The School of Pathology and Laboratory Medicine has a strong research program and offers a wide variety of Honours projects, located both in-house at the QEII Medical Centre and in various locations around Perth including Royal Perth Hospital, Fremantle Hospital and the CSIRO.

If you intend to enrol in Honours in 2014, this booklet will provide you with an overview of the interests of each of the research groups as well as the specific projects on offer this year.

These projects can be taken with several enrolments, including

Honours in Microbiology
Honours in Pathology
Honours in Biomedical Science
Bachelor in Medical Science
Graduate Diploma
M.Sc. Preliminary

This book includes information about application procedures and project choices.

You can contact the co-ordinators or the individual supervisors with any queries.

Honours Co-ordinators

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## Table of Contents

### Introduction to Honours

<table>
<thead>
<tr>
<th>Projects</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ID No.</strong></td>
<td><strong>Project Title</strong></td>
</tr>
<tr>
<td>Beilharz 1</td>
<td>Immunotherapy for the Treatment of Cancer</td>
</tr>
<tr>
<td>Beilharz 2</td>
<td>The effect of topical tea tree oil on UV-induced DNA damage</td>
</tr>
<tr>
<td>Bentel 1</td>
<td>Interaction Between RMND5 Proteins and Ubiquitin-40s Ribosomal Protein S27a</td>
</tr>
<tr>
<td>Bundell 1</td>
<td>Temporal (giant cell) arteritis</td>
</tr>
<tr>
<td>Chakera 1</td>
<td>Analysis of the biological effects of bacterial pathogens known to cause peritoneal dialysis-related peritonitis on mesothelial cell function</td>
</tr>
<tr>
<td>Dilley 1</td>
<td>Biomaterials approaches to developing patch grafts for eardrums</td>
</tr>
<tr>
<td>Dilley 2</td>
<td>Hearing loss</td>
</tr>
<tr>
<td>Dilley 3</td>
<td>Promoting tissue formation through angiogenesis</td>
</tr>
<tr>
<td>D’Orsogna 1</td>
<td>The impact of public T cell receptor responses on the alloreactive T cell repertoire</td>
</tr>
<tr>
<td>D’Orsogna 2</td>
<td>Tolerance induction using overexpression of FoxP3 within T cells recognising foreign transplanted tissue</td>
</tr>
<tr>
<td>Fisher 1</td>
<td>Targeted immunomodulation of tumor resident regulatory T cells as an effective therapy to treat disseminated melanoma</td>
</tr>
<tr>
<td>Fisher 2</td>
<td>Targeting regulatory T cells to improve combination chemo-immunotherapy treatment of solid tumours</td>
</tr>
<tr>
<td>French 1</td>
<td>Isotype diversification of IgG antibodies and activation of antiviral accessory cell responses in the control of HIV infection</td>
</tr>
<tr>
<td>Inglis 1</td>
<td>Therapeutic efficacy of novel antimicrobials against infectious disease agents</td>
</tr>
<tr>
<td>Johansen 1</td>
<td>Characterisation and application of monoclonal antibodies to flavivirus NS1 proteins</td>
</tr>
<tr>
<td>Kahler 1</td>
<td>Outer membrane biogenesis in Gram negative bacteria</td>
</tr>
<tr>
<td>Kahler 2</td>
<td>Endotoxin synthesis in <em>Neisseria</em> sp.</td>
</tr>
<tr>
<td>Kahler 3</td>
<td>A molecular epidemiology approach to understanding meningococcal disease in Western Australia</td>
</tr>
<tr>
<td>Kahler 4</td>
<td>Protein folding in the periplasm of Gram negative bacteria</td>
</tr>
<tr>
<td>Marshall 1</td>
<td>Longitudinal molecular epidemiology of <em>H. pylori</em> using whole genome sequencing</td>
</tr>
<tr>
<td>Meehan</td>
<td>Sensitive Blood-Based Monitoring of Breast Cancer</td>
</tr>
<tr>
<td>Peacock 1</td>
<td>Investigation into using whole genome shotgun metagenomic data to investigate bacterial virulence factors in otitis media</td>
</tr>
<tr>
<td>Peacock 2</td>
<td>The impact of low level in utero arsenic exposure on susceptibility to infection</td>
</tr>
<tr>
<td>Redwood 1</td>
<td>Distinguishing HCMV strains in human breast milk</td>
</tr>
<tr>
<td>Redwood 2</td>
<td>Within-host competition, the battle of the viruses</td>
</tr>
<tr>
<td>Redwood 3</td>
<td>Do viruses hunt in packs, a search for viral complementation</td>
</tr>
<tr>
<td>Redwood 4</td>
<td>Immune evasion function of the cytomegalovirus gene m15</td>
</tr>
<tr>
<td>Riley 1</td>
<td><em>Clostridium difficile</em> Research</td>
</tr>
<tr>
<td>Robinson B 1</td>
<td>Use of gene sequencing to identify key tumour antigens for therapy</td>
</tr>
<tr>
<td>Robinson C 1</td>
<td>Investigation of the heterogeneity of mesothelioma cell line responses to chemotherapy</td>
</tr>
<tr>
<td>Schoep 1</td>
<td>New uses for old drugs: repurposing drugs to treat childhood brain cancer</td>
</tr>
<tr>
<td>Schoep 2</td>
<td>microRNA and childhood brain tumours</td>
</tr>
<tr>
<td>Tan 1</td>
<td>Improving protective anti-bacterial immune responses in COPD patients by blocking co-inhibitory T-cell receptors</td>
</tr>
<tr>
<td>Walton 1</td>
<td>T cell responses in <em>H. pylori</em> infected hosts</td>
</tr>
<tr>
<td>Xu 1</td>
<td>Molecular Pathology of Skeletal Diseases</td>
</tr>
</tbody>
</table>

### How to apply

**Preference Form** | 27
GOALS AND BENEFITS OF HONOURS

Honours and B. Med. Sci. programmes are offered to BSc graduates with a mark of 65% or better in a major subject from the field of pathobiology, microbiology, immunology or medical genetics. It is the usual path to a PhD programme and a career in research. It may also be undertaken as an isolated experience in research or to gain the edge in a career as a hospital scientist or research assistant.

The course includes a full-time supervised research project, which is presented for evaluation as a thesis. Additional prescribed work includes a research proposal, one seminar on another topic in genetics, pathobiology or immunology, plus a seminar and an oral defence of the findings. Students will be expected to undertake an orientation programme designed to provide a comprehensive introduction to the conduct of original scientific research.

Students should achieve the following on completion of the course:

a. knowledge of a designated area of research and an ability to design further experiments.

b. mastery of experimental techniques, including the skills to record and collate the results of the experiments in an accurate and systematic manner.

c. an ability to analyse and interpret data effectively.

d. an ability to evaluate the relevant literature to assemble a logical background for a study and discuss the significance of the data obtained.

e. an ability to communicate scientific findings with clarity in a thesis and in seminars.

f. an ability to think with originality in the area studied and evaluate the approach taken.

The course will require long hours of work, with little time for other activities. However at the end you will have a thesis that you can show with pride. You will know your own skills, strengths and potential.

We suggest you speak to some of the investigators to help you make the right choice. Then select projects that interest you and write a few brief notes about your choice – ranking the projects as first to fourth choices. The form is at the back of this booklet.

Vacation Projects are available in some laboratories. Scholarships may be available through the groups directly. Although many students continue Honours in the same laboratory, this is not essential.
SUMMARY OF THE HONOURS PROGRAMME

1. Attendance at scientific seminars of general interest is highly recommended.

2. Participation in one week introduction to scientific techniques is compulsory, in the week prior to semester one. The associated short written assignments will be marked.

3. Writing of the Honours project research proposal. This is to be 6-7 pages long and include clear statements of aims, hypotheses to be tested, background, research plan (including a brief description of methods), the significance of the work and references. The proposals will also be presented as oral seminars. The seminars will not be marked.

4. Presentation of one seminar on a topic outside the student’s research programme. These will be in the student’s field of study (pathobiology, microbiology, immunology or genetics). The topics will be provided by student supervisors in consultation with the course co-ordinators.

5. Students should prepare and submit the literature review for their thesis as a pdf file. This will be marked with the thesis at the end of the year but its submission mid year is highly recommended.

6. Students will present a seminar summarising their thesis results towards the end of Second Semester on final completion of their written thesis. This seminar will provide the students with an opportunity to defend their work in front of a scientific audience. The thesis markers do not contribute to the grading of the seminar.

7. There will be a short oral defence of the thesis after the written work and seminars have been marked. This will be attended by the supervisors and markers only.

ASSESSMENT SUMMARY
(This may be varied in MSc preliminary projects)

Research Skills (12 points).
Research Proposal 30%
Assignments based on Orientation week 30%
Seminar outside the field of research (with a summary) 40%

Research Project (36 points)
Thesis 67%*
Final Seminar 13%
Oral defence (incorporating marks from supervisor) 20%

The final Honours grade will be a weighted average of the mark from the two units.
ASSOCIATE PROFESSOR
MANFRED BEILHARZ
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Research Interests: New anticancer therapies involving immunotherapies and/or natural product based therapies.

PROJECTS

1. Immunotherapy for the Treatment of Cancer
   Supervisors: A/Prof Manfred Beilharz

Over the last six years the Beilharz laboratory has developed a new triple immunotherapy against subcutaneous mesotheliomas in mouse models (Kissick et al 2012, 2010a, 2010b, Ireland et al 2012, Needham et al 2006). The triple immunotherapy can cure tumours completely in 100% of Balb/c mice and some 50% of C57BL/6J mice. We have recently demonstrated that once cured, both strains of mice show immunological memory in that the tumour cannot be regrown in these cured mice. Whilst the triple therapy is targeted at the regulatory T cell system, our most recent data suggests a B cell involvement as the sera of cured mice contains IgG antibodies which appear to be tumour specific.

The project will involve: 1. Dendritic cells isolated during triple therapy as these cells are thought to orchestrate both the T and B cell responses. 2. Definition of the role of antibody in triple therapy success. 3. Assessment of autoimmune reactions during triple therapy. A wide variety of immunological, in vivo and molecular techniques will be employed in this project including ELISA, FACS and in vitro tissue culture.

2. The effect of topical tea tree oil on UV-induced DNA damage
   Supervisors: A/Prof Manfred Beilharz

Research carried out in the Beilharz laboratory has shown that topical tea tree oil (TTO) is a promising alternative to current topical chemotherapies. However, the ultimate advance in skin cancer treatment would be to prevent their development. UV-induced inflammation and DNA damage in the skin are early initiating events of precancerous lesions and skin cancers (Halliday, 2005, Berhane et al., 2002). The aim in this project is to investigate TTO’s potential to protect and/or repair against UV damage.

The most frequent measure of UV-induced DNA damage is the formation of cyclo primidine dimers (CPDs), of which the most commonly occurring are thymine dimers. Histological staining and ELISA analysis of extracted DNA from skin of UV irradiated and TTO treated mice will show if any changes in CPD numbers result from treatments applied before or after UV exposure. The tumour suppressor gene, p53, has an important role in the repair and abortion of DNA damaged cells by inducing cell cycle arrest or apoptosis (Burren et al., 1998). qRT-PCR of RNA from skin exposed to UV/treatments will be performed to identify changes in p53 gene expression as it may be a mechanism of increased DNA repair. Additionally, the gene expression levels of matrix metalloproteinase-1 (MMP1), a gene expressed at elevated levels in the skin following UVR exposure will also be quantified as an additional marker of UV damage.

This project will involve:
   1. Examination of a variety of topical TTO formulations to prevent UV-induced DNA damage in Balb/c mice by pre-treating with a selection of TTO formulations prior to a single dose of acute UV exposure
   2. Examination of a variety of topical TTO formulations to repair UV-induced DNA damage in Balb/c mice by exposure to a single dose of acute UV exposure immediately followed by treatment with a selection of topical TTO formulations.

Techniques to be used include immunohistochemistry, RNA and DNA extractions, ELISA, and qRT-PCR.
Hormone Dependent Cancers Group

Our group is interested in the hormone dependent cancers, prostate and breast cancer. These are the most commonly diagnosed cancers of men and women, respectively and although quite distinct, they share common mechanisms of hormone-stimulated growth that are influenced by the activity of other signalling pathways and transcription factors. For example, androgens, acting via the androgen receptor, regulate the growth of both prostate and breast cancers, however the same pathway stimulates the growth of prostate cancers but inhibits the growth of breast cancers. Androgen activity is regulated by the Hedgehog, MAPK and AKT signalling pathways that are highly active in subsets of prostate and breast cancers. In addition to these pathways, prostate and breast cancers express highly specialised factors that regulate the growth of cells in a tissue-specific manner. These include NKX3.1, a prostate-specific homeodomain protein, expression of which is reduced in prostate tumours. Several projects addressing these areas of research are available for 2014.

The Honours project described below and others available in the laboratory may appeal to prospective Honours students with a background in Molecular Biology, Biochemistry, Pharmacology or Pathology. Students will be exposed to a range of techniques including cell culture, RNA/DNA extraction, RT-PCR, DNA cloning, protein expression and analysis, bacterial culture and immunofluorescence microscopy.

PROJECT

1. Interaction Between RMND5 Proteins and Ubiquitin-40s Ribosomal Protein S27a:
RMND5A and RMND5B are two novel proteins of similar structure that we have shown in our laboratory to function as E3 ubiquitin ligases in prostate cancer cells. E3 ubiquitin ligases are enzymes that add ubiquitin to target proteins, signalling their degradation or processing, depending on the number or type of linkage of the ubiquitin molecules. RMND5A and RMND5B interact with the important tumour suppressor of the prostate, NKX3.1 and induce its degradation. Additional studies in our laboratory have shown that RMND5A and RMND5B also interact with Ubiquitin-40s Ribosomal Protein S27a (Ub-S27a), a regulator of cell responsiveness to DNA damage. This project will investigate the interaction of Ub-S27a and S27a with RMND5 proteins and will characterise their effects on RMND5 protein E3 ubiquitin ligase activity and NKX3.1 ubiquitination and function.
Clinical immunology

PROJECT

1. Temporal (giant cell) arteritis

Giant cell arteritis (GCA) is a chronic vasculitis of large- and medium-sized vessels. The prevalence of GCA has been estimated to be 1 in 500 individuals.

Although GCA is characteristically a systemic illness and vascular involvement may be widespread, symptomatic blood vessel inflammation most frequently involves the cranial branches of the arteries that originate from the aortic arch. The most feared complication of GCA, visual loss, is one potential result of the cranial arteritis associated with this disease.

The manifestations of GCA are protean and highly variable from patient to patient. In addition, some symptoms are transient, making the clinical diagnosis difficult.

The gold standard diagnostic tool remains a temporal artery biopsy. Negative temporal artery biopsy occurs in up to 40 percent of patients suspected of GCA who are referred for biopsy. In addition, a biopsy is often difficult to obtain in a timely manner.

Although some laboratory tests such as a high ESR aid the clinical diagnosis, this test is rather non-specific. Recently, antibodies against ferritin heavy chain have been shown to be associated with giant cell arteritis and have been proposed as a novel reliable diagnostic test for the disease.

PROJECT Outline

Improving the diagnosis of temporal arteritis

1. Development of an anti-ferritin ELISA for the diagnosis of temporal arteritis in Western Australia
2. Critical evaluation of such an ELISA with testing of patients with biopsy proven temporal arteritis and a range of controls (which includes other vasculitides, patients with SLE, fibromyalgia, Rheumatoid arthritis and healthy age and gender matched controls).
3. Testing of Cytokine profiles in the above patient groups to find if this adds to diagnostic algorithm of temporal arteritis
4. Presentation of the finding at a national Pathology meeting and preparation of a manuscript in a Pathology journal

Learning objectives:
Establishment of a laboratory test and its critical evaluation
ELISA testing
Cytokine bead array and Luminex technology
Preparation of manuscript and presentation at a national meeting
Translational Renal Research Group (TRRG).

PROJECT

1. Analysis of the biological effects of bacterial pathogens known to cause peritoneal dialysis-related peritonitis on mesothelial cell function

Primary supervisors  Dr. Aron Chakera, Dr. Amanda McGuire
Other supervisors  Dr. Gary Lee, Dr. Sally Lansley

Project description

Peritoneal dialysis (PD) is the dialysis modality of choice for many patients with end-stage renal disease. One of the major complications of therapy is the development of peritonitis, which is associated with significant morbidity and economic costs, and may be a contributing factor in the deaths of up to 16% of patients on PD (ISPD 2010 PDI 2010; 30:393). Even in patients who recover from peritonitis, infections can result in shortened modality survival or modality failure. Currently, PD peritonitis occurs on average, once in every 27 months of peritoneal dialysis therapy in Western Australia, with the most commonly isolated bacterial pathogens being Staphylococci species and members of the Enterobacteriaceae family. Approximately 27% of patients are unable to continue with PD, usually due to the development of adhesions and ultrafiltration failure.

Organisms that are capable of infecting the peritoneum usually possess an array of virulence factors, including exotoxins, endotoxins and adhesins that may damage the peritoneum and initiate a cascade of inflammatory events. For example, the binding of S. aureus to human mesothelial cells depends on adhesins such as the cell wall lipoteichoic acid, teichoic acid, and protein A (Finlay and Falkow, 1997), while Streptococcus pyogenes pyogenes binds using its surface lipoteichoic acid and a fibronectin binding protein. Facilitating these interactions are cell surface molecules on the mesothelial cells that serve as receptors for the bacterial determinants. These include toll-like receptors, monosialyl containing receptors and a variety of glycoproteins (Ohtsuka et al., 1997). Once attached to the mesothelial cell surface, pathogenic bacteria damage the host cells, which may then detach from the basement membrane leading to desquamation and denudation of the surface, potentiating the inflammatory response.

Few studies have studied the effect of bacteria on mesothelial cells in a systematic fashion. From a range of small studies, it had been shown that exposing peritoneal mesothelial cells to heat killed E. coli results in increased IL-6, IL-8, RANTES and MCP-1 expression, whereas treatment with heat killed S. aureus increases IL-6 and IL-8 but has no significant effect on RANTES or MCP-1, suggesting different signalling cascades activated by lipoteichoic acids or lipopolysaccharides (Kinnaert et al., 1996). Activation of different signalling pathways was also observed in a separate study comparing the IL-8 response of peritoneal mesothelial cells treated with live or heat killed S. aureus, Staphylococcus epidermidis or E. coli (Visser et al., 1995). S. aureus may also induce VEGF production by mesothelial cells in a concentration-dependent manner, causing a decrease in electrical resistance of mesothelial monolayers and enhanced protein permeability across mesothelial cell monolayers (Mohammed et al., 2000).

Despite the importance of PD-peritonitis, the biology of the interactions between bacteria and the peritoneum remain poorly understood. This project will employ a variety of techniques to study the biological responses of peritoneal mesothelium to bacterial exposure, including cell culture, cytokine ELISAs, flow cytometry, adhesion, migration and viability assays. Using pure cultures of bacteria known to cause PD peritonitis, as well as mutant strains missing specific proteins or with altered surface properties, we will be able to improve our understanding of the mechanism(s) by which different bacterial species damage the mesothelium and influence the resultant cellular response. This knowledge may translate into new targets and approaches for the management of PD-peritonitis and will have direct relevance to patients with renal failure who are on PD.

The Translational Renal Research Group, consisting of the group Head and a Research Assistant Professor, are part of the UWA School of Medicine and Pharmacology and are physically located within WAIMR, the Western Australian Institute for Medical Research at the QEII Medical Centre. The group has close ties with the Sir Charles Gairdner Hospital Renal Unit.
ADJ ASSOC PROF
RODNEY DILLEY,
DR ROBERT MARANO,
ADJ PROF R EIKELBOOM,
PROF P FRIEDLAND,
PROF M ATLAS

Molecular and Cellular Otolaryngology Laboratory
Ear Science Institute Australia
School of Surgery UWA, Sir Charles Gairdner Hospital, Nedlands WA
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PROJECTS

Defects in the auditory system are amongst the most often treated ailments. Research at Ear Sciences Centre aims to improve knowledge in this area and develop new treatments for ear health.

The main areas of research are:

1. Biomaterials approaches to developing patch grafts for eardrums

Failure to heal a tympanic membrane perforation is relatively common and requires new approaches to surgical repair. We have developed a tissue engineering method that improves healing rate and rapidly restores hearing. Projects for Honours and PhD students are based around this model and provide opportunities for BScHons and PhD students.

Specific projects include:

- Optimizing scaffold material for integration and degradation on the tympanic membrane.
- Regulating epithelial repair and angiogenesis through scaffold material design and implant protocols.
- Examination of vibroacoustic properties of tympanic membrane grafts using laser vibrometry.
- Identifying stem cells in the tympanic membrane and their potential surgical use for regeneration.
- Effects of scaffold stiffness on angiogenesis in tissue engineering
- The role of biofilms in the development of chronic tympanic membrane perforations.

2. Hearing loss

Inner ear pathology research indicates that there are multiple mechanisms which contribute to age-related and sudden sensorineural hearing loss. We are investigating hormonal and cardiovascular mechanisms related to these conditions.

- A novel hormonal mechanism for age related hearing loss – cochlear organ culture models
- Investigating the relationship between cardiovascular disease and hearing loss:
  - New mechanisms based on atherogenic environments and neurovascular interactions.
  - Epidemiological approach to identifying a role for cardiovascular disease in hearing loss.
- Vascular mechanisms for sudden sensorineural hearing loss.

3. Promoting tissue formation through angiogenesis

Mesenchymal stem cells are substantial sources of growth factors. They are being investigated for their ability to support and promote tissue formation in tissue engineering and tissue repair. The contribution of various adult stem cell populations to promoting wound healing and angiogenesis in engineered tissues and their regulation with matrix scaffold materials will be investigated.

- Role of mesenchymal stem cells in tympanic membrane repair
- Utilizing stem cell paracrine activity for engineering significant volumes of vascularized tissue
Clinical Immunology and Transplant Biology Laboratory

Kidney Transplantation Research

Kidney transplantation is a life saving procedure for many patients however immune responses against the transplanted organ can lead to organ rejection and graft failure. These immune responses are mediated by T cells which recognise foreign proteins (HLA molecules) present on the surface of foreign cells. Improving transplant patient survival requires a better understanding of immune mechanisms involved with T cell mediated rejection and devising methods to predict and/or prevent organ rejection, with the ultimate aim of tolerance induction. Techniques used include virus specific T cell cultures, T cell receptor studies, flow cytometry including studying immune responses with the use of tetramers, state-of-the-art HLA typing, cytokine assays and others. Research studies are performed using samples obtained from transplant recipients.

PROJECTS

1. The impact of public T cell receptor responses on the alloreactive T cell repertoire

Kidney transplantation is a life saving procedure for many Australians however immune-mediated graft rejection and chronic allograft nephropathy continue to cause significant patient morbidity and can lead to graft loss. Virus infection, particularly CMV, is associated with acute rejection and impaired long-term renal graft survival. Work by our group and others makes it increasingly clear that alloreactivity (graft rejection) by virus-activated memory T-cells explains the high frequency of T-cell responses to the foreign graft. Study of alloreactivity by “public” T-cell responses, in which T cells bearing identical T-cell receptors (TCRs) are observed to dominate the response to the same antigenic epitope in multiple individuals, may be useful to define unacceptable or “taboo” HLA mismatches in clinical kidney transplantation, thereby preventing rejection.

2. Tolerance induction using overexpression of FoxP3 within T cells recognising foreign transplanted tissue

Kidney transplantation is a life saving procedure for many Australians however T cell mediated immune responses can damage the graft. Graft rejection is caused by alloreactive T cells whereas naturally occurring regulatory T cells express FoxP3 and can control the immune response to foreign transplanted tissue. The aim of the current project is to stably transfect FoxP3 into an alloreactive T cell line with the expectation that overexpression of FoxP3 in the alloreactive CD4 T cells may induce regulatory T cell function in the cells that recognise the foreign graft. Induction of allo-specific regulatory T cell function may have important implications for cellular immunotherapy for tolerance to transplanted organs.
Targeting T regulatory cells in cancer

PROJECTS

1. Targeted immunomodulation of tumor resident regulatory T cells as an effective therapy to treat disseminated melanoma

Melanoma is an aggressive cancer with poor prognosis if left untreated. Suppression of anti-tumour immunity by regulatory T cells (Treg) significantly limits the efficacy of many anti-tumour immunotherapies. Finding immunotherapy agents that specifically target Treg without affecting other immune cell populations has been problematic. Here, we propose to use a strategy that will enable us to identify and target tumour resident Treg with the aim of generating effective therapy in a pre-clinical model of malignant melanoma.

We will first identify markers that are unique to the suppressive Treg and use antibodies that specifically target these markers to deplete the tumour resident Treg. We will then assess whether this strategy is sufficient to induce strong tumour-specific immune responses that are also effective against non-treated tumours, located at other sites in the body to mimic metastatic disease. Importantly, this strategy utilises reagents that are either currently approved for individual clinical use (ipilimumab; anti-CTLA-4 mAb) or are being tested in clinical trials (anti-OX40 mAb) for the treatment of melanoma.

Aim 1: Assess the expression profile of co-signalling receptors on tumour resident antigen-specific regulatory T cells. We will identify surface markers that discriminate between tumour resident Treg and effector lymphocytes so they may be used as targets for immunotherapy. **Hypothesis:** Tumour resident Treg express high levels of T cell co-signalling receptors OX40 and CTLA-4 relative to other infiltrating lymphocytes.

- We will use multi-parametric flow cytometry to assess the expression profile of co-signalling molecules on T cells isolated from several sites (blood, tumour draining and non-draining lymph nodes, spleen and tumour) from tumour bearing mice.
- We will measure OX40 and CTLA-4 expression on B (B220) and T (CD3) lymphocytes, as well as CD4+, CD8+ and Treg (CD4+FoxP3+CD25+) T cell subsets.

Aim 2: Assess the anti-tumour efficacy of the immunomodulatory mAbs αCTLA-4 + αOX40 on distal untreated tumour. We will investigate the efficacy of immunomodulatory antibodies directed toward the best combination of surface markers that identify tumour resident Treg identified in Aim 1 as an effective therapy for disseminated disease. **Hypothesis:** Triple therapy (adjuvant + αCTLA-4 + αOX40) at a single tumour site promotes systemic, long lasting anti-tumour immunity.

- Mice will be inoculated with 2 separate melanoma tumours (1 primary tumour, and 1 distal tumour to mimic metastasis).
- The primary tumour will be treated with the best treatment combination identified in Aim 1 (ie. anti-CTLA4 and/or anti-OX40 to remove tumour resident T regs, in conjunction with a strong adjuvant, ie. CpG.)
- We will see if treating the primary tumour with this treatment combination is enough to induce a strong, systemic, anti-tumour response, by monitoring the growth of the untreated distal tumour.
Once we have identified an effective treatment modality (i.e. adjuvant+ αCTLA-4+αOX40) we will perform additional experiments to optimise delivery route and dose of targeting antibodies.

Any surviving mice will be assessed for immunological memory by either intravenous (to mimic lung metastases) or s.c. rechallenge on the left flank slightly above where the original distal tumour was inoculated.

This project will involve:
Development of basic laboratory skills associated with tissue culture, tissue preparation/immune cell isolation and flow cytometry acquisition and analysis.
Animal handling skills: general maintenance and monitoring. Handling, tumour and immune cell inoculation, administration of therapeutic drugs, vivisection.

2. Targeting regulatory T cells to improve combination chemo-immunotherapy treatment of solid tumours

Combination chemo-immunotherapy is a promising treatment option for many solid malignancies, although it is not 100% effective. This project is based on the recent observations that chemotherapy can stimulate an anti-cancer immune response but this response cannot destroy the tumour in patients because it is 'restrained' by regulatory T cells (Treg). This presents an exciting therapeutic opportunity - to unmask effective anti-cancer immune activity by turning off these restraining T cells. This could generate effective therapy for established solid cancers, such as malignant mesothelioma (MM) and restore effective anti-cancer immune surveillance and thus prevent treatment relapse and additional tumour development.

Our research incorporates the use of two novel strategies to inhibit Tregs during treatment with first line chemotherapy agents to assess the role of Tregs in “restraining” the development of anti-cancer immune responses. Furthermore, the proposed research utilises three distinct murine tumour types, to assess the efficacy of Treg removal in different tumour models. The combination of distinct tumour models in conjunction with the use of novel strategies to inhibit Treg provides a useful platform with which to assess novel therapeutic strategies to improve the treatment of solid cancers.

Hypothesis:
Targeted removal of regulatory T cells will enhance anti-tumour immunity and lead to greater efficacy of chemo-immunotherapies used to treat solid cancers.

Aim 1: Identify which chemotherapy is most efficient in the absence of Treg in different tumour models.
- Treat tumour-bearing BALB/c-FoxP3.dtr transgenic mice with individual chemotherapies (eg. gemcitabine, cisplatin) and assess treatment efficacy based on tumour growth and survival
- Compare ABI-HA (mesothelioma), CT44 (colon carcinoma) and Renca HA (renal carcinoma) tumour lines. These tumour cells have been transfected with the influenza ‘HA’ antigen, which acts as a tumour ‘neo’-antigen to allow us to look for tumour-specific immune responses.
- Tumour growth & survival as primary read out.

Aim 2: Determine optimal dose / schedule for best combination treatment identified in aim 1.
- Optimise Treg depletion schedule using the best chemotherapy + Treg depletion combination for each tumour model.
- Compare neoadjuvant (before chemotherapy) and adjuvant (after chemotherapy) Treg depletion with concurrent combination therapy.
- Tumour growth & survival as primary read out.

Aim3: Determine immunological mechanisms that promote enhanced anti-tumour immunity following combination therapy
Treat tumour-bearing animals with optimised chemo-immunotherapy.
- Harvest tissues (lymph nodes, spleen, tumour) at specific time points to characterise anti-tumour response (ie. T cell activation, proliferation)
- Adoptive transfer of HA-specific lymphocytes to determine tumour-specific response
- Look for memory T cells or signs of established immunological memory in animals where the tumour has completely regressed (ie. ‘cured’).

This project will involve:
Development of basic laboratory skills associated with tissue culture, tissue preparation/immune cell isolation and flow cytometry acquisition and analysis.
Animal handling skills: general maintenance and monitoring. Handling, tumour and immune cell inoculation, administration of therapeutic drugs, vivisection.
1. Isotype diversification of IgG antibodies and activation of antiviral accessory cell responses in the control of HIV infection

Background

Some people with HIV infection are able to control the infection without the use of drug therapy, and are referred to as HIV controllers. These individuals produce a ‘protective’ immune response against proteins of the HIV core that are encoded by Gag. CD8+ T cell responses restricted by ‘protective’ HLA-B alleles play a dominant role but other immune responses, including antibodies, may also contribute. Elucidating the mechanisms of immune control in HIV controllers may facilitate the production of vaccines for treatment and prevention of HIV infection.

We have shown that IgG antibodies to the HIV Gag protein p24, that have undergone isotype diversification, are more prominent in HIV controllers than non-controllers. We hypothesised that these antibodies are more effective in activating antiviral accessory cell responses, particularly those mediated by plasmacytoid dendritic cells (pDCs). To investigate this, we have established a novel assay to assess activation of a pDC cell line (Gen2.2) by antibodies to Gag proteins and also established a repository of sera from Australian and American HIV controllers. This project will continue on-going studies.

Research plan

1. To assess isotype diversification of IgG antibodies to HIV p24 further, sera from HIV controllers and non-controllers will be assayed for the magnitude and breadth of IgG4 antibodies to p24, using native p24 and p24 peptides as antigens in ELISAs, and compared with IgG1 antibodies.

2. To investigate the effects of IgG antibodies to HIV Gag proteins on pDC activation, sera from controllers and non-controllers will be incubated with HIV core particles and then phagocytosed by Gen2.2 cells and interferon-alpha levels assayed in culture supernatants by ELISA.

Techniques

This project will provide experience in cell culture and ELISAs.

Further reading


PROJECT

1. Therapeutic efficacy of novel antimicrobials against infectious disease agents
(3 year project funding under development, commencing 2014)

Background: Antibiotic resistance is beginning to erode the efficacy of some of our most valuable antibiotics. Resistance to the carbapenems and extended spectrum cephalosporins is starting to establish untreatable strains of common hospital bacteria such as Klebsiella and Acinetobacter. Hospital outbreaks have caused closure of intensive care units. New antimicrobials are urgently needed, with actions that are radically different from those already in use. We have an opportunity to work on a new family of synthetic antimicrobial compounds that target a group of bacterial proteins called immunophilins, associated with cell invasion. This new antimicrobial family targets bacteria as diverse as Burkholderia, Legionella, Klebsiella, Salmonella, Yersinia and Mycobacteria. This project has potential to be converted into a full PhD programme, subject to progress during a preliminary Honours year and will involve collaboration with an international research group, PaLM and PathWest. This new mode of antimicrobial action demands new methods of antimicrobial susceptibility measurement based on integrated systems biology (multiple Omics).

In this project the student will:
1. Establish a working bacterial culture collection of Klebsiella pneumoniae, Burkholderia pseudomallei, Burkholderia thailandensis, Yersinia enterocolitica, Salmonella enterica and Mycobacterium fortuitum.
2. Perform disk diffusion, broth microdilution and plate incorporation methods.
3. Develop advanced susceptibility methods using acoustic focused flow cytometry and gene expression systems to demonstrate immunophilin effects on bacterial viability, membrane integrity and bacterial physiology.

Methods and techniques: conventional, cell culture and intracellular antimicrobial susceptibility tests, acoustic flow cytometry, bacterial cell biology, expression PCR and RNA, enhanced biosafety methods

References:
ASSOC. PROFESSOR CHERYL JOHANSEN, ASSOC. PROFESSOR ALLISON IMRIE
Rm 1.31 L Block, Arbovirus Surveillance and Research Laboratory, School of Pathology and Laboratory Medicine UWA, QEII Medical Centre, Nedlands. Email: cheryl.johansen@uwa.edu.au; allison.imrie@uwa.edu.au Telephone: 9346 4656

PROJECT

1. Characterisation and application of monoclonal antibodies to flavivirus NS1 proteins

Background:
Members of the mosquito-borne Japanese encephalitis serological complex are genetically very closely related, which often translates to serological cross-reactivity in assays designed to detect infection with these viruses. This is particularly the case for Murray Valley encephalitis virus (MVEV), Japanese encephalitis virus (JEV) and Alfuy virus (ALFV), making serological diagnosis of infection in sentinel animals and humans difficult, especially where these viruses co-circulate. This project aims to develop diagnostic tools and virus-specific serological assays by assessment of monoclonal antibodies (Mabs) raised to purified recombinant NS1 proteins from Murray Valley encephalitis virus, Japanese encephalitis virus and Alfuy virus. By using purified antigen as an immunogen and in the down-stream immunoassays, it is envisaged that these diagnostic reagents will facilitate detection of infection with, and discrimination between, infections with these closely related flaviviruses in sentinel animals and in serum from clinical cases of disease.

Project description:
Purified NS1 of MVEV, JEV and ALFV from recombinant baculoviruses will be used as antigens for immunological challenge of mice (for production of Mabs) and for use as a diagnostic reagent in down-stream virus-specific ELISAs. Mouse immunisations and production of hybridomas will be performed at the WA Monoclonal Antibody Facility (WAIMR). Hybridomas will be cloned and expanded and Mabs to MVEV, JEV and ALFV will be characterised (immunoglobulin class/subclass, epitope structure) and tested for specificity in the Arbovirus Surveillance and Research Laboratory (UWA). Optimised concentrations of Mabs, antigens and conjugated antibodies will be used to test the antibody-blocking capacity of each Mab, and test each Mab against a panel of sera from laboratory-infected rabbits, chickens and pigs with MVEV, JEV and/or ALFV antibodies to investigate specificity and sensitivity of the blocking ELISAs. Established assays using the Mab 3H6 (which detects all flavivirus infections) and 10C6 (MVE-specific assay which may also be inhibited by antibodies to JEV and ALFV) will be used as controls. The most suitable candidate Mabs (ie those most specific, with no or limited cross-reactivity) will be tested against a large panel of sera from naturally infected chickens in a serum bank at UWA from the mid-1990s that were positive in the MVEV-specific (10C6) blocking ELISA. Pre-seroconversion, acute phase and ‘convalescent’ sera will be tested from sentinel chickens in years when both MVEV and ALFV were concurrently isolated from mosquitoes (5 yrs), as well as years when no ALFV activity was detected in mosquitoes (7 yrs), and years when ALFV was the most commonly isolated flavivirus in northern WA (1 yr). Human JEV vaccination sera and sera from patients serologically diagnosed with MVE infection at PathWest Laboratory Medicine WA will also be tested for specificity. Alternative ELISA formats will also be investigated. New assays will be compared with the epitope blocking ELISAs currently used and the ‘gold-standard’ neutralisation assay. It is envisaged this project will greatly improve specificity of serological assays for detection of flavivirus-specific infections for surveillance and diagnosis.

Methods to be used include cell culture (hybridoma, insect and mammalian), ELISAs, Western Blot analyses neutralisation assays.
Bacterial Pathogens Causing Sepsis

Neisseria meningitidis and N. gonorrhoeae are two closely related obligate human pathogens. N. meningitidis is the causative agent of epidemic meningococcal meningitis and septic shock. It colonises mucosal surfaces of the nasopharynx and in susceptible individuals, particularly children under the age of two years, the bacterium becomes systemic resulting in fatal bacteremia. Despite the continued sensitivity of the meningococcus to multiple widely available antibiotics, including penicillin, the case-fatality ratio for meningococcal disease remains around 10%–14% (CDC, unpublished data, 2004). Vaccines have been developed based on the polysaccharide capsules to prevent community spread and therefore have become an effective means of reducing meningococcal disease. However, in the West Australian community, type B remains prevalent and there is no vaccine against this organism since the type B polysaccharide is a poor immunogen.

Neisseria gonorrhoeae on the other hand, is the causative agent of the sexually transmitted disease (STD) gonorrhoea. Comparatively, the rate of disease in developing nations is approximately ten times that of developed countries and globally approximately 20-60 million new cases are reported per annum (WHO). In males, gonococcal infection is generally acute and resolves rapidly with treatment. However, higher morbidity is seen in women as the infection remains asymptomatic and without treatment progresses to pelvic inflammatory disease (PID) resulting in infertility in approximately one third of patients. Unlike meningococci, this organism is increasingly resistant to antibiotics with a recent report of the emergence of a “superbug” resistant to all antibiotics. To date no successful vaccine strategies have been developed for this organism, primarily because the cell surface proteins expressed by this organism are highly antigenically variable, thus eliciting limited immunological protection against other strains. As a result individuals can contract the disease multiple times throughout their lifetime.

My group is interested in three different facets of these important human pathogens:

a. Endotoxin is the primary toxin that results in septic shock and death of the patient. We are interested in understanding the biosynthesis pathway and regulatory networks controlling the production of this important toxin.

b. The regulatory networks within these pathogens that are triggered during attachment to the human nasopharynx.

c. Understanding the invasive mechanisms used by these bacteria using models of invasion into tissue culture cell lines.

Prospective Honours students with a background in Molecular Biology or Biochemistry or Microbiology are particularly encouraged to apply.

The projects below are descriptions of basic projects that can be undertaken as is or can be modified to suit your skills and interests.

PROJECTS

1. Outer membrane biogenesis in Gram negative bacteria

Neisserial endotoxin (or lipopoligosaccharide [LOS]) is a glycolipid related to E. coli lipopolysaccharide and contains lipid A attached to a conserved outer core of sugars. Although the complete biosynthetic pathway of this structure has been determined, very little is known about transportation of this structure across the inner and outer membranes of Gram – negative bacteria. In an effort to understand the LOS transport pathway, we will examine whether the LOS biosynthesis enzymes form an interactome for efficient biosynthesis and transport of LOS. To do this we will clone a number of LOS biosynthesis enzymes and tag them with known epitopes which will allow us to specifically detect each protein. The location of each tagged protein in the cell will be determined by cell fractionation and Western Immunoblot. Candidate proteins that may interact intracellularly
will be assessed using a protein two-hybrid system. With this information, we hope to eventually understand enough about how Gram negative bacteria make LOS that we can then develop anti-bacterial agents against this unique process.

2. Endotoxin synthesis in *Neisseria sp.*
   *This project is conducted in collaboration with Dr Keith Stubbs and Dr Alice Vrielink.*

*N. meningitidis* and *N. gonorrhoeae* produce similar endotoxins or lipooligosaccharide (LOS) structures. We are interested in understanding the structure and function of the enzymes involved in this pathway (Figure 1). We are currently working on NmLptA which is a phosphoethanolamine transferase which adds phosphoethanolamine to the LOS in the periplasm. To understand how this enzyme works we have crystallised the protein with Dr Vrielink’s lab. Dr Stubbs is developing enzyme substrates so that we can assess how the enzymes work. My lab is making mutant versions of the proteins which we will examine for function in the bacteria. We have designed small molecule inhibitors against these enzymes and are assessing whether these molecules could have anti-bacterial activity.

3. A molecular epidemiology approach to understanding meningococcal disease in Western Australia
   *With Dr Tim Perkins, Dr David Speers (PathWest), Dr Tony Keil (PathWest)*

Since the neisserial surface proteins are antigenically diverse, vaccine design using protein antigens for serogroup B meningococci and gonococci has so far not been successful. Recent advances in vaccine design have led to the identification of four antigens for inclusion in a vaccine which should be protective against all meningococci causing disease (2). However, these antigens are variable in composition and it is known that some disease causing meningococci do not have these antigens at all (3). Currently it is unknown what percentage of strains in Western Australia does not express these antigens and hence would be resistant to this vaccination approach. It is also unknown how variable the antigens presented by isolates in Western Australia could be.

The aim of this project is to start analyzing a 10 yr collection of disease causing meningococcal strains from Western Australia to understand the genetic diversity in these isolates.


4. Protein folding in the periplasm of Gram negative bacteria.

All proteins are made in the cytoplasm of bacterial cells. However, some are sent to the periplasm and a few others are secreted. These proteins often contain special bonds, called disulphide bonds, to keep them stable and to prevent degradation by proteases. The disulphide bonds are donated to the proteins by enzymes called the oxidoreductases (DshA). We have completed the structural analysis of the oxidoreductases and know how they work enzymatically. We also know the identity of some of their substrates. In Neisseria, the oxidoreductases are important for the proper folding and function of the pili which are important for attachment to host cells. We are particularly interested in how the oxidoreductases recognise their substrates and if we can interfere with this process. This project seeks to understand how the oxidoreductases interact with pilin structure and function in Neisseria.


Dependent upon the projects you pick, you will learn the following techniques:

1. Bacterial genetics including culture and mutagenesis
2. Molecular biology techniques including DNA/RNA extraction, PCR, cloning, transformation
3. Protein manipulation including purification, development of enzyme assays, immunoassays
4. Microscopy techniques including confocal microscopy
5. Cell biology techniques including culturing immortalised cell line
Molecular Epidemiology

Professor Barry Marshall continues to treat patients for Helicobacter pylori infection at Sir Charles Gairdner Hospital. Almost 100 patients are referred to his clinic annually after failing an initial H. pylori treatment therapy. Tailored follow-up treatment regimes include substantial isolate phenotyping and storage of culturable strains. Prof Marshall’s strain collection is unique, containing isolates from patients dating back to 1998. Whole genome sequencing can now be performed more cheaply than multi-locus sequence typing (MLST) but can provide more information as to the provenance of each strain.

1. **Longitudinal molecular epidemiology of H. pylori using whole genome sequencing**
   
   *With Assist. Professor Tim Perkins* (email: tim.perkins@uwa.edu.au, tel 9287 6214) and *Dr Alfred Tay* (email: alfred.tay@uwa.edu.au, tel 9346 4817).

   *H. pylori* molecular epidemiology has recently been used to trace the putative human migratory patterns since dispersal “out of Africa” 60,000 years ago (60kya)(1). This model is heavily based on the observation that transmission generally occurs vertically (parents to progeny) and that strains, despite high intra-species variability, exhibit very little inter-strain recombination. Environmental transmission, or horizontal transmission, is not considered the most frequent mode, however, it remains important to consider. Perth has recently undergone a rapid expansion in population, harbouring a diverse population of immigrants, many of whom are likely to arrive in Australia infected by phylogeographically diverse strains.

   This project will combine the *H. pylori* genomics with robust patient metadata to analyse the composition of the isolate collection over a ten year period.

   The project will involve development of the following skillsets –

   - Culturing and genomic DNA isolation of a large number of strains from the present collection
   - PCR and high-throughput DNA sequencing
   - Metadata collation and analysis
   - Basic bioinformatics

   Examples of hypotheses tested as follows –

   - The epidemiological profile has of recent *H. pylori* isolates differs from those isolated 10 years previously.
   - Particular phylotypes are only found in Perth, WA.
   - *H. pylori* infection is environmentally acquired in Perth, WA.
   - Particular phylotypes are specific to areas of Perth.

References

PROJECT

1. Sensitive Blood-Based Monitoring of Breast Cancer

Background:
Breast cancer is the most common malignancy, and one of the leading causes of death in women. Therapeutic strategies include surgery, chemotherapy, radiotherapy and hormone therapies. Despite optimal therapy being administered, disease relapse or recurrence occurs in up to 30% of patients. This is a major cause of morbidity and mortality. Currently, standard monitoring following treatment includes physical examination, imaging and measurement of serum biomarkers. These are generally non-specific and lack the sensitivity to detect disease relapse sufficiently early to implement alternative treatments. Therefore, there is a need for better markers to monitor response to treatment and for the early detection of recurrent disease.

In this project the primary aim is to define a set of breast cancer specific mutations. This robust data set will then be the basis for development of a blood-based tests that will specifically identify biomarkers unique to the patient's tumour. The ultimate objective is to advance the ability to identify patients that are at high risk of relapse following therapy and thereby offer more intensive therapy at the time of diagnosis.

In this project the student will use next generation sequencing (NGS) to individually profile 50 key oncogenes and tumour suppressor genes that are frequently mutated in breast cancer.

- DNA will be sequenced from 25 tumour specimens obtained from breast cancer patients.
- DNA sequences will be analysed to generate a list of DNA mutations that are specific for the breast cancer, by comparison with each patient’s constitutional (germline) DNA obtained from buccal swabs.

Methods and techniques: NGS (DNA extraction, library construction, chip loading), bioinformatics.
Pathogen Immunogenomics

Research undertaken by my group focuses on the interaction of the pathogen with the host. We utilize molecular biological and genomic methodologies to investigate a range of pathogens in an effort to understand the mechanisms that induce pathogenicity in the host. Our current focus is on the intra macrophage pathogen *Leishmania* and metagenomics of common bacterial infections.

**PROJECTS**

1. **Investigation into using whole genome shotgun metagenomic data to investigate bacterial virulence factors in otitis media**  
   Supervisors A/Prof Christopher Peacock ([christopher.peacock@uwa.edu.au](mailto:christopher.peacock@uwa.edu.au)) Dr Tim Perkins (School of Pathology and Laboratory Medicine), Dr Sarra Jamieson (TICHR).

Otitis media (OM, middle ear infection) is the most common reason for antibiotic prescription and the second most common reason for a child to undergo surgery. In the Australian Aboriginal population OM is almost universal, with the highest reported rates of OM worldwide. The burden on health services is immense with an estimated cost of $380 million in Australia alone in 2008. Antibiotic treatment is usually ineffective due to the persistence of antibiotic-resistant bacteria, often surviving in biofilms. Metagenomics is the genetic study of the complete microbial community in a sample. While it is often applied to environmental samples there are numerous technical issues that make it more challenging in the clinical sample. Currently, most metagenomic studies in human samples are limited to amplification of variable regions of the 16S rRNA locus. While this informs on the diversity of bacteria in the sample, it provides no information on the potential virulence factors involved. This study will compare the sequence data from children with severe OM to those that are resistant to developing the disease. Bacteria in a clinical environment commonly exist in complex communities, including those in the middle ear during OM. In addition there is increasing evidence that some bacteria found as commensal organisms can protect against the colonization and proliferation of pathogens. This project will attempt to identify those that may contribute to resistance to OM with the aim of developing a novel probiotic treatment. The mode of action of such bacteria will be investigated using a microfluidic imaging system.

The project will involve
(a) Collection and processing of samples  
(b) Molecular biological techniques including, DNA extraction, qPCR.  
(c) Preparation of sequencing libraries.  
(d) Analysis of high throughput sequence data.

This project will provide the student with experience in both the molecular laboratory area and computational work with bioinformatic analysis of large amounts of metagenomic sequence data. The project will be undertaken both with PALM and the Telethon Institute for Child Health.
2. The impact of low level *in utero* arsenic exposure on susceptibility to infection

*With Dr Graeme Zosky (Telethon Institute of Child Health)*

Arsenic is a ubiquitous element that has contaminated water supplies in many countries leading to millions of people suffering from a range of diseases and illnesses. In the Indian subcontinent exposure in drinking water due to contaminating environmental arsenic has led to the largest ever recorded instance of mass poisoning with many millions affected over 3 decades. It is becoming increasingly apparent that arsenic also affects the immune system. However the mechanisms, which appear to be diverse and complex, have not been well defined. *Importantly, there is increasing evidence that while the direct effects of arsenic are significant, a more serious issue relates to exposure* *in utero* *which represents a period of increased sensitivity*. There is recent data showing that maternal processing of inorganic arsenic interferes with epigenetic programming of the fetal genome during the defining period of embryogenesis. Critically, unlike adult exposure, this appears to require only low levels of arsenic, similar to those found in many regions affected by environmental contamination. *This group has recently demonstrated that pregnant mice exposed to low levels of arsenic in the drinking water have significant effects on the regulatory epigenetic reprogramming that occurs in utero producing offspring that are more susceptible to respiratory infection than adult mice exposed to higher doses*. This project will utilize a defined animal model and *Leishmania*, a pathogen that has intricate interactions with the innate and acquired cell mediated immune response and in particular macrophages and neutrophils, to determine the mechanisms involved. In Australia there is environmental groundwater contamination of inorganic arsenic in large areas of Western Australia, Northern Territory and South eastern regions of Australia. The critical question being addressed by this research relates to the poorly documented effects of low level exposure *in utero* on human health, specifically the impact on the sensitive reprogramming of the epigenome during embryonic development. While the effects of high level chronic and acute exposure in adults are well documented, the more subtle but potentially serious effects on the genome during gestation are not. This study could have far reaching implications in the way inorganic arsenic is seen in relation to human health.

The project will involve
(a) Isolation and culture of mouse cells and *Leishmania* parasites.
(b) Molecular biological techniques including, RNA extraction, rtPCR, ELISA.
(c) A range of data analysis of both expression data and the output from infection experiments.
(d) Flow cell cytometry.

This project will provide a range of laboratory experience in cell culture, molecular biology and analysis of gene expression. The project will be undertaken both with PALM and the Telethon Institute for Child Health.

References:

Cytomegalovirus Research Group

The Cytomegalovirus Research Group is interested in the investigation of all aspects of growth and pathogenesis of these fascinating betaherpesviruses. Murine cytomegalovirus (MCMV) is a mouse-specific betaherpesvirus that is commonly used as a model for human cytomegalovirus (HCMV) infection. MCMV has similar growth characteristics to HCMV and induces similar disease states. Our group is interested in the natural genetic variation found in strains of MCMV that have been isolated from wild mice and the effect of genetic variability on the pathogenicity of the virus. In addition we are interested in the complex interactions that occur between these genetically distinct strains of MCMV when more than one strain infects the same mouse. This is because it is becoming increasingly clear that many, and possibly most, infections are caused by multi-strain infections. Multi-strain infections have the potential to alter disease outcome in quite profound ways, including increasing pathogen transmissibility, enhancing the acquisition of drug resistance or increasing the evolution of virulence. We are using MCMV to address some of these potential outcomes.

Additionally, we have demonstrated over many years that MCMV is a remarkably effective vector for the delivery of foreign antigen to vaccinated animals. A number of immunogenic antigens such as ovalbumin, haemagglutinin, and various reproductive antigens have been incorporated into the viral genome, and rapid and long-lived immune responses have been induced. Currently, we are investigating the efficacy of the recombinant vaccines in species other than mice.

Recently, we have entered into collaboration with the neonatal intensive care nursery at Princess Margaret Hospital and the Human Lactation Research Group situated at the School of Biochemistry and Chemistry, UWA to investigate human cytomegalovirus in breast milk. This research is hoped to reveal the criteria for infection risk in very premature (<30 week gestation) babies and to provide realistic treatment options to limit disease in this vulnerable population.

The Cytomegalovirus Research Group is located in state of the art laboratories within the Marshall Centre for Infectious Disease Research and Training in the School of Pathology and Laboratory Medicine. Professor Shellam has more than 30 years experience using MCMV to model human disease and has been responsible for significant scientific discoveries in this field.

PROJECTS

1. Distinguishing HCMV strains in human breast milk
   
   **Supervisor(s): Dr Megan Lloyd and Prof Geoffrey Shellam**

   **Description of Project:**
   The infection of very premature babies (<30 weeks) with HCMV via maternal breast milk may have devastating medical consequences, although the clinical significance and long term sequelae of postpartum HCMV infection are unclear. The number of strains of HCMV present over time in breast milk has not previously been well defined. This project will use PCR technology to characterise the presence of HCMV in breast milk samples and will use heteroduplex mobility shift and quantitative PCR assays to determine the number of strains of HCMV present in breast milk samples. Additionally, HMCV strains will be isolated from breast milk using cell culture...
techniques and preliminary characterisation of the isolated HCMV strains will be undertaken. HCMV-specific maternal antibodies present in breast milk will be assessed and correlated to virus presence. The project will involve:
1. DNA extraction of breast milk samples, PCR analysis using both standard and quantitative methodologies
2. Development and evaluation of multiplex PCR analysis to detect multiple HCMV strains
3. Cell culture and immunohistochemistry techniques to isolate HCMV strains from breast milk.
4. ELISA avidity assays to determine maternal HCMV-specific IgG and IgA titres in breast milk.

References:

2. Within-host competition, the battle of the viruses

Supervisor Dr Alec Redwood

Multiple MCMV infection in certain strains of mice leads to competition between the viruses. This competition is profound and prevents the shedding of certain “sub-dominant” strains of MCMV. Unexpectedly this competition appears to be mediated entirely by natural killer (NK) cells, the first such demonstration that these cells can mediate competition. This level of competition is unprecedented but the means by which NK cells can mediate clearance, of what would normally be a persistent infection, is unknown. This study will investigate this phenomenon in more detail. We will determine how NK cells are able to mediate such competition, what effect this competition has on the co-infecting, “dominant” strains of MCMV and how this competition appears to promote re-infection of the host. This project would suit a student interested in immunology and virology and will involve such techniques as flow cytometry, tissue culture, animal studies and real time PCR.

3. Do viruses hunt in packs, a search for viral complementation

Supervisor Dr Alec Redwood

Multi-strain infection can lead to profound within host competition between viral strains. However, from an evolutionary perspective it is likely that complementation between viral strains would best suit the replication and transmission of cytomegaloviruses. In humans multi-strain HCMV infection has been linked to enhanced disease in immunosuppressed patients, in particular in sold organ transplant recipients. We now have the opportunity to study competition between multiple strains of MCMV in outbred mice that are used for the Gene Mine project headed by Prof. Grant Morahan. Complementation between the viral strains will be assessed as enhanced disease or viral titres in mice infected with more than one MCMV strain. We will also assess the capacity of MCMV strains to replicate in multi-strain infected mice by qPCR to determine if competition also occurs in outbred mice. Finally, because these mice are a component of the Gene Mine project, collection of genetic material from the mice may ultimately enable us to determine which host genes are responsible for resistance to CMV infection. This project would suit a student interested in immunology, virology and molecular genetics and will involve such techniques as flow cytometry, tissue culture, animal studies and real time PCR.

4. Immune evasion function of the cytomegalovirus gene m15

Supervisor Dr Alec Redwood

Like all herpesviruses, MCMV persists for the life-time of the host. To do this MCMV encodes a large number of genes that directly targets the host immune response. The function of most of these genes remains unknown and is a fertile area for research in the fields of virology and immunology. We have recently demonstrated that the MCMV gene, m15, inhibits the function of natural killer (NK) cells by a mechanism that is yet to be determined. NK cells are at the front line when it comes to defending the host from infections and MCMV encodes at least 4 other genes that target these cells. However, m15 appears unique, as its effects are seen during chronic infection, the other 4 genes all enhance acute viral replication. Using gene knockout strains of virus we have demonstrated that m15 enhances viral replication in the salivary gland (the organ of transmission) and inhibits NK cell proliferation, production of IFNγ and NK cell maturation. This project will seek to investigate m15’s mechanism of action. This project would suit a student interested in immunology and virology and will involve such techniques as flow cytometry, tissue culture, animal studies, real time PCR and molecular biology.

References relevant to projects 2-4:
Clostridium difficile Research Group

Our group is interested in the epidemiology of C. difficile infection (CDI), an anaerobic bacterium that colonises the gut of young animals and is the most common cause world-wide of infectious diarrhoea in hospital patients. CDI is also thought to be the number one healthcare related infection in the USA costing >US$3 billion annually. C. difficile produces spores which allow it to survive outside the body and resist common disinfectants including alcohol hand rubs, so an infected patient can readily contaminate their surrounding environment and spread the infection. Since 2002, there has been an escalation in rates of CDI and infection has become more severe, with an attributable mortality of >10% in those aged >60 years. In North America and Europe, outbreaks of an epidemic PCR ribotype (RT 027) were largely responsible. With the introduction of strict infection control procedures in parts of Europe, RT 027 rates have reduced. The strain remains a problem in North America and is now considered endemic in some settings.

Toxigenic C. difficile usually produce two toxins, toxin A and toxin B that are thought of as the major virulence factors. Toxin B and the gene encoding this toxin are the two main targets for laboratory diagnosis of CDI in symptomatic patients. PCR ribotype 027 produces 16 times more toxins A and B due to mutations in a regulatory gene tcdC, and an additional toxin, binary toxin, not considered important until now. The third important feature of these strains is that they are resistant to fluoroquinolone antibiotics, including later generation fluoroquinolones, and excessive fluoroquinolone use appears to be driving the recent outbreaks. There is evidence of intercontinental spread and it has also been suggested that there is now more community-acquired CDI. There were three separate known introductions of RT 027 into Australia in 2008 and two in 2010, although the strain has not established here possibly because of Australia’s conservative policies regarding community-acquired infection. The rates of CDI in Australia have been increasing since mid 2010, with some seasonality, thought to be due to a RT 027-like strain, RT 244. RT 244 shares the toxin profile of RT 027 but is mostly associated with community-acquired infection. It is unknown how these patients are being exposed to this organism in the community but transmission directly or indirectly from infected livestock is hypothesised.

We have been conducting state-wide surveillance of CDI since 2010. These isolates form part of a vast collection of isolates from humans (carriers and patients with symptomatic infection), animals, and the environment, locally, nationally, and internationally sourced and dating back to the 1990s. This collection can be used to define the evolutionary relatedness of strains, to explore the molecular and clinical epidemiology of C. difficile, to develop, improve, or validate methods for the laboratory diagnosis of infection or for surface decontamination, and to investigate virulence-associated characteristics such as antibiotic resistance, heat resistance of spores, sporulation and germination rates, and toxin production. An Honours project with the C. difficile research group would appeal to prospective Honours students with a background in Microbiology or Molecular Biology. Students will be exposed to a range of techniques including PCR, ribotyping, pulsed-field gel electrophoresis, sequencing, phase contrast microscopy, anaerobic culture, tissue culture, and antibiotic susceptibility testing depending on the choice of project.

PROJECT

1. Use of gene sequencing to identify key tumour antigens for therapy

*Background:* The strongest immune responses against tumour cells are those directed against mutated antigens. It has been hard to identify these because they are different in each patient. However, next generation sequencing allows for the identification of these mutations which, when combined with peptide prediction and testing, enables investigators to determine exactly which tumour antigens the immune system is ‘seeing’. It also means that we can determine how this changes with therapy e.g. the selection of resistant cells by loss of antigens or the unmasking of previously hidden ‘submarine’ antigens by chemotherapy. Ultimately, it will be possible to make personalised vaccines to ‘drive’ the patient’s immune response to attack tumour cells bearing these key mutations.

This project involves the sequencing of mouse tumour exomes and evaluation of immunoreactivity to mutated proteins. It will also evaluate changes in these reactivities with time and therapy.

In this project the student will:

1. Obtain gene sequences (exomes) from mouse tumours
2. Use computer algorithms to predict MHC-binding peptides.
3. Evaluate CD8 T cell reactivity to those peptides (to determine which ones the host immune system ‘sees’ when tumours grow
4. Evaluate these responses as the tumour grows (to determine if the reactivities get tolerised)
5. Evaluate the effects of chemotherapy on these responses (to see if new reactivities are unmasked)

*The research group:* This work will be done within the Tumour Immunology Group of the School of Medicine. This group has led the way in a number of aspects of tumour immunology, has won >$19 million in NHMRC grants and has a strong track record of translating its research findings into novel therapies for patients.
Background
Mesothelioma is a cancer that arises in the thoracic and abdominal cavities and is predominantly caused by asbestos. Consistent with many other types of cancer, response to chemotherapy varies considerably from patient to patient. We have set out to measure the extent of this heterogeneity of response, using a bank of mesothelioma cell lines and a wide range of chemotherapies. Preliminary data show that different cell lines have diverse levels of sensitivity to the same chemotherapy, despite a uniform method of cancer induction using asbestos.

PROJECT

1. Investigation of the heterogeneity of mesothelioma cell line responses to chemotherapy

This honours project extends this study by investigating the mode of cell death (ie apoptosis or necrosis) for each cell line treated with the range of chemotherapeutic drugs. In addition, the release of inflammatory cytokines and markers will be examined in each case, given that the immune system is known to have a role in efficacy of chemotherapy. By building up a comprehensive database of drug sensitivity, cell death pathway and immune response to chemotherapy for our mesothelioma cell lines, we will form a library of data, which can be correlated with the molecular signature of the cell lines. We hope this will provide an insight into what causes the wide variability in chemo responsiveness amongst patients.

In this project the student will:

1. Develop quantifiable assays to measure cellular proliferation, apoptosis, cytokine release, inflammatory cytokine release.
2. Test a panel of cell lines in these assays
3. Assess responses to chemotherapies for each cell line and each parameter
4. Correlate data with gene analysis performed separately on each cell line and carry out validation experiments

Methods and techniques: Cell culture, proliferation and apoptosis assays, ELISA, FACS analysis.
Dr Tobias Schoep  
Dr Peter Dallas  
BRAIN TUMOUR RESEARCH PROGRAM  
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Brain Tumour Research Program

Survival rates for childhood brain cancers are poor and are not improving. We focus on understanding both the basic biology of childhood brain cancers and developing new therapies for the treatment of these cancers. The Brain Tumour Research Program is a collaborative group comprising clinicians, neurosurgeons and senior scientists. Our expertise includes: cancer biology, drug discovery and bio-therapeutics development, analysis of cancer pathways, animal model development and treatment studies in animal models of cancer.

PROJECTS

1. **New uses for old drugs: repurposing drugs to treat childhood brain cancer**  
   *With Dr Tobias Schoep (tschoep@ichr.uwa.edu.au)*

   The survival rates for childhood brain cancers have plateaued and as a result they are the major cause of childhood cancer related mortality. For the treatment of young children radiotherapy is avoided as it severely damages the developing nervous system. However, chemotherapy treatment alone is rarely effective, with the tumour growing back in many cases. There is a clear need for the development of novel therapies. We are taking drugs used to treat other diseases, and repurposing them for the treatment of childhood brain cancer. We have performed a high-throughput biology based screen to identify drugs that inhibit cancer cells. The aim of this project is to examine the effectiveness of repurposed drugs in combination with conventional therapies on cancer biology in cell culture and animal models of childhood brain cancer that we have developed in our laboratory. This study will provide the first step towards developing a new therapy for the treatment of these devastating cancers. As well as gaining a greater depth of understanding of cancer biology, you can expect to gain skills in: i) drug discovery methodologies ii) tissue culture and associated assays, iii) molecular engineering and vector production, v) biochemical and immunological techniques and assays, iv) animal work and downstream analyses such as histological analyses. You will also connecting and working with a network of national and international collaborators.

2. **microRNA and childhood brain tumours**  
   *With Dr Peter Dallas (pdallas@ichr.uwa.edu.au)*

   MicroRNAs (miRNAs) are small non-coding RNA molecules with important regulatory roles in mammalian development. Concomitant with their functional importance, deregulated miRNA expression has been linked to the pathogenesis of many cancers, including medulloblastoma (MB), the most common type of malignant brain tumour affecting children. Four molecular sub-types of MB have been described providing an opportunity to devise new treatments that are sub-type specific, more effective, and less damaging to normal cells. We have identified several deregulated miRNAs and their putative mRNA targets that are associated with the more aggressive MB subtypes. The aims of this project are to investigate and confirm these miRNA/mRNA interactions, and assess their functional significance using our panel of MB cell lines. Ultimately, this research may open up new avenues for the treatment of MB.

We would like to discuss projects further with you. Please feel free to contact us.

The Telethon Adventurers Children’s Brain Cancer Training Award is available for an outstanding candidate
1. Improving protective anti-bacterial immune responses in COPD patients by blocking co-inhibitory T-cell receptors

Background
Chronic Obstructive Pulmonary Disease (COPD) is the most common respiratory cause of morbidity and mortality worldwide. COPD is characterized by a progressive decline in lung function and chronic irreversible inflammation which are not ablated by current treatment strategies. A proportion of COPD patients (~10%) experience acute exacerbations (AECOPD), defined as significant worsening of COPD symptoms requiring treatment and/or hospitalisation. Most episodes of AECOPD (up to 50%) are associated with pulmonary bacterial infections [predominantly non-typeable Haemophilus influenzae (NTHi) and Streptococcus pneumoniae (Sp)]. Impaired Th1 responses may limit the clearance of infections and so promote AECOPD.

We have shown that AECOPD patients have increased expression of T-cell co-inhibitory molecules including cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed cell death-1 (PD-1) and the ecto-nucleotidase CD39. We hypothesised that these molecules compromise protective Th1 responses against bacteria in COPD patients and so promote AECOPD. Hence, we would like to investigate the role of T-cell co-inhibitory molecules in the context of an immune response to bacteria in patients who experience AECOPD. Ultimately, we seek to examine the potential of blocking these anti-inflammatory signals to improve bacterial-specific responses to prevent exacerbations.

Research plan
2. To assess cytokine responses of T-cells by flow cytometry and ELISA following stimulation with bacteria

3. To measure phagocytosis of NTHi or Sp labelled with a dye (pHrodoTM) that is only visible after bacteria are taken into acidic phagolysosomes. It is detected in monocytes using flow cytometry.

4. To determine whether the blocking of CTLA-4, PD-1 or CD39 using monoclonal antibodies improves bacterial-specific immune responses as measured in 1 & 2.

Techniques
This project will provide experience in cell culture, flow cytometry and ELISA.

Further reading
PROJECT

1. T cell responses in *H. pylori* infected hosts

**Description of Research Project:**

*Helicobacter pylori* (*H. pylori*) a ubiquitous gram-negative, extracellular bacterium, first discovered by Nobel Laureates Marshall and Warren in 1984, colonizes the human gastric mucosa. Encounter with the bacteria generally leads to asymptomatic chronic gastritis but in 10 -15% of patients *H. pylori* infection can cause gastric ulcers and in approximately 1% gastric cancer. Interestingly recent findings from our group and others unveiled a strong negative correlation between *H. pylori* infection and development of allergic disorders such as asthma in human and mice. We were further able to demonstrate that the underlying mechanism of protection lies in the ability of *H. pylori* to modulate adaptive immune responses to secondary challenges. We are currently investigating in more details the immune suppressive functions of the bacterium.

Our understanding of the anti- *H. pylori* immune response and immune evasion mechanisms exploited by the bacteria to allow for lifelong persistence at the site of infection are still very basic. Hence in this study we focus on *H- pylori*-specific CD4 T cells, major immune players in the control of the bacteria. To augment the otherwise difficult to detect T cell response we will infect our mice with *H. pylori* mutant strains expressing the Ovalbumin protein, a well-defined T cell antigen. Adoptively transferring OVA-specific T cells isolated from a T cell receptor transgenic mouse prior to infection will allow the tracking of bacteria-specific T cells in the context of *H. pylori* infection. In this project we will generate OVA-expressing *H. pylori* mutant strains depleted of genes previously indicated to modulate T cell response and investigate the impact of gene deletion on the consecutive *H. pylori* specific CD4 T cell response in a mouse model.

**Aims:**

- Investigating the impact of *H. pylori* infection on T cell responses generated in response to a secondary challenge.
- Investigating the impact of bacterial genes on the *H. pylori*-specific CD4 T cell response.

**Techniques:**

The proposed project will include a wide variety of techniques ranging from molecular biology (PCR, Western Blot) to culturing of bacteria (*H. pylori, E. coli*) as well as mammalian cells and even *in vivo* and *ex vivo* experiments using laboratory mice if desired by the prospective student (ELISA, flow cytometry).
PROJECT

1. Molecular Pathology of Skeletal Diseases

Current research activities are focused on gene discovery, molecular mechanisms of osteoclast functions and the intercellular communication between osteoclasts and osteoblasts, which have significant implication in bone and joint diseases; including osteoporosis, osteoarthritis, and tumour metastasis to bone. The team utilises microarray, proteomics and ENU-induced mutant mice models to identify novel molecules that regulate bone resorption, formation and repair. Integrated molecular and genetic approaches help to unveil molecules that play a key role in bone homeostasis and pathogenesis.

Main research areas that can be selected for a honour project include:

- RANK Ligand (RANKL) signalling transduction pathways and transcription program
- Screening of natural compounds for the treatment of osteoporosis
- Deciphering the molecular basis of bone diseases using ENU-induced mutant mice
- Molecular mechanism of intercellular communication in bone microenvironment

Key techniques to be employed include:

- RT-PCR, real time PCR
- Cell culture
- Western blot
- Fluorescent microscopy
- Luciferase reporter gene assay
- Cloning, DNA Transfection
- Protein expression and purification
- Histology
- MicroCT analysis

Recent selected publications derived from our research projects include:

HOW TO APPLY

UWA Applicants: If you completed your undergraduate studies at UWA you should lodge an on-line application via StudentConnect by clicking on the Apply for Honours link. Applications will open online on Monday 14th October and close on Tuesday 17th December 2013.

Non-UWA Applicants: If you have not previously been enrolled at UWA, you apply through one of the following centres, depending on your circumstances. The link is the following: http://www.studyat.uwa.edu.au/courses-and-careers/honours

HONOURS PROJECT PREFERENCE FORM

All applicants must complete the Honours Project Preference Form and return it to OLGA ELEY, office 1.4G, 1st floor, M Block (email: olga.eley@uwa.edu.au) by Monday 2nd December 2013.

I am interested in a Honours/Grad. Dip. Courses starting in Feb 2014 □

1. CONTACT DETAILS

Name ........................................................................................................................................................................


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Phone No. (during same period) ..............................................................................................................................

Mobile No. (during same period) ............................................................................................................................

Email address ...........................................................................................................................................................

2. PROJECT PREFERENCES (for Project booklet please see www.pathology.uwa.edu.au)

In order of preference (please use the project IDs given in the Project booklet’s Table of Contents):

1 Project No. ....................................................................................................................................................... Supervisor has been contacted yes/no

2 Project No. ....................................................................................................................................................... Supervisor has been contacted yes/no

3 Project No. ....................................................................................................................................................... Supervisor has been contacted yes/no

Please describe briefly why you have made this choice:

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Signature ............................................................................................................................................................. Date .................

The Faculty's end-on Honours on-line application form must be completed by December 18th 2013. Students who have submitted this project preference form and who are eligible to enroll in the course will be emailed a confirmation of eligibility as soon as exam results are known (approximately 18 December), and allocation of projects will be advised as soon as possible after this. Student Administration will send you an Authority to Enroll in January 2014.