. This binding activates the T4SS and hence the secretion of virulence factors including the highly immunogenic and oncogenic protein, CagA, into stomach cells. ‘Injected’ CagA then interacts with host signalling molecules and triggers activation of a suite of host responses. Interestingly, our recent findings suggest that CagL can also directly modulate host cell functions. The precise mechanisms by which CagL functions both as a host-activated sensor of the *H. pylori* T4SS and as a direct activator of aberrant host responses remain to be fully understood.

Our team uses multi-disciplinary state-of-the-art approaches to study the molecular mechanism of *H. pylori* type IV secretion and *H. pylori*-host interactions. We aim to understand the molecular basis of how *H. pylori* induces stomach cancer, with the ultimate goal of providing knowledge for a better treatment and/or prevention of *H. pylori*-associated stomach diseases. Projects are available to address the following key questions:

- How does *H. pylori* trigger inflammation and carcinogenesis through the virulence functions of CagL and CagA?
- Can cagL and cagA genotypes predict gastric cancer risk and therefore help pinpoint cancer-prone patients for early treatment?
- How do CagL function as a host-activated sensor during type IV secretion?
- How does CagL and CagA modulate host cell signalling during chronic *H. pylori* infection?
- Can we utilise the type IV secretion of *H. pylori* for delivery of therapeutic proteins?

The available honours projects will enable one to gain experience with the important techniques of molecular cloning and mutagenesis, bacterial culture, eukaryotic cell culture techniques, mouse infection models, CRISPR, RNAi, immunostaining, Western blotting, ELISA, confocal laser scanning microscopy, live cell imaging, etc. Someone who is enthusiastic in learning about the exciting secrets of bacteria-host interactions, infectious cancer biology and bacterial pathogenesis is strongly encouraged to apply.
My lab focuses on re-development of ‘old’ polymyxins as the last-line therapy against Gram-negative ‘superbugs’ (namely *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*). There has been a marked decrease in the discovery of novel antibiotics over the last two decades. As no novel class of antibiotics will be available for Gram-negative ‘superbugs’ for many years to come, it is crucial to optimise the clinical use of ‘old’ polymyxins using systems pharmacology and to develop novel, safer polymyxins. My major research programs include: (1) optimising clinical use of polymyxins and their combinations using pharmacokinetics/pharmacodynamics/toxicodynamics (PK/PD/TD) and systems pharmacology; (2) elucidation of mechanisms of antibacterial activity, resistance, and toxicity of polymyxins; and (3) discovery of novel, safer polymyxins against multidrug-resistant (MDR) Gram-negatives. My lab is funded by the US National Institutes of Health (NIH) and Australian NHMRC.
Elucidating the polymyxin resistome of Gram-negative ‘superbugs’

Professor Jian Li

*A. baumannii* is a clinically important bacterial pathogen that can cause pneumonia, bacteraemia and meningitis. Many clinical isolates are multidrug-resistant and polymyxins are a last-line treatment for life-threatening infections caused by MDR *P. aeruginosa*, *A. baumannii* and *K. pneumoniae*. This project will employ targeted transposon mutant screening to identify genes important for survival during polymyxin treatment. Our approach combines the latest high-throughput sequencing techniques with traditional molecular biology methods to screen the entire bacterial genome. The results of this study are of significant importance for the understanding of polymyxin mode of action, and the pursuit of novel antimicrobial targets.

Pulmonary toxicity of novel polymyxin combination therapies

Dr Mohammad Azad and Professor Jian Li

Current dosing recommendations of parenteral polymyxins are suboptimal for treatment of respiratory tract infections due to poor drug exposure at the infection site. Moreover, nephrotoxicity is the dose-limiting factor and can occur in up to 60% of patients. Pulmonary delivery of polymyxins in combination with other antibiotics has offered a great promise for bacterial eradication in the respiratory tract. Unfortunately, there is a lack of experimental data on the pulmonary toxicity of polymyxins and their combinations with other antibiotics. This multi-disciplinary project aims to investigate the effect of polymyxins and their combinations with another five key classes of antibiotics on human lung epithelial cells, using fluorescence activated cell sorting (FACS), metabolomics and cutting-edge imaging techniques. The outcome of this project will provide the much-needed pharmacological information for safer and more efficacious use of polymyxin inhalation therapy against life-threatening lung infections.
Molecular mechanisms of bacteriophage resistance

Dr Jeremy Barr and Professor Trevor Lithgow

By 2050 antimicrobial-resistant (AMR) infections will kill >10 million people every year. While new antibiotics will help slow this trend, the discovery of adjunct treatments that will not further promote AMR is essential if we are to avoid the devastating impact of AMR superbugs. Our collaborative research projects are aimed at characterization of adjunct treatments including bacteriophage therapy and the repurposing of drugs that promote immune function: bacteriophages and immune cells do not discriminate between antimicrobial-resistant and antimicrobial-sensitive bacteria, they kill both.

Unlike antibiotic resistance, which can be broadly mobilized and transferred across species, the development of phage resistance is unique to the specific phage and bacterial host. If bacteria become phage-resistant it comes at a significant fitness cost including reduced virulence, growth, and increased antibiotic-susceptibility. Thus, phage treatment of multidrug-resistant bacterial pathogens both reduces bacterial carriage and selects for phage-resistant bacteria that will be less fit, virulent and in specific cases re-sensitized to antibiotics. There is a limited understanding of the underlying molecular mechanisms and repeatability of phage-resistance evolution, so the aim of this project will be to isolate and characterize novel phages targeting multidrug-resistant Enterobacteriaceae spp., including Escherichia coli and Klebsiella pneumoniae. These initial studies will be followed by phenotypic and molecular characterizations of phage-resistant bacterial mutants. This project will involve the use of molecular technologies, microbial culturing, phenotyping and sequencing.
Targeting host factors in bacterial infections

Dr Thomas Naderer and Professor Trevor Lithgow

Superbugs are not only resistant to current antibiotics but they are also highly effective in evading immunity. This causes several human diseases that are increasingly difficult to treat. Thus, there is an urgent need to develop alternative approaches to antibiotic therapy. Rather than killing the bacteria, targeting host-factors that promote pathogen survival has emerged as a promising strategy in infectious diseases. To develop this further we need a better understanding about how superbugs evade immunity on the molecular and cellular levels.

To identify new host-pathogen interactions we follow infections by live-cell imaging. This enables the identification of host cell responses on the single cell level in a high-temporal resolution. In addition, we employ super-resolution imaging to uncover how pathogens target host factors in immune cells. Screening host genome libraries identifies the host factors that enable superbugs to survive immune attack. This has led us to new therapeutic approaches by re-purposing existing drugs to kill infected host cells (Speir et al, Nature Micro, 2016).

The aim of the project is to identify host factors that enable the superbugs *Staphylococcus aureus* and *Neisseria gonorrhoeae* to evade innate immunity and to cause disease. For this, a whole genome CRISPR library will be screened to identify mutant immune cells that resist bacteria mediated killing. Identified genes will be further validated in infections that depend on transgenic stem-cell derived human immune cells. This will utilize live-cell imaging, super-resolution imaging, immunological and biochemical assays with the aim to characterize new host-pathogen interactions.
Understanding the host immune response to *Clostridium difficile* infection

**Professor Dena Lyras, A/Professor Helen Abud and Dr Steven Mileto**

*Clostridium difficile* is recognised as the major cause of nosocomial diarrhoea in Australian hospitals and in hospitals worldwide. Chronic colitis syndromes caused by this organism are a significant cause of morbidity in hospitals with control and treatment costs rapidly escalating. The recent emergence of hypervirulent strains has increased the severity of disease and hence the urgency with which the mechanism of disease needs to be understood. The pathogenesis of *C. difficile*-associated diseases involves the production of numerous toxins and other virulence factors. We have developed a mouse model of infection which closely mimics human infection. This project will use the mouse model of *C. difficile* infection to assess the host immune response to *C. difficile* infection, in particular using specific mutants of clinically relevant *C. difficile* strains. Our primary focus will be on the ability of *C. difficile* toxins to modulate specific immune responses during disease and we will extend these studies into a broader exploration of the pathways involved downstream of these responses, including the effect of *C. difficile* infection on repair capacity of the gut and stem cells.

Genetic and phenotypic analysis of pathogens in antibiotic-associated diarrhoeal disease

**Professor Dena Lyras and Dr Grant Jenkin**

The treatment of bacterial infections in humans and animals has largely relied on the use of antibiotics for over 70 years. One consequence of the use of these drugs is antibiotic resistance, which is now one of our most serious health threats worldwide. Another complication is antibiotic-associated diarrhoea (AAD), which results from the unintended disruption of the protective resident gut microbiota. This disruption can lead to opportunistic infection, subsequently leading to diarrhoeal disease. Using a multidisciplinary approach, and with the involvement of clinical colleagues, this project aims to gain new insights into the mechanisms of pathogenesis and the subversion of host processes by AAD-causing bacteria. Animal models of infection will be used, together with specific mutants, to study virulence factors and host interactions, allowing us to gain a mechanistic understanding of how these bacteria interact with, and damage, the host.
Analysis of toxin secretion in the large clostridial toxin (LCT) producing clostridia
Professor Dena Lyras, Dr Sheena McGowan and Dr Milena Awad

The LCT-producing clostridia are an important group of pathogens that cause severe disease in both humans and animals. In most cases the diseases caused by these organisms are at least partly mediated by the production of potent exotoxins known as the large clostridial toxins (LCTs), which are mono-glycosyltransferases that irreversibly inactivate members of the Rho family of small GTPases. These toxins are structurally related and reside within a genomic region known as the Pathogenicity Locus (PaLoc) that also encodes several accessory proteins thought to be involved in the control of toxin production and secretion.

None of the LCTs have any recognisable signal peptides or export sequences, suggesting that they are secreted by a novel mechanism, potentially involving holin-like proteins. The aim of this project is to further define the mechanism by which the LCTs are secreted from the cell. This will be achieved through the construction of isogenic mutants in the LCT-producing clostridia and subsequent phenotypic testing, as well as through the purification and testing of proteins hypothesised to be involved in the export process.

Understanding the role of bacterial structures in the transfer of antibiotic resistance genes during conjugation
Professor Dena Lyras, Dr Yogitha Srikhanta and A/Professor Priscilla Johanesen

Antibiotics are a precious and diminishing resource. There is a desperate need to reduce or replace the use of antibiotics to treat bacterial infections, which is an important veterinary and medical pursuit in this new age of antibiotic-resistant “superbugs”. The treatment of bacterial infections in animals and humans has relied on the use of antibiotics. One consequence of the use of these drugs is antibiotic resistance, which is now one of our most serious global health threats. Bacteria can become resistant to antibiotics through lateral gene transfer of resistance genes, which are often located on mobile elements such as plasmids and transposons. This project will focus on one such mechanism, conjugation, which is a process by which one bacterium transfers genetic material to another through direct cell-to-cell contact. Apart from the conjugation apparatus, very little is known about the role that bacterial structures play in conjugation. Here, we will examine the role of numerous structures in DNA transfer efficiency using molecular technology and microbial genetics, which may provide new targets and strategies through which the transfer of antibiotic resistance genes may be prevented.
Understanding Human Cytomegalovirus assembly and egress

HCMV is a herpesvirus that infects over 60% of the adult population. HCMV is a significant cause of morbidity and mortality in immuno-compromised individuals, such as organ transplant recipients. Additionally, the largest burden of disease occurs from intrauterine transmission during pregnancy. This occurs in greater than 1% of pregnancies worldwide, and can cause permanent hearing loss, vision impairment, and mental retardation. There is no vaccine currently available, and discovery of new antivirals is urgently required. Importantly, the process by which infectious virus is packaged and released is not well understood, and this presents a novel molecular axis to develop antiviral therapeutics.

Research Strategy

Research in our laboratory uses a multidisciplinary approach to better understand host defense mechanisms, and identify cellular pathways that are hijacked by HCMV. We work at the interface between cell biology, virology and quantitative proteomics, and have proprietary virus libraries and reagents to make unique discoveries. All projects have an opportunity to learn standard (tissue culture, western blotting, immuno-precipitation, confocal microscopy, RNAi, CRISPR) and advanced (liquid chromatography, mass spectrometry, bioinformatics, electron microscopy) laboratory techniques and skills.

Biogenesis of the HCMV viral assembly complex

Dr Rommel Mathias and Dr Svenja Fritzlar

HCMV is a large double-stranded DNA virus whose 236 kbp genome is known to code for at least 150 proteins. The HCMV virion comprises a nucleocapsid that houses the DNA genome, and is surrounded by a proteinaceous tegument layer, and glycoprotein-containing lipid envelope. During infection, exactly how the virion is assembled and released (egress) remains unknown. However, infection causes extensive organelle remodeling in infected cells (see image above), and produces a structure known as the viral assembly complex (vAC). It is currently thought that the vAC facilitates virion assembly and maturation.

We have a library of mutant viruses that contain a transposon to disrupt each of the 150 open reading frames in the HCMV genome. We use these mutant viruses to identify the essential viral proteins needed to generate the vAC. Using a confocal microscopy-based assay, we screen for defects in Golgi ring, endosome clustering, and secondary envelopment of the virion. Ongoing efforts in the lab have revealed nine candidate viral proteins that regulate secondary envelopment. Many of these are novel viral proteins with uncharacterized functions that await further investigation.
Hijacking of host exosome pathways by HCMV

Dr Rommel Mathias and Dr Yea Seul Shin

HCMV is a master at manipulating existing host pathways to benefit completion of the life cycle. It is known that maturing nucleocapsids bud into host membrane-derived structures to acquire the outer virion envelope. However, the origin of the membrane is unknown, and the precise molecular mechanisms remain elusive. In our lab, proteomic sequencing identified a strong enrichment of host exosome proteins in the virion. Exosomes are small nanovesicles (50-200 nm) secreted by almost all cell types. Therefore, we hypothesize that HCMV hijacks this pathway for viral egress.

We use CRISPR to knock-out various host exosome proteins in cells, and measure the functional impact to virions by assaying viral titre released from these ‘edited’ cells. Incorporation of host exosome proteins into infectious virions is also validated by immuno- gold EM. Thus far, our investigations have unearthed the tetraspanins as a family of host proteins that facilitate virion assembly, and will be explored further.
The McGowan laboratory is interested in characterising new drug targets. The lab has a strong research focus in the design of novel anti-malarial drugs as well as other parasitic and bacterial diseases. Primarily we are a structural microbiology laboratory using techniques in protein crystallography, biochemistry and molecular biology to analyse drug targets of interest. We use this mechanistic information to design inhibitors or analogues with potential applications in human medicine. The laboratory has close connections with both the Department of Biochemistry and the Monash Institute of Pharmaceutical Sciences (in Parkville).

The general projects are outlined below and interested students are encouraged to contact Sheena with any questions or to discuss further.

The malaria parasite *Plasmodium falciparum* employs metallo-aminopeptidase enzymes (*Pf*MAP) that are required for parasite survival. Three of these enzymes, M1, M17 and M18, are validated and attractive drug targets. Agents that inhibit the activity of these enzymes thus represent leads for the development of new anti-malarial drugs. These project(s) will develop detailed structural and functional models of the mechanism of action of the aminopeptidases and use this information in the design and development of dual and triple *Pf*MAP inhibitors as novel pre-clinical drug candidates.

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**Development of Phage Lysins as Novel Antimicrobials**

*Dr Sheena McGowan*

The growing problem of antibiotic resistance underlies the critical need to develop new treatments to prevent and control resistant bacterial infection. Exogenous application of bacteriophage lysins to dormant and actively growing cell cultures results in rapid cell death. Understanding the mechanism of action will allow the development of lysins as a next generation antimicrobial agent.

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**Development of New Drug Targets for Malaria**

*Dr Sheena McGowan and Dr Nyssa Drinkwater*

The malaria parasite *Plasmodium falciparum* employs metallo-aminopeptidase enzymes (*Pf*MAP) that are required for parasite survival. Two of the *Pf*MAPs remain uncharacterised. This project will investigate the structure and function of the two Prolyl aminopeptidases identified in *P. falciparum*. The development of detailed structural and functional data will allow development of novel inhibitors of these enzymes and assessment of their suitability as drug targets.
Viruses pose one of the grand challenges to human and animal health globally and within Australia. Viral disease progression is critically dependent on the formation of specific interaction networks between viral proteins and host cell factors, which enable viral subversion of important cellular processes such as antiviral immunity and cell survival. We use advanced cellular/molecular biology approaches including quantitative proteomics, structural biology, functional genomics, immune signalling assays, and live-cell/super-resolution imaging to elucidate these interactions at the molecular and cellular level, and viral reverse genetics and in vivo infection models to define their functions in disease.

Our major focus is on highly lethal human viruses including rabies, Australian bat lyssavirus, Nipah, Hendra, and Ebola, as well as a number of agriculturally significant and potentially zoonotic animal viruses. The overarching aim of the research is to identify novel targets and strategies for the development of new vaccines and therapeutics for currently incurable viral diseases.

The research involves extensive collaborations within Monash University and other leading national (e.g. University of Melbourne, CSIRO-AAHL high-containment facility) and international institutes (e.g. Pasteur Institute and CNRS, Paris; Gifu and Hokkaido Universities, Japan; Dundee University (UK)), enabling access to unique resources and technologies including novel and highly pathogenic viruses.

Elucidating the Rabies Virus P protein Axis

Dr Greg Moseley and Dr Celine Deffrasnes

Rabies is a currently incurable disease that has the highest fatality rate of known infectious diseases. The etiological agents of rabies are lyssaviruses, such as rabies virus and Australian bat lyssavirus. Despite a very limited genomic capacity these viruses are able to mediate replication, assembly and budding, while simultaneously arresting potent control over the infected cell and host immune system. Central to this is the expression of multifunctional proteins including P protein, which resides at the core of the virus-host interface where it forms a myriad of interactions with viral and host proteins. We showed that by inhibiting such interactions, we can prevent otherwise invariably lethal rabies disease, identifying the P protein ‘axis’ as a therapeutic target. However, the molecular/structural mechanisms by which this small protein coordinates/regulates its diverse interactions remain unresolved, leaving major gaps in knowledge concerning fundamental processes in a lethal human disease.

The project will seek to define the specific molecular surfaces mediating key interactions of P protein, and to analyse their function using mutagenesis. This will contribute to the elucidation of the structural organisation and regulatory mechanisms of the virus-host interface and help to define novel mechanisms by which viruses efficiently co-regulate host cell subversion and replication. These findings have the potential to redefine our understanding of the relationship of viruses and their hosts, and to provide critical tools and data for the development of new vaccines and antivirals.
Viral reprogramming of host cell signalling

Dr Greg Moseley and Dr Celine Deffrasnes

Central to the spread of pathogenic viruses is their capacity to interfere with host immunity, in particular the antiviral system mediated by cytokines such as the interferons. It is well known that many viruses target signalling by antiviral type I interferons to shut down the expression of interferon-stimulated genes. However, our recent work has indicated that the interaction of viruses with cytokine signalling pathways is much more complex and intricate than previously assumed.

In particular, we and our collaborators have found that rabies virus, the cause of c. 60,000 human deaths/year, interacts with multiple signalling pathways, including those initiated by interleukin-6 and interferon-a/ß using a number of mechanisms including viral interactions with and remodelling of cellular structures of the cytoskeleton and nucleus. Importantly, using mutagenic analysis and viral reverse genetics, we found that altering viral targeting of these pathways profoundly inhibits pathogenesis in vivo, indicative of critical roles in disease.

We are currently seeking to delineate the precise mechanisms by which viruses interfere with and modulate cellular pathways, not only to inhibit antiviral signalling, but also to reprogram specific signalling pathways toward ‘pro-viral’ responses, a novel concept in viral biology.

Can rabies cure Alzheimer’s?

Dr Greg Moseley and Dr Celine Deffrasnes

Neuroinflammation is a major factor in human pathologies such as stroke, Alzheimer’s disease (AD), and traumatic brain injury (TBI). Viruses such as the lyssaviruses rabies and Australian bat lyssavirus, paramyxoviruses Nipah, Hendra and measles, and the filovirus Ebola, have evolved powerful mechanisms to shut down inflammatory signalling as part of their strategies for immune evasion. We aim to discover the molecular ‘tricks’ used by viruses to subvert host immunity, and to exploit these mechanisms to develop new methods to efficiently prevent the inappropriate immune responses underlying neuroinflammatory disorders.

We have made major advances in understanding how viruses achieve immune evasion, including defining the specific virus-host interactions involved, and the molecular basis of these interactions. Using this knowledge, and established models of stroke, TBI, AD and Parkinson’s disease, the project will investigate the potential of harnessing viral immune evasion to combat immune disorders.

Super-resolution Analysis of the Virus-Host Interface

Dr Greg Moseley and Dr Toby Bell

Viruses are experts at remodelling the infected cell, and can fundamentally alter cellular biology to transform host cells into efficient virus factories. Although molecular/biochemical evidence indicates that certain viral proteins can functionally modify structures such as the mitochondria, cell membranes, nucleus, and cytoskeleton, understanding of the physical effects on these structures is limited due to the poor resolving power of standard cell imaging approaches. Using single molecule localization techniques to surpass the physical diffraction limit of visible light, we have developed methods to observe and quantify the effects of viral proteins on cellular structures at super-resolution, enabling us to directly measure viral remodelling of the subcellular environment. Using this approach, we demonstrated that virus protein targeting of the cytoskeleton correlates with the capacity to cause lethal disease in vivo. The project will apply state-of-the-art single molecule localization techniques such as 3D dSTORM to define viral effects on cellular structures in unprecedented detail; this will provide new insights into the ways that viruses co-opt cellular function to cause disease.

Why do cytoplasmic RNA viruses target the nucleolus?

Dr Greg Moseley and Dr Stephen Rawlinson

Many diverse viral proteins have evolved independently to target the nucleolus but this phenomenon had been largely overlooked, particularly for RNA viruses that replicate within the cytoplasm. Following the development of advanced ‘systems-biology’ approaches to analyse nucleolar biology, it has become clear that the nucleolus, previously viewed solely as a factory for ribosome production, is in fact a complex, dynamic, and highly multifunctional machine that coordinates many critical cellular processes including immunity and cell survival. This has redefined our understanding of the nucleolus and suggests that the virus:nucleolar interface might represent a central hub for viral hijacking of cellular processes, important to viral replication and pathogenesis.

Using nucleolar proteins from the highly pathogenic RNA viruses rabies virus and Hendra virus, we are investigating in molecular detail the mechanisms by which viruses can reprogram the nucleolus to alter cellular biology. These studies are identifying for the first time specific nucleolar functions for RNA virus proteins. The project will advance this work, utilizing techniques including molecular biology, proteomics, confocal/super-resolution microscopy, virus replication and gene expression assays, and siRNA/CRISPR/Cas gene knockout approaches to delineate the precise events underlying cellular dysfunction caused by virus-nucleolus interaction. [This project will be in collaboration with the CSIRO-AAHL PC4 high-containment laboratories in Geelong.]
MECHANISMS OF PATHOGENESIS OF HOSPITAL-ACQUIRED ORGANISMS

Impact of antibiotic resistance on immune recognition of *Staphylococcus aureus*

Dr Jhih-Hang Jiang and Professor Anton Y. Peleg

*S. aureus* is one of the most common human bacterial pathogens, and is able to cause a wide range of life-threatening infections in the community and hospital setting. As a consequence of the rising rates of methicillin-resistant *S. aureus* (MRSA), agents such as vancomycin and daptomycin have been increasingly relied upon. Unfortunately, reduced susceptibility to these agents has now emerged. By using large-scale, whole-genome sequencing of clinical *S. aureus* isolates, whereby the first isolate is susceptible and the paired isolate is non-susceptible, we have been able to describe the genetic evolution of antibiotic resistance in patients. Interestingly, we have also identified, using both mammalian and non-mammalian model systems that these resistant strains have altered host-pathogen interactions, and appear to be more persistent.

PROJECT 1

The aim of this project is to characterise the mechanisms of MRSA adaptation and evasion to antibiotic and host innate immune attack. The work will comprehensively identify genetic mutations that confer a survival advantage to MRSA under daptomycin pressure in the context of an immune response. This will be achieved by exposing clinically relevant MRSA strains to both antibiotic and host immune selection pressure, and apply a novel sequencing approach to characterise the full repertoire of mutations in specific phospholipid biosynthesis genes known to be important for antibiotic resistance. The significance of the identified mutations will be assessed by making independent mutants using our well-developed targeted mutagenesis system. The impact of individual mutations on antibiotic resistance, staphylococcal virulence, bacterial membrane biogenesis and host immune responses, will be assessed. This project will combine exciting bacterial genetic techniques and advanced biochemical approaches together with novel infection model systems.
PROJECT 2

In collaboration with Professor Meredith O’Keeffe (Dept. of Biochemistry)

The aim of this project is to characterise the activation of pathogen recognition receptors by our paired susceptible and resistant clinical isolates. This will be achieved by studying one of the key first responders of our immune system; dendritic cells. Different dendritic cell types differ in their expression of pattern recognition receptors and hence the types of pathogens that they recognise. They also differ markedly in their subsequent innate response to pathogens, with discrete dendritic cell subsets specialised in the production of different cytokines and interferons. This project will focus on the differences in pathogen recognition and the subsequent immune activation between clinically important and drug-resistant S. aureus strains. Using established S. aureus mutants, we will also determine the impact of changes in bacterial surface characteristics on activation of pathogen recognition receptors.

Characterising novel virulence mechanisms in the emerging hospital-acquired pathogen; Acinetobacter baumannii

A/Professor John Boyce, Dr Faye Morris and Professor Anton Peleg

Small RNA (sRNA) molecules play important roles in the regulation of a wide range of bacterial phenotypes including virulence. Together with Dr Gerald Murray we have previously determined which A. baumannii sRNA molecules are expressed in vivo during a mouse infection model. We predict that sRNAs expressed at high levels in vivo will have a role in regulating A. baumannii virulence factors. We have generated an assortment of individual sRNA mutants and in this project, we will select those that are highly expressed in vivo and complement the mutants by generating individual constructs expressing the relevant sRNA from different promoters. By comparing our repertoire of strains (ie sRNA mutants, complements and overexpression strains) we will analyse the effects on a range of virulence associated phenotypes (growth in human serum, biofilm formation, and mouse infection models), with a view to identify and confirm the sRNA specific targets using high-throughput proteomics and RNA sequencing of sRNA-mRNA duplexes. Where inactivation of an sRNA affects virulence, we will design and construct sRNA inhibitors and test these as novel antimicrobials.

Note: Working with animals is not compulsory for any of the advertised projects.
**Interaction between two-component systems in *Clostridium perfringens***

**Dr Jackie Cheung and Professor Julian Rood**

*C. perfringens* is the Gram-positive, spore-forming causative agent of clostridial myonecrosis and food poisoning. Since these diseases are toxin-mediated the elucidation of the mechanism by which toxin production is regulated is of significant importance. Previous studies show that the production of many toxins in *C. perfringens* is regulated by two-component signal transduction systems and recent work in this laboratory has shown that the novel RevSR two-component regulatory system is involved in controlling the virulence of *C. perfringens*. Our current studies indicate that the RevSR system may interact with other two-component systems in the cell to regulate the expression of toxins or other potential virulence factors that are important in the disease process. The aim of this project is to expand our knowledge of the RevSR network by attempting to identify two-component systems that interact with the RevS sensor histidine kinase or the RevR response regulator. New interacting partners of RevSR will be identified using the bacterial two-hybrid system and further analysed with bio-layer interferometry and surface plasmon resonance. This project will involve the use of molecular biology, microbial genetics and protein biochemistry.
Dissecting architecture of high torque bacterial motor

The bacterial flagellar motor is a remarkable nanoscale molecular engine. *H. pylori* evolved to be highly motile in the very viscous mucous layer of the stomach, and its flagellar motor is specialised for locomotion in viscous liquids – it produces a significantly higher torque (turning force) than, for example, enteric bacteria. Preliminary cryo-electron tomography reconstruction of this motor revealed a unique protein cage that supports a wider power-generating ring allowing it to sustain the larger torque. Our aim is to unravel the make-up of this cage and the structural basis for its ability to recruit more force-generating units.

How do bacteria sense environmental cues?

Many bacteria are motile. Chemotaxis, mediated by chemoreceptors, plays an important role in bacterial survival and virulence. In this project, we shall investigate what ligands such receptors recognize and why some molecules are attractants and some repellents, how binding to the receptor leads to signalling, how mutations in the sensor domain affect ligand specificity and, building on this, how bacterial chemoreceptors can be redesigned to recognize and respond to non-native ligands for innovative applications in biotechnology and bioengineering.

Applications are welcome from students with a strong interest in structural biology, X-ray crystallography, the biology of *H. pylori*, or protein biochemistry.
Projects based at affiliated institutions
**Helicobacter pylori** interactions with the innate immune system: The impact of these interactions on inflammation and stomach cancer

A hallmark of *H. pylori* infection is the chronic inflammation that precedes the development of severe diseases, including stomach cancer. The major research theme in our laboratory is focused on understanding how *H. pylori* induces inflammation and how this promotes stomach cancer in some individuals. This theme is addressed through the study of host-pathogen interactions using various in vitro and in vivo models. We are particularly interested to understand the mechanisms by which *H. pylori* engages with the innate immune system to not only cause inflammation, but to also maintain tissue homeostasis. For this, our research has centred on members of a family of cytosolic innate immune molecules, known as the NOD-like receptors (NLRs), which are able to sense the presence of both endogenous and exogenous “danger” signals. Although NLRs were first identified for their ability to mediate host defence responses against infection by microbial pathogens, it is now clear that these proteins have much more diverse functions.

Regulation and biological functions of a novel NLR protein, NLRC5, in *H. pylori* infection

Professor R. Ferrero and Dr L. Ying

The new NLR family member, NLRC5, is a key transcriptional coactivator of genes required for MHC class I presentation and has been reported to play a role in innate immune responses to several intracellular pathogens. Interestingly, it was recently shown that low levels of NLRC5 expression are associated with poor patient prognosis in cancer, leading to the suggestion that NLRC5 may also be important in tumour surveillance. Data from our laboratory suggest that *H. pylori* bacteria regulate NLRC5 expression thereby dampening inflammation and stomach cancer development. The overall aim of the project is to investigate NLRC5 expression and its downstream signalling functions in response to *H. pylori* infection. This will be addressed in both in vitro and in vivo models, including Nlrc5 knockout mice. The project will involve a variety of techniques, including the culture of primary cells, cell transfection, mouse infection, histological analyses, cytokine ELISA and quantitative PCR.
Innate immune and oncogenic properties of bacterial membrane vesicles

Professor R. Ferrero and Dr L. Ying

The release of small membrane vesicles (MVs) is a property that has been conserved by both multi- and unicellular organisms during evolution. One of the major functions of these MVs is to facilitate intercellular communication and transport of molecules. The release of outer MVs by prokaryotes was first described nearly 40 years ago, yet the biological significance of these structures is only beginning to be appreciated. We and others have shown that MVs from Gram-negative bacteria, including *H. pylori*, are potent modulators of host cell responses. The aim of the project is to investigate the immunogenic and oncogenic properties of *H. pylori* MVs using *in vitro* and mouse models. These models will be used to identify the immune and oncogenic signalling pathways that are induced by MVs. This project will involve cell culture, mouse models, fluorescence imaging, flow cytometry, cytokine ELISA and quantitative PCR.

Role of the *H. pylori* cag pathogenicity island in regulating host immune responses

Professor R. Ferrero and Dr L. Ying

A major virulence determinant of *H. pylori* is a type IV secretion system, encoded by the cag pathogenicity island (cagPAI). The *H. pylori* T4SS interacts intimately with host epithelial cells and delivers factors to these cells, resulting in the induction of oncogenic and pro-inflammatory signaling cascades. Clinical and animal infection studies have shown that cagPAI+ *H. pylori* strains which have a functional T4SS generally induce more inflammation and tissue damage in infected hosts than cagPAI- strains. *H. pylori* strains harboring a cagPAI are therefore generally thought to be more pro-inflammatory. Recent data from our laboratory, however, suggest that the cagPAI may encode factor(s) which can restrict or modulate these inflammatory responses, thus facilitating establishment of a chronic infection. The aim of the work is to determine the mechanisms whereby these factors dampen host immune responses. This project will involve molecular biology techniques (i.e. cloning, PCR, sequencing, mutant construction), cell culture and mouse infection studies, cytokine ELISA and quantitative PCR.
Innate Responses to Bacterial Infection

The subversion of host cell processes by microbial pathogens is an intrinsic part of the host-pathogen interaction. Many bacterial pathogens have the ability to transport virulence proteins, called effector proteins, into host cells via specialised protein secretion systems. We work on a range of virulence effectors from pathogenic E. coli, Shigella and Salmonella that interfere with host innate immune signalling pathways and block inflammation and cell death. The aim of this work is to investigate the manipulation of host cell signalling by effector protein families to understand their influence on host cell function, inflammatory signalling and the innate immune response. In this way effector proteins can be used as tools to understand the innate responses important for control of the pathogen.

Many bacterial pathogens have acquired the capacity to replicate inside human cells. Legionella and Burkholderia are environmental organisms that cause the life-threatening opportunistic infections known as Legionnaire’s Disease and Melioidosis respectively. A feature of both pathogens is the capacity of the bacteria to replicate within human cells through the manipulation of host cell biology. This depends on the ability of the pathogens to inject multiple virulence effector proteins into the host cell during infection. Our goal is to identify and characterise effectors that interact with cell intrinsic innate immune pathways.

Suppression of the innate immune response during Burkholderia infection

Professor E. Hartland and Dr S Ong

To replicate inside human cells, intracellular pathogens must avoid host innate and cell intrinsic immunity. We are interested in understanding how intracellular pathogens avoid activation of the inflammatory response in human cells. Burkholderia has three type III secretion systems that translocate virulence effector proteins into the host cell during infection. However, unlike many pathogens with a type III secretion system, very few effector proteins have been identified. We have discovered that Burkholderia inhibits activation of innate immune signaling pathways in macrophages and that this does not involve any known virulence factors. This project will begin to define the bacterial factors required for the suppression of the innate immune response. We hypothesize that this inhibition occurs through the translocation of one or more effector proteins. This project will involve molecular biology, bacterial mutagenesis, protein expression studies, confocal microscopy, cell culture-based assays and in vitro bacterial infections.
Host cell signaling pathways modulated by bacterial enteric pathogens

Professor E. Hartland and Dr C Giogha

Bacterial pathogens including species of Salmonella and Shigella, and enteropathogenic Escherichia coli (EPEC) and enterohaemorrhagic Escherichia coli (EHEC) are significant causes of gastrointestinal disease worldwide. During infection these bacteria stimulate host immune responses due to recognition of their pathogen-associated molecular patterns (PAMPs). However, to successfully establish infection, replicate and then disseminate, these pathogenic bacteria must also evade the host immune response. These pathogens have evolved specialised secretion systems that allow them to inject bacterial proteins directly into the host cell cytoplasm to subvert various host cell processes. We have recently shown that several of the secreted proteins of EPEC block innate immune signaling including the pro-inflammatory NF-κB pathway, and apoptotic and necroptotic cell death signaling pathways. These secreted bacterial proteins are often enzymes that mediate novel post-translational modifications to inactivate or modulate the activity of host cell protein targets. Although significant advances have been made in elucidating how gastrointestinal pathogens modulate host cell signaling pathways to dampen the immune response, there are still many secreted bacterial proteins that are not well characterised. The aim of this project will be to identify the host cell targets of a specific secreted bacterial protease of Shigella flexneri, and understand the role of this protease during infection. This project will involve molecular biology, mass spectrometry, cell culture-based assays and in vitro bacterial infections.

Stressed out! Host-cell responses to Legionella Infection

Professor E. Hartland and Dr K. McCaffrey

Bacteria use diverse and ingenious mechanisms to evade our immune system and establish infection. The study of complex microbes such as Legionella, which reproduce inside our cells, allow us to identify novel ways by which they exploit cell function and subvert immune responses. The most striking feature of Legionella infection is the formation of a large Legionella-containing vacuole (LCV) from endoplasmic-reticulum (ER) membranes within the host cell. We hypothesize that the formation of the LCV causes ER stress and activates ER-stress signalling via the unfolded protein response (UPR). This project will analyse the activation of key UPR markers during Legionella infection using semi-quantitative PCR, Western blotting and confocal microscopy techniques. The determinants of UPR activation will then be investigated using mutant variants of Legionella as well as chemical inhibitors of the UPR. These studies aim to evaluate the UPR as a potentially novel drug target during Legionella infection.
The focus of our research is to determine the mechanisms by which bacterial pathogens interact with their host and to then use this information to understand the specific immune responses that are essential for fighting infections and maintaining immune homeostasis in the body.

Our overarching goal is to make discoveries on the fundamental mechanisms of immunity to infections and to inform the future development of therapeutics for bacterial infections and also, dysregulated immune responses in inflammatory diseases.

The model pathogen that we use in our research is *Salmonella enterica*. These pathogens are able to colonise a wide range of human and animal hosts and in humans, can cause disease ranging from gastroenteritis to systemic disease, depending on the Serovar of the bacteria. *S. enterica* serovar Typhi and Paratyphi are human restricted and are known as the ‘Typhoidal’ *Salmonella*. These serovars cause serious illness as the bacteria disseminates systemically, causing severe fever, nausea, diarrhoea, abdominal cramping and headaches, and if left untreated, death can occur. Other non-typhoidal *Salmonella* serovars (NTS) include *S. enterica* serovars Typhimurium and Enteritidis, both of which account for the majority of gastrointestinal disease (salmonellosis) in immunocompetent individuals worldwide. In immunocompromised people however, some NTS strains cause invasive disease and thus more serious illness.

### Understanding the biochemical mechanisms of *Salmonella* virulence proteins

**Dr Jaclyn Pearson and Professor Elizabeth Hartland**

Pathogenic serovars of *Salmonella* are the causative agents of a spectrum of disease states, including typhoid fever, self-limiting gastroenteritis, and invasive bacteremia. Australia has one of the highest incidences of Salmonellosis in the developed world. Pathogenesis is dependent on the activity of two distinct type III secretion systems (T3SS), encoded by genetic regions termed *Salmonella* pathogenicity islands (SPI). The SPI-1 T3SS is associated with bacterial invasion as well as activation of innate immune signaling, and the SPI-2 T3SS is associated with intracellular survival in immune and epithelial cells, replication and systemic infection. While the importance of the SPI-1 T3SS to *Salmonella* pathogenesis is well established, the function of many SPI-2 encoded effectors remains unknown. This project aims to investigate the role of a subset of relatively uncharacterised SPI-2 effectors in *Salmonella* virulence. Overall this project will
provide critical insights into the pathogenic mechanisms of an important public health issue and provide the basis for potential future therapeutic development.

Host cell death signaling and susceptibility to *Salmonella* infection

Dr Jaclyn Pearson and Dr Kate Lawlor

Enteric bacterial pathogens such as *Salmonella* spp. and enteropathogenic *E. coli* deliver “effector” proteins directly into host cells via specialised secretion systems which exert specific enzymatic activity on host proteins to subvert host responses and prolong infection. Our recent work characterised an effector protein from pathogenic *E. coli* as a cysteine protease that cleaves and inactivates all mammalian RIP homotypic interaction motif (RHIM) proteins including RIPK1, RIPK3, TRIF and DAI. RHIM proteins are key immune signaling factors that mediate inflammation, apoptosis and necroptosis. Dysregulated immune responses and cell death form the basis of much human disease pathogenesis. This study aims to understand the role of RHIM proteins in controlling *Salmonella* and other enteric infections.
Environmental and Public Health Microbiology Laboratory – EPHM Lab

The EPHM Lab is located within the Civil Engineering Department at Monash University, Clayton, and has a specific focus on health-related urban water microbiology; some of our projects include: (1) understanding the pathways pathogens follow in urban environments, (2) development of novel antimicrobial filtration media and methods for stormwater treatment, (3) understanding the human health risks caused by pathogens, such as Campylobacter, Klebsiella and Salmonella in the Yarra River and (4) understanding microbial community dynamics in complex environments.

Pathogens in the Yarra River estuary: tracking the sources?

Dr David McCarthy, Dr Rebekah Henry (Dept. Civil Engineering), Mr Scott Coutts and A/Professor John Boyce (Dept. Microbiology)

Faecal microorganisms are the leading cause of microbial pollution in recreational waters. Sources of these bacteria include rivers and creeks, human wastewater (via overflows, leakages and cross connections) river bed sediment and direct deposition by wildlife. Melbourne’s Yarra River has come under scrutiny in national media due to reports of high faecal contamination. However, quantitative assessment of the pathogen levels within this river, in particular in recreational hot spots such as Warburton and Warrandyte, has not been conducted. Furthermore, there has been no study that directly compares the sources of faecal contamination to determine their relative contributions and quantitatively assess microbial risk to the recreating public.

This project will identify sources of faecal contamination in the Yarra River. In particular, you will undertake field and laboratory work to determine the significance of direct faecal deposition from wildlife as a source of contamination. Water quality, sediment and faecal samples will be collected from various locations along the Yarra River. You will analyse these samples using metagenomic and 16S bacterial community analysis. Metagenomics is a broad term used to describe the analysis of genetic material from a microbial population directly from an environmental niche without culturing. This technique will be conducted using Illumina® high-throughput ‘Next-Generation’ sequencing technologies. You will use a range of DNA extraction and molecular biology techniques to prepare samples for the metagenomic analysis. Bioinformatic analysis will be conducted to generate a genus-level microbial profile for the input source, which will be compared to control water samples to identify pathogens that may have entered the waterway as a result of direct deposition.
from wildlife. Further work will be conducted to characterise specific pathogens identified during the metagenomic analysis, to species- and serovar-level using directed PCR methods.

The project will allow you to engage in and gain experience across multiple disciplines, including engineering, microbiology, molecular biology and bioinformatics. This project presents a unique opportunity to work in conjunction with important industry partners such as Melbourne Water. This research will contribute to future mitigation strategies for the prevention of microbial contamination into the Yarra River.

Campylobacter in Melbourne’s recreational waters: what’s the risk?

Dr David McCarthy, Dr Rebekah Henry, Dr Dieter Bulach, Scott Coutts and A/Professor John Boyce

Campylobacter spp. are the highest cause of reported acute gastroenteritis in the developed world surpassing both Salmonella spp. and Escherichia coli [1]. Since 1996 the number of reported Campylobacteriosis cases in Victoria has been steadily climbing. Campylobacter is ubiquitous in the gut of various wild and agricultural animals, both of which are sources of faecal contamination for environmental waters. Most importantly, Campylobacter are also prevalent in high concentrations in human effluent, which can contaminate these environmental waters via overflowing or failing sewerage infrastructure.

The Yarra River catchment is an important water resource for agricultural and recreational users as well as providing an important ecosystem for many native animals. A recent Qualitative Microbial Risk Assessment (QMRA) identified knowledge gaps in the microbial ecology of the river.

Since the QMRA, extensive investigation involving several sampling sites along the river has revealed ongoing high concentrations of Campylobacter spp. at several of these sampling sites. But there is limited understanding of the source and the pathogenicity of these Campylobacter isolates.

Multilocus Sequence Typing (MLST) is a molecular method that uses highly conserved DNA sequences to identify trackable genetic markers. This information can be used to infer a strain host but more importantly it can link strains to disease and an origin.

This project aims to understand the risks derived from Campylobacter found in the Yarra River. The student will have the opportunity to isolate Campylobacter from a range of environmental sources, prepare samples for whole genome sequencing and perform bioinformatic and MLST analysis on the nation’s largest environmental Campylobacter database. The outcome of this project will directly influence new policy and guidelines for recreation in Victorian water systems.
White biotechnology (also known as industrial biotechnology) is the application of biotechnology in industrial processes. This cross-disciplinary emerging area of science is well placed to play a key role in a sustainable future through (i) overcoming dependence on non-renewable feedstocks such as crude oil, (ii) new environmental remediation technologies and (iii) a greatly reduced environmental footprint from manufacturing. Broadly applicable across a range of market sectors, many large companies are seeking to develop products and processes that are inspired by biological systems and use renewable feedstocks such as terrestrial biomass to ensure their long-term future. Some example applications of white biotechnology already proven at an industrial scale include production of 1,3-propandiol (DuPont), polylactic acid (NatureWorks LLC) and more recently isoprene (Dupont, Goodyear) to name but a few. Not only are these products derived from renewable feedstocks, but the processes typically require less energy input, less solvents and result in reduced levels of toxic by-products.

Our research centres on industrial biotechnology and aims to use microbial systems to specifically modify highly functional small molecules which can then be used as starting materials in a diverse array of chemical syntheses. Using microbiological and molecular techniques, the student will characterise microorganisms and enzymes that can be used to produce synthetically useful compounds. Through the manipulation of growth conditions in both batch and continuous culture, the student will use a range of analytical techniques (e.g. NMR and mass spectrometry) to identify and characterise metabolic products that are potential chemical synthons. Of particular interest is the conversion of eucalyptus oil into chemicals that can be used to improve the properties of polymers. This project will provide the student with the opportunity to engage with varied scientific disciplines including industrial microbiology, molecular biology and organic chemistry and also an opportunity to work in CSIRO’s state-of-the-art Recombinant Protein Production Facility.
Developing vaccines against malaria

Malaria is one of the world’s leading causes of death and illness, particularly among young children. There remains a strong need for highly effective vaccines to reduce the burden of malaria and progress towards eventual malaria elimination. To date, most vaccines have achieved only modest levels of efficacy, emphasising the need for novel approaches in vaccine design that can induce potent immune responses.

This project will focus on identifying key antigens and specific epitopes that are targets of protective immunity against malaria and understanding the mechanisms mediating immunity, which includes antibodies and cell-mediated responses.

This knowledge is crucial for the development of effective vaccines against malaria. The project will also involve using knowledge of immunity to malaria for informing vaccine design, and the expression and testing of novel vaccine candidates. These studies will use novel approaches in molecular biology, cell biology and immunology to address these aims, and will build on recent major advances generated from our malaria vaccine program.

The project will primarily involve laboratory-based research, including western blotting, imaging, standard immunoassays, functional immunoassays (e.g. neutralisation assays, cell-mediated immunity), flow cytometry, cell culture and protein expression. The project could also include bioinformatics, structural modelling of vaccine antigens, or modelling vaccine impact depending on the student’s interest. The specific activities and focus of the project will be refined to suit the interests and training background of the student. Interested students should contact Arzum, arzum.cubuk@burnet.edu.au

Discovering the mechanisms and targets of immunity against malaria

Antibodies are an important component of acquired immunity against malaria, as demonstrated in pivotal studies in which immunoglobulin G (IgG) from immune adults was transferred to malaria-infected children and resulted in clearance of infection. The mechanisms of protection and specific target epitopes of protective immunity are not well understood. In recent studies, we have begun to uncover important roles for antibodies that can directly inhibit host-cell infection, interact with immune cells to kill and clear malaria, or recruit complement to neutralise infection.

The aims of this project include identifying the key targets of specific mechanisms mediating immunity. The project may combine detailed studies of immune responses with clinical and population studies in Africa, Asia, and Papua New Guinea. It will examine how immune responses protect
Currently, very little is known about the interactions between malaria infection rates and patterns and malaria immunity in populations, and how these interact. Malaria control programs face the challenge that as malaria transmission declines, malaria immunity also declines, which places the population at higher risk of malaria transmission and rebound epidemics.

This project will investigate the impact of malaria immunity on malaria infection rates and transmission of malaria in populations. The student will analyse various parameters to define the patterns of infection and immunity, with a particular focus on defining the interaction between immunity and malaria transmission.

The findings of this project will be highly relevant to informing malaria elimination efforts and understanding the value of incorporating vaccines into elimination strategies. Skills acquired may include established high-throughput immunoassays and assays that quantify the functional activity of immune responses (e.g. flow cytometry, Fc-receptor mediated immunity, complement activation, western blots, ELISA, neutralisation assays). This could be expanded to include modelling of the interaction between infection and immunity, and how this may impact on malaria elimination and control. Interested students should contact Arzum, arzum.cubuk@burnet.edu.au

Understanding malaria transmission and immunity to inform malaria elimination

Malaria transmission in populations involves interactions between infection rates and prevalence that drive transmission, and the presence of malaria immunity that has the potential to reduce transmission. Malaria immunity can act to reduce infection rates and levels of malaria parasitemia, and specific components of immunity can also function to directly block transmission of malaria. This is known as transmission-blocking immunity.

The studies would particularly focus on using innovative approaches to understand how antibodies neutralise and clear malaria parasites in the blood, including interactions with monocytes/macrophages and dendritic cells, and identifying specific epitopes targeted by protective antibodies. Skills may involve assays of functional immunity, cell culture, isolation and analysis of immune cells, flow cytometry, western blotting, ELISA, and epitope mapping. The project will be tailored to best match the student’s interests and training background. Interested students should contact Arzum, arzum.cubuk@burnet.edu.au

children from malaria or protect pregnant women and their developing babies from the devastating consequences of malaria in pregnancy.

The studies would particularly focus on using innovative approaches to understand how antibodies neutralise and clear malaria parasites in the blood, including interactions with monocytes/macrophages and dendritic cells, and identifying specific epitopes targeted by protective antibodies. Skills may involve assays of functional immunity, cell culture, isolation and analysis of immune cells, flow cytometry, western blotting, ELISA, and epitope mapping. The project will be tailored to best match the student’s interests and training background. Interested students should contact Arzum, arzum.cubuk@burnet.edu.au
Impact of microbiota metabolites on cervicovaginal mucosa and HIV susceptibility

**Professor Gilda Tachedjian, Dr Anna Hearps, Dr Raffi Gugasyan and Dr Joshua Hayward**

Epithelium lining the lower female reproductive tract (FRT) acts as a barrier to pathogens and is the sentinel of the innate immune response. In particular, inflammation and breaks in epithelium integrity at the genital mucosa can promote HIV infection. While there are several studies describing the immune modulatory effects of short chain fatty acids (SCFAs) and lactic acid in the context of the gut and cancer, respectively, little is known regarding their impact on immune mediators released by vaginal, ecto- and endo-cervical epithelial cells of the FRT. Lactobacilli are associated with eubiosis (healthy microbiota) and maintain a non-inflammatory environment while dybiosis [e.g. bacterial vaginosis (BV)] promotes inflammation. We hypothesise that lactic acid, produced by vaginal lactobacilli, apart from its direct antimicrobial activities (Aldunate et al 2013 J Antimicrob Chemother 68:2015), has immune modulatory effects on cervicovaginal epithelial cells that decreases HIV susceptibility (Hearps et al 2017 Mucosal Immunol). This study aims to determine the effects of lactic acid informed by RNASeq analysis of cervicovaginal cells treated with lactic acid alone and in the presence of TLR ligands that mimic pathogen-associated molecular patterns. These studies could lead to the development of strategies to treat and prevent vaginal inflammation and consequently susceptibility to HIV, other sexually transmitted infections, as well as adverse reproductive health outcomes.

Immune modulatory effects of microbiota metabolites and female sex hormones on cervicovaginal epithelial cells

**Professor Gilda Tachedjian, Dr Anna Hearps and Dr Raffi Gugasyan**

Epithelium lining the lower female reproductive tract (FRT) acts as a barrier to pathogens and is the sentinel of the innate immune response. In particular, inflammation and breaks in epithelium integrity at the genital mucosa can promote infection with sexually transmitted infections (STIs) including HIV. Vaginal eubiosis, characterised by the presence of lactobacillus-dominated microbiota is noninflammatory while vaginal dysbiosis, typified by a high load and diversity of anaerobic bacteria, promotes a proinflammatory cervicovaginal environment that increases susceptibility to HIV and other STIs. We have
shown that lactic acid, produced by beneficial lactobacilli, elicits an anti-inflammatory effect that inhibits production of proinflammatory cytokines and chemokines induced by components of virus and bacteria in a cervicovaginal epithelial transwell model (Heaps et al 2017 Mucosal Immunol). However, the impact of female sex hormones on the immune modulatory effects of lactic acid on these epithelial cells is unknown. This study aims to determine if female sex hormones augment or attenuate the anti-inflammatory effects of lactic acid that could potentially modulate infection with STIs including HIV.

**Characterisation of bat intracellular restriction factors**

**Professor Gilda Tachedjian and Dr Joshua Hayward**

Bats are a major reservoir of viruses such as Hendra virus and Ebola virus that are pathogenic in humans but not in bats. The reason why bats can coexist with these viral pathogens is unknown. However, one explanation is that the coevolution of bats and their viruses has led them to an effective “peace treaty”, a biological equilibrium in which viral replication is regulated such that both host and virus can co-exist without undue antagonism. The role of the host’s immune system is to control infections and intrinsic intracellular restriction factors are the front line of the innate immune response that targets viruses. These factors have originally been discovered to restrict retroviruses such as HIV; however, some of these factors e.g. tetherin can also restrict paramyxoviruses, coronaviruses and filoviruses. We are currently characterising the intracellular restriction factor repertoire of megabats (Hayward et al 2018 Mol Biol Evol Mar 29) and microbats by mining their genomes and transcriptomes, validating the expression of these factors in bat tissues and evaluating their inhibitory activity against viral pathogens. This study will focus on characterising a class of restriction factor found in megabats and microbats, their antiviral activity and mechanism of action compared to human homologues.

*This is a joint project between the Tachedjian Lab and CSIRO Australian Animal Health Laboratory.*

**Characterisation of a bat retrovirus**

**Professor Gilda Tachedjian and Dr Joshua Hayward**

Bats are a major reservoir for many viral pathogens however retroviruses are yet to be isolated from bats. Retroviruses such as gammaretroviruses have simple genomes in contrast to HIV and are known to infect a wide variety of species including mice, cats, koalas and non-human primates, and cause leukemias, lymphomas, neurological diseases and immunodeficiencies in these species. Retroviruses are found in the genome of mammals and can be transmitted vertically through the germ line (e.g. endogenous) or transmitted horizontally (e.g. exogenous). Endogenous retroviral sequences are present as a critical part of eukaryotic genomes and normally represent the fossil record of extinct viruses. Our analysis of the genome of bat species has revealed the presence of retroviral sequences that, at the amino acid level, demonstrate homology to extant (currently existing) gammaretroviruses (Cui et al 2012 J Virol 86:4288) and betaretroviruses (Hayward et al 2013 Retrovirology 10:35) indicating that retroviruses have circulated in bats. However, whether exogenous retroviruses are currently circulating in bats are unknown. We have discovered retroviral sequences in bat scat that we have engineered into a replication competent virus. This study aims to characterize this virus including determining the cell host range and receptor usage. This study will increase our fundamental understanding of bat retroviral biology and will provide insight into the transmission of pathogens that affect Australian native animals as well as providing evidence if this virus is potentially a public health concern.

*This is a joint project between the Tachedjian Lab and CSIRO Australian Animal Health Laboratory.*

**Discovery of a new drug class for HIV treatment and prevention**

**Professor Gilda Tachedjian, Dr George Mbogo and Dr David Chalmers**

There is a real threat that drug resistance, toxicity and intolerance will eventually lead to exhaustion of antiretroviral drug options for both HIV treatment and prevention, especially since there is little in the way of new drug classes in the pipeline. We have initiated a drug discovery program targeting HIV reverse transcriptase (RT) to identify compounds that inhibit this essential viral enzyme. We are using an innovative and validated paradigm for drug discovery called fragment-based drug design (FBDD) that uses very small compounds called “fragments” to find inhibitors with novel mechanisms of action against HIV RT. Using FBDD means screening far fewer compounds than conventional drug screens since fragments are so small and can be developed into more potent drugs. Two screens have identified promising fragments that inhibit HIV-1 RT. We have discovered fragments with mechanisms that are distinct from drugs used in the clinic (La J, Latham C et al 2015 PNAS 112:6979). The aim of this study is to progress one of the fragment hits into more potent RT inhibitors or drug leads. Compounds that are structurally related to the fragment hit will be evaluated for their ability to bind and inhibit wild-type and drug-resistant RT, as well as inhibit HIV-1 replication. This study will identify leads for the development of a novel class of RT inhibitor for use in HIV treatment and prevention.

*This is a joint project between the Tachedjian Lab and Monash Institute of Pharmaceutical Sciences.*
Characterising the milieu of hepatitis B spliced variants associated with advanced liver disease and liver cancer

A/Professor Peter Revill and Dr Margaret Littlejohn

Hepatitis B virus (HBV) is one of the most important human pathogens, infecting 257 million people worldwide, including 239,000 Australians. We have previously shown that splice variants of Hepatitis B virus are associated with liver cancer, the 5th most prevalent cancer worldwide (Bayliss et al, J. Hepatol, 2013), and that splice variants are more diverse than previously appreciated (Betz-Stablein et al., 2016). Yet the complexity of splice variants associated with advanced liver disease and liver cancer is unknown. This project will utilize next generation sequencing, cloning, and in vitro cell culture studies to identify and characterize novel splice variants associated with advanced liver disease and liver cancer, across different HBV genotypes, and determine their replication phenotype. Techniques utilized will include cell culture, real-time PCR/digital PCR, next generation sequencing, Southern, northern and western blotting, and quantitative serology. This project will make a major contribution to our understanding of the role of HBV splice variants in liver disease progression.
Monash Microbiology Honours 2019

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Please return this form to Associate Professor John Boyce as soon as possible, but no later than Friday 16 November 2018