Honours projects in Biochemistry, Genetics, Microbiology and Physiology 2018

Immunology: Parasite Antigen Discovery

Supervisors: Dr Nick Andronicos (UNE), Dr Peter Hunt (CSIRO)

The aim of this project is to define the antigens that are liberated from the surfaces of parasites by granulocytic cells during the innate immune response.

Techniques:

- Granulocyte isolation from the blood of parasite resistant and susceptible sheep using density centrifugation.
- Co-culturing of granulocyte cells with exsheathed *T. colubriformis* parasites.
- Proteomic identification of antigen released from the parasite using mass spectroscopy.
- Cloning of antigen in preparation of recombinant protein expression.

Immunology: Mast Cell Engineering

Supervisor: Dr Nick Andronicos (UNE), Dr Peter Hunt (CSIRO)

The aim of this project is to use tissue engineering to immortalise mast cells thereby enabling these cells to grow in vitro indefinitely.

Techniques:

- Recombinant protein engineering (Cloning and expression of cytokine genes).
- Mast cell genetic modification and cloning
- Cell differentiation and activation studies
- Immunocytochemistry and flow cytometric analysis

Immunology: Meta-analysis of gastrointestinal inflammation

This project is designed for external students.

Supervisors: Dr Nick Andronicos (UNE), Dr Richard Charlesworth (UNE)

The aim of this project is to perform meta-analyses of publically available transcriptomic datasets of intestinal inflammation diseases such as coeliac disease, IBD, colitis and intestinal cancers to define disease-specific signatures.

Techniques:

- Meta-analysis of human and rodent publically available transcriptomic datasets of intestinal inflammatory diseases.
- Definition of classification algorithms using discriminant analysis.

Meta-analysis: Gastrointestinal microbiome meta-analysis

This project is designed for external students.

Supervisors: Dr Nick Andronicos (UNE), Dr Rob Hart (UNE)

The aim of this project is to perform meta-analyses of publically available metagenomics, metabolomics and transcriptomic datasets to define disease signatures that may be used to classify disease severity.

Techniques:

- Meta-analysis of publically available phenotypic, metagenomics, metabolomics and transcriptomic datasets.
- Definition of classification algorithms using discriminant analysis.

Microbiology: Microbiome-Host cell interactions

Supervisors: Dr Nick Andronicos (UNE), Dr Gal Winter (UNE)

The aim of this project is to establishment of an in vitro GIT-microbiome culture model.

Techniques:

- Isolation of microbes
- Culture of gut epithelial and immune cells with microbes
- Metagenomic identification of cultured microbial species
- Analysis of proteomics associated with the co-culture.

Genetics: Genetic diversity monitoring in gastrointestinal parasite *Haemonchus contortus* populations

Supervisors: Dr Nick Andronicos (UNE), Dr Marielle Babineau (CSIRO)

Background: *Haemonchus contortus* (*H. contortus*) is a gastrointestinal nematode parasite of ruminants. It has a global distribution in tropical/subtropical zones and is one of the most important ruminant parasite. It causes anaemia, oedema, lethargy, weight loss and production trait losses (wool, milk) and deaths in infected animals especially in young sheep. Anthelmintic drugs are the best options for controlling this parasite, however, repeated use of the same chemical mode of action (MoA) has led to the evolution of anthelmintic drug resistance in many *H. contortus* populations worldwide. In Australia, field *H. contortus* populations resistant to amino-acetonitrile (AAD), benzimidazole (BZ), tetrahydropyrimidine, macrocyclic lactone (ML), organophosphate (OP) and salicylanilide-phenol (SAL) with some populations showing resistance to multiples MoA. A better understanding of the genetic variation and allelic diversity among and between populations of this parasite showing different phenotype to drugs would allow for better resistance preventive and diagnostic tools and lead to more efficient management strategies.

Techniques:

- Perform DNA extraction on adult worm individuals from conserved specimens originating from four populations of *H. contortus* showing variations in their phenotype to anthelmintic drugs.
- PCR, qPCR and sequence analysis of drug target genes.
- Identify the frequency of single nucleotide polymorphism (SNP) in three drug target genes sequences B-tubuline-1 (BZ-target), pgp-A (ML-target) and mptl-1 (AAD-target) for the different nematode populations.
- Population genetics analysis to define anthelmintic drug resistance evolution strategies of *H. contortus*.

Genetics: Anthelmintic drug target gene evolution through time in *H. contortus*: pre-existing variation vs selection?

Supervisors: Dr Nick Andronicos (UNE), Dr Marielle Babineau (CSIRO)

Techniques:

- Use conserved specimens of *H. contortus* collected before and after the introduction of anthelmintic drug Monepantel in 2010.
- Extract DNA, quantify, amplify and sequence the nicotinic acetylcholinesterase (nACH) target gene(s) for monepantel in order to elucidate the timing of resistance-conferring SNPs.

- Establish the alleles number and frequency in nACH before the introduction in Monepantel

Genetics: Spatial distribution of anthelmintic drugs resistance in *H. contortus* in Australia.

Supervisors: Dr Nick Andronicos (UNE), Dr Marielle Babineau (CSIRO)

Techniques:

- Detect alleles (SNP, indels) frequency from *H. contortus* field populations throughout Australia (> 30 populations) from target drug gene.
- Determine spatial autocorrelation between the field population location and drug resistance phenotype.
- Determine the effect of resistance level on spatial distribution
- Determine the effect of multiple resistance phenotype
- Generate a map that would help plan more efficient resistance management strategies and "hot-spots" of resistance.
- Determine if hot-spots are associated with abiotic factors.

Genetics: Fitness cost of anthelmintic drug resistance in *H. contortus*

Supervisors: Dr Nick Andronicos (UNE), Dr Marielle Babineau (CSIRO)

Background: *H. contortus* shows a low tolerance for lower temperature at the egg stage. Eggs will not survive temperatures below 8-10˚C. A cost of resistance could be that, in the absence of a selection from drug use, resistant population's eggs will die at even higher temperature than the minimum 8-10˚C for the species in general, or could hatch necessitating more time. Alternatively, a resistance benefit could be that eggs from resistant populations can hatch at lower temperatures or can hatch needing less time.

Techniques:

- Use geographically close-by populations of *H. contortus* showing variation in response to one or more anthelmintic drug resistance.
- Using climate chambers at various temperatures (-2 to 12˚C), determine the hatch rate of the different populations.
- Generate hatch-temperature and hatch-time curves and compared population for significant differences.
- Assess the presence of a fitness cost, benefit or neutral fitness response.

Genetics: Survey of anthelmintic resistance in parasite species in vitro

Supervisors: Dr Nick Andronicos (UNE), Dr Peter Hunt (CSIRO)

Background: *H. contortus* develop in the abomasum of sheep from larval stage 3 (L3) to sexually mature adults that produce eggs. The sensitivity to anthelmintics may be lifestage specific. Therefore the aim of this project is to compare the stage-specific resistance/susceptibility of *H. contortus* to anthelmintics.

Techniques:

- In vitro culture of different developmental stages of *H. contortus*.
- Anthelmintic LD50 analysis of *H. contortus*
- DNA extraction
- PCR amplification and sequence analysis of anthelmintic target genes from *H. contortus.*

Gut feeling – The gut-brain axis

Drs Gal Winter, Adam Hamlin, Lily Pereg

The huge significance of the gut microbiome to human health and well-being is widely accepted. New studies are constantly being published, reinforcing the role of the gut microbiome in modulating digestion, immunity, behaviour and more. Our research is focused on the identification of beneficial microbes from different environments and how these effect the brain to influence behaviour.

Projects:

- The effects of Chronic Early Life stress on the gut microbiome in animal populations
- The effect of medications on the gut microbiota
- Microbial pathways from soil to gut

Microbiology

Assoc. Prof Lily Pereg

1. Pathogen suppression by probiotic/biocontrol microbes with applications in Aquaculture, Agriculture and Medicine
2. Plant-Microbial Interactions and Soil microbiology
3. Ecosystem recovery following traumatic events

Genetics/Biochemistry

Dr Mary McMillan

Although depression represents a major personal and community health burden, the efficacy of current treatments for depression is low. It has been suggested that the efficacy of treatment could be greatly improved by providing 'personalised' treatments - preferably by identifying different "subtypes" of depression that have distinct symptoms, mechanisms, and treatment requirements. It is therefore important to identify genetic and other biological markers associated with these subtypes of depression.

Projects include the identification of potential biological and genetic markers of the different subtypes, and of total depression, and to identify genetic factors that may contribute to depression. Students will gain skills in a range of molecular biology techniques including extraction of nucleic acids, PCR, qPCR, RFLP's, DNA sequence analysis, and ELISAs.

More information on potential projects and the research group can be found at: une.edu.au/bbrg

Physiology

Dr Robert Hart

Lifestyle diseases and conditions, such as obesity and type 2 diabetes mellitus, are prevalent in western populations and are becoming increasingly significant burdens in developing countries. Potential projects will use animal models to investigate the mechanisms involved in the development or management of these conditions. Interdisciplinary projects will be considered. Potential projects include:

- Leptin in the Digestive Tract
- Physiology (e.g. endocrine or immune parameters) and the gut microbiome
- The effects of traditional medicines on glucose metabolism

Physiology

Dr Tom van der Touw

Abnormal respiratory muscle function in patients with respiratory disease

Respiratory muscle function is often abnormal in patients with respiratory disease. For example, the inspiratory muscles do not fully relax between breaths in asthmatics. Few studies have systematically examined whether this behaviour occurs in the accessory inspiratory muscles of the neck in asthmatics, and there is an opportunity for a suitable Honour's candidate to investigate this during airway challenges which induce carefully controlled episodes of reversible airway obstruction in asthmatics. This research will also provide an Honours student with a range of valuable respiratory diagnostic skills which can prove useful if s/he chooses to seek employment as a hospital respirologist.

Daily body energy expenditure to evaluate physical impairment

It has recently become possible to indirectly and unobtrusively measure daily body energy expenditure in people using a small noninvasive monitor which is worn by the subject for extended periods and continuously records ECG based heart rates and body motion to provide accurate estimates of body energy expenditure on a minute-by-minute basis over entire week long periods. Such measurements would be useful for quantifying the degree of physical impairment in many debilitating chronic disorders. However, such work first requires collecting a data base of daily body energy expenditure from healthy individuals and there is currently an opportunity for a suitable Honours candidate to carry out this research.

Immunological function and stress biomarkers in obstructive sleep apnoea (OSA)

There is growing interest in examining the clinical potential of biomarkers (e.g. serum/plasma levels of fibrinogen, heat shock proteins, interleukin 8, leptins, uric acid) for diagnosing OSA. Assaying techniques for these potential biomarkers are available in the School of Science and Technology. The *Armidale Private Hospital* has a large *Respiratory Failure and Sleep Disorders Unit* for diagnosing and treating OSA patients, and Dr van der Touw has in recent years carried out a number of studies with this patient cohort. There is currently an opportunity for a suitable Honours candidate to carry out research in immunological function and stress biomarkers in OSA patients with Dr van der Touw. This research would be carried out in collaboration with Dr Nick Andronicos who has a well established UNE laboratory for examining inflammatory biomarkers and immune function.

Influence of exogenous phospholipids on the function of antigen presenting cells.

The amphipathic nature of phospholipid molecules enables them to adhere to cell surfaces and alter the physico-chemical properties of these surfaces. This can result in a wide range of biological effects which may have significant respiratory and immunological implications. There is currently an opportunity for a suitable Honours candidate to carry out a study with Dr van der Touw on the effects of phospholipid adherence on the function of antigen presenting cells. This research would be carried out in collaboration with Dr Nick Andronicos who has a well established UNE laboratory for examining inflammatory biomarkers and immune function. A primary objective of this research would be to explore whether coating molecular pattern recognition receptors on antigen presenting cells (human macrophages) with exogenous phospholipid (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) impairs their ability to take up and respond to danger associated molecules.

Plant Biochemistry/Physiology

Dr Heather Nonhebel

Cereal crops provide approximately 95% of starchy foods worldwide and form staple food of almost every country. Climate change is reducing both the quality and quantity components of cereal grain yield. Reproductive growth, particularly the events immediately before and after pollination are the most sensitive to heat stress. In my laboratory we are investigating the signalling processes that control early grain development and the initiation of starch and storage protein production.

Investigating the role of cell wall invertase inhibitor OsINVINH3 in regulating sugar signalling during early grain development

Cell wall invertase GIF1 is an enzyme shown to be essential for grain fill whose role and regulation appears to have changed during rice domestication. OsINVINH3 is an invertase inhibitor expressed solely in early grain fill. The role of OsINVINH3 in fine tuning the activity of cell wall invertase during early grain fill will be investigated.

Role of auxin in chalk formation in heat stressed grains

Chalk formation results in a decrease in value and quality of rice grains grown under high temperature. Our recent work indicates that the plant hormone may be involved. This project will monitor the effects of heat stress on the auxin content and auxin-related gene expression in developing grains.