School of Biomedical, Biomolecular and Chemical Sciences

Honours Projects 2010

MICROBIOLOGY & IMMUNOLOGY
Welcome to Honours
School of Biomedical, Biomolecular and Chemical Sciences

2010 Honours

We hope that you will enjoy this event and that it will serve as a good introduction to the range of Honours projects offered in the School for 2010.

If you are interested in doing an Honours year at UWA, you maybe are already asking about the exciting prospects available within each of the Disciplines and sub-disciplines within the School. These are Biochemistry and Molecular Biology, Biomedical Science, Chemistry, Forensic Chemistry, Genetics, Medical Science, Microbiology and Immunology, Pharmacy, Physiology and Structural Biology. This Honours Projects book will enable you to further explore the possibilities and talk to staff that will be on hand. If you intend to enrol in Honours in 2010, this booklet will provide you with a comprehensive overview of the interests of each of the research groups within Microbiology and Immunology as well as outlining suitable Honours projects. The Honours Expo is designed to showcase the depth and diversity of research being undertaken in the School. Here, you will be able to talk to staff who will be available to explain their research in much detail.

Enjoy!
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Head of School

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Virus Pathogenesis and Vaccine Group

Research by this group aims at limiting viral infection and associated disease processes. Our knowledge of hepatitis C virus (HCV) is particularly poor because it could not be grown in culture until recently and it does not infect laboratory animals. This virus infects about 3% of the population producing a lifelong infection in many which can progress to fibrosis, cirrhosis, hepatocellular carcinoma and end stage liver disease.

Due to the persistent nature of the infection it can re-infect donor tissue transplanted to replace failing liver (1). Other viral infections are controlled by administration of antibody but this is not an option for hepatitis C patients. One arm of our studies is to produce an antibody which will prevent infection of the new tissue. Neutralising antibodies have been described in some, but not all, patients and have been correlated with recovery from acute infection (2-4). Treatment of chimpanzees with polyclonal anti-HCV has been found to prevent or delay infection (5-7) although treatment of liver transplant patients was not successful, probably due to the low titre of HCV-specific antibody (8,9). Our group has developed novel systems for testing the efficacy of antibody preparations in preparation for producing materials for clinical use. This project will advance the process by selecting the most appropriate antibodies for the formation of anti-HCV antibody producing cell lines.

Neutralising HCV Infection

Supervisors: Dr Jane Allan and Professor Jim Flexman

Immunoglobulin will be purified from the serum of selected HCV seropositive patients and tested for recognition of HCV, by western blotting and immunofluorescent staining, and also by functional activity. We will be looking for sera that can neutralise HCV infection using a reporter cell line. Finally selected sera will be tested for protection in vivo using a recombinant viral vector to express HCV proteins in the mouse. This part of the project can be limited to the extraction of tissues for DNA analysis or the participant can become involved in all aspects of the project. Training in the safe handling of infectious materials and in the autopsy of mice will be provided. Techniques used in this project include analysis by quantitative PCR, use of a reporter cell line, immunofluorescent antibody staining, western blotting and tissue culture to maintain a stable reporter cell line and to produce and titrate viruses.

REFERENCES

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Research Interests

Cancer Immuno and Complementary therapies
One major aspect of research conducted in the Beilharz laboratories focuses on immunotherapy of cancer based on regulatory T cell concepts. The second major aspect is the recent discovery of the anticancer properties of tea tree oil, a natural renewable Australian product.

Type I Interferon and Influenza
This area has been central to the molecular/ medical interests of the Beilharz laboratory and has culminated in a phase IIb, WA Health Department sponsored clinical trial for winter colds and flus here in Perth.

Fermentation in Wine Production
A more recent addition to our research team is the wine research group. This group is exploring microbial and molecular aspects to stuck and sluggish ferments which place a significant economic burden on the productivity of Australia’s burgeoning wine industry.

PROJECTS

1. Immunotherapy for the Treatment of Cancer
Supervisors: Assoc Prof Manfred Beilharz, Dr Sara Greay (sara.rooney@uwa.edu.au) and Dr Demelza Ireland (demelza.ireland@uwa.edu.au)

Description:
Regulatory T-cells (Tregs) are essential in maintaining peripheral immune tolerance. Dysfunction of this cell population is associated with autoimmune diseases, persistent bacterial and viral infections, and inhibition of the immune response to cancer (Sakaguchi, 2004). Homeostasis of this cell population is tightly regulated and typically, Tregs account for around 5-10% of the CD4+ T-cell population. However, in tumour bearing hosts, Treg homeostasis is manipulated by the tumour to assist in evasion of the immune system. Our research and that of others has shown increased populations of Tregs in both the tumour, and the periphery of tumour bearing hosts beyond the normal homeostatic range (Curiel et al., 2004, Needham et al., 2006). Previous work from this laboratory has found that depletion of the Treg, from the tumour results in immune mediated clearance of the tumour cells (Needham et al., 2006, Kissick et al., 2009). However, our research in mice and that of others in humans (Barnett et al., 2005) has found that following Treg depleting treatments, the Treg population re-accumulates in the tumour which coincides with the resumption of tumour growth. A novel approach to improving the efficacy of anti-CD25 mAb treatment may be to inhibit the re-accumulation of Treg in the tumour. This project will assess the migration patterns of Treg in tumour bearing mice following various treatments by adoptively transferring fluorescently labelled cells. Additionally, the efficacy of various treatment approaches in inhibiting Treg migration to the tumour will be assessed.

2. Complementary Tea tree oil Treatment of Cancer
Supervisors: Assoc Prof Manfred Beilharz, Dr Sara Greay and Dr Demelza Ireland

Description
We have previously identified topical tea tree oil as effective against subcutaneously implanted B16 melanoma and AE17 mesothelioma tumours. This antitumour effect appears to involve activation of dendritic cells which leads to tumour growth inhibition and tumour regression. Furthermore, we have also identified that tea tree oil and its major component terpinen-4-ol induces low level apoptotic cell death, primary necrotic cell death and cell cycle arrest in G1 phase (Greay 2009). However, the mechanism(s) of action of how tea tree oil and
terpinen-4-ol may elicit cell death or arrest remains to be elucidated. This project will aim to characterize the role of specific genes and proteins involved in the known cell death and cell cycle processes.

3. Interferon and Influenza

Supervisors: Assoc Prof Manfred Beilharz, Dr Sara Greay and Dr Demelza Ireland

Description:
The persistence of highly pathogenic avian influenza within wild bird populations has forged interest in control measures to limit a possible human pandemic. We therefore investigated the efficacy of low dose oral administration of IFN-α as a potential therapy against influenza infection in a murine model. We have identified an optimal low oral dose of IFN-α that when delivered daily as prophylactic therapy protects C57BL/6J mice from a lethal challenge with mouse adapted human influenza virus A/PR/8/34 H1N1. These results provide strong support for the application of low dose type 1 IFN pretreatment to human influenza control. These results have been i) published in BBRC (Beilharz et al, 2007) and ii) internationally verified by researchers in the Trudeau Institute (USA and the Friedrich Loeffler Institute (Germany). We are currently conducting a phase IIb human clinical trial using low dose oral IFN-α. This project will involve analysis of human clinical trial data and basic work in the mouse model. Specifically, the role of dendritic cells in transmission of the oral IFN-α signal will be examined.

4. Stuck and Sluggish Wine Fermentations

Supervisors: Assoc Prof Manfred Beilharz, Dr Sara Greay and Dr Demelza Ireland

Description:
Hexose transporters play a critical role in the wine fermentation process by importing the hexoses glucose and fructose into the yeast cell for fermentation (Luyten et al., 2002). It has been observed that the process of hexose transport is the rate limiting step in the fermentation pathway (Kruckeberg et al., 1993) thus implicating expression of the genes encoding the hexose transporter proteins as a potential cause of problem ferments. Research conducted in our laboratory comparing yeast gene expression in both a normal and a sluggish commercial wine ferment has shown overexpression in the sluggish case suggestive of competitive inhibition. We have identified a bacterium (L. kunkei) that may be responsible. This project will undertake small scale laboratory simulations to verify the hypothesis that competitive inhibition underlies sluggish fermentations. Such verification will be important economically for the wine industry worldwide.
Molecular Bacteriology

The first research area in my laboratory concerns the biology and genetics of the bacterial viruses known as bacteriophages (phages), and their use as biocontrol agents in human disease and in aquaculture. Research has included the isolation of *Vibrio harveyi* phages and an examination of their use in aquaculture (in collaboration with Dr David Sutton), genome sequencing of a phage of *Clostridium difficile* (with Prof Tom Riley) and a study of the role of phages in virulence of *Aeromonas* (with Dr Harry Sakellaris).

The second general area is in molecular studies of virulence mechanisms of bacterial pathogens including *Aeromonas* spp., *Burkholderia* spp., *Clostridium difficile*, *Moraxella catarrhalis*, *S. maltophilia* and *Vibrio* spp.

PROJECTS

1. **Virulence determinants of Western Australian Aeromonas pathovars**
   With Dr Tim Inglis, PathWest

*Aeromonads* are ubiquitous aquatic Gram-negative bacteria that cause disease in amphibians and fish. Three species of *Aeromonas*, *A. hydrophila*, *A. caviae* and *A. veronii* biovar *sobria*, are human pathogens. They are associated with gastrointestinal infections and a variety of extra-intestinal infections such as septicaemia, wound infections, soft tissue infections, and occasionally meningitis, peritonitis and haemolytic-uraemic syndrome. A large number of putative virulence factors have been identified in *Aeromonas*, including the production of pili and other adhesins, O-antigens and capsules, lateral flagella, exotoxins such as haemolysins and enterotoxins, and extracellular enzymes such as proteases, amylases and lipases. A type III secretion system gene cluster has been identified in *A. hydrophila*, although the role of proteins secreted by this system and how they interfere with host cellular processes is not yet known. In addition, a phage-associated genomic island has been reported, although no role in virulence is yet proven. Some *Aeromonas* isolates have the ability to invade human epithelial cells in a process which has not been completely characterised, but which has been shown to involve actin polymerisation.

There have been several clusters of invasive *Aeromonas* sp. infection in Western Australia in recent years. The reason for this has not been fully elucidated, but a comprehensive cataloguing of *Aeromonas* spp. in WA is now under way in our laboratories. Carefully characterised *Aeromonas* isolates will be available for virulence factor analysis using nucleic acid amplification to probe key gene sequences, DNA sequencing to identify potential mutations, an expression PCR to analyse possible variability in virulence expression. If rapid progress is made, fluorescent hybridisation will be used to explore gene expression in a cellular model of infection. Together with the molecular work, a phenotypic analysis of selected virulence factors will be performed. In particular, adhesion to and invasion of human epithelial cell lines will be examined. This project will lead to a better understanding of the multifactorial nature of *Aeromonas* pathogenicity, and a determination of the association of putative virulence genes with clinical invasiveness in WA isolates.

**References:**


2. *Aeromonas* phages and their role in *Aeromonas* virulence and biology
*With Dr Harry Sakellaris*

There is currently renewed scientific interest and rapid development world-wide in the study of phages. This has been partly fueled by the emergence of multiple antibiotic resistance in bacterial pathogens and the development of alternative antibacterial therapies, including phage therapy. However it is also becoming increasingly clear that phages play a major role in bacterial evolution and specifically in the virulence of many bacterial pathogens. For example, phages often encode toxins and other virulence factors of bacterial pathogens, such as the ctx phage of *Vibrio cholerae* which encodes cholera toxin. Little is known about *Aeromonas* phages and whether they have any role in disease. Thus the aim of this project is to determine the role of selected temperate phages in virulence of *Aeromonas* and in the biology of their host strains.

We have a collection of 19 *Aeromonas* phages, and have been able to construct a number of strains lysogenic for some of these phages. The role of phages in virulence will be studied by assaying lysogens (harbouring phage genomes or prophages) and their parental non-lysogenic strains using *in vitro* assays such as haemolysis, haemagglutination, adhesion to and invasion of human cell lines such as HEp-2 cells, cytotoxicity and serum resistance. Other phenotypes examined will include growth characteristics and metabolic functions. Phage DNA isolation and pulsed-field gel electrophoresis, in order to determine the size of the phage genomes, may be undertaken in order to further characterise the phages. Southern hybridisation using DIG-labelled phage DNA probes will be used to determine the number and location of phage genomes within lysogens, and wild-type *Aeromonas* isolates will be screened to determine the presence of related phages; correlation with source and virulence of strains can then be determined. Another molecular approach will be to use PCR to amplify regions of known virulence genes of *Aeromonas* using as templates the isolated phage genomes, as well as DNA from lysogens and parental strains. This project will increase our understanding of the contributions made by *Aeromonas* phages to their hosts.

**References:**

NB Also see project co-supervised with Dr David Sutton
Clinical Microbiology

Recent research by my laboratory group has focused on bacteria that cause human infections including the Burkholderias, Legionella, Mycobacteria, Listeria and other facultative intracellular species. Current work spans bacterial survival strategies, the role of secondary metabolism in mechanisms of disease, bacterial taxonomy, molecular diagnostic and molecular epidemiological methods and the cell biology of bacterial-eukaryote interactions. Recent highlights include participation in an international whole genome sequencing project, the discovery of bacterial species that are new to Australia, the development of new molecular epidemiological methods and work on direct molecular detection of bacteria in early bloodstream infections. There may be late changes to the projects available: interested students should contact Dr Inglis.

PROJECTS

1. **The laboratory Diagnosis of Legionella longbeachae infection**
   With Drs Charlene Kahler and Gerry Harnett

   In most of the world, including much of Australia, the majority of cases of Legionnaire’s Disease are caused by *Legionella pneumophila* serotype 1. However, in Western Australia *L. pneumophila* is an unusual cause of infection. Much more common is *Legionella longbeachae*, which is associated with exposure to potting mix and other garden products. It is not clear whether this apparent difference is due to real differences in the epidemiology of Legionella infection in WA, or due to differences in the way laboratory diagnostic tests are used and interpreted. *Legionella* spp are quite difficult to grow in the diagnostic laboratory, requiring the use of special selective agar and experienced staff. In recent years, *L. pneumophila* infections have benefited from PCR-based molecular diagnostic methods and an easily performed urinary antigen test (UAT). A UAT is not available for *L. longbeachae* and PCR methods are of variable specificity. There is a pressing need to improve the currently available laboratory tests for *L. longbeachae* infection.

   The project will combine a review of currently used laboratory methods for *L. longbeachae* infections, and seek to improve the PCR-based method by designing a new set of PCR primers from sequence data that has recently been uploaded to GenBank. Sera from patients with confirmed Legionella infection will be tested by Western blot to help differentiate antibody responses to *L. pneumophila* and *L. longbeachae* antigens. Suitable candidate antigens for differentiation of *L. longbeachae* infections from other causes of Legionnaires’ Disease will be characterized and preliminary purification attempted. If time permits, a polyclonal antibody preparation will be raised in laboratory animals for use in a prototype urinary antigen test, and evaluated on clinical samples from patients with suspected Legionella infection.

References:
Methods used:
- Selective culture of *Legionella* species
- Nucleic acid amplification by PCR
- Serodiagnostic methods for Legionella infection
- Western blot
- Polyclonal antibody development in laboratory animals
- Antigen characterisation, purification and detection in clinical specimens

2. **The intracellular survival of Burkholderia species**
   With Drs Avram Levy ([a-levy@cyllene.uwa.edu.au](mailto:a-levy@cyllene.uwa.edu.au)) and Gerry Harnett

The Burkholderias are a group of soil-dwelling environmental Gram negative bacteria that include human pathogens such as *B. mallei*, *B. pseudomallei* and *B. cepacia* as well as a large range of non-pathogenic near-neighbour organisms that inhabit the upper layers of the soil. Disease-causing members of this genus have a capacity to invade eukaryotic cells such as tissue macrophages and can remain dormant there for a very long time. We have shown that these bacteria can persist in naturally occurring eukaryotic inhabitants of the upper soil layer such as free-living amoebae and mycorrhizal fungi and have proposed that the ability to invade human cells is as a result of an intracellular lifestyle in the wider environment. It is possible that this environmental niche is also a means of exposure to infection by disease-causing species such as *B. pseudomallei*. We have collected a library of soil samples from the north of Western Australia and isolated *Burkholderia* species from these.

A previous Honours project measured the biodiversity of Burkholderias from these samples but stopped short of recovering them from amoebic cysts or mycorrhizal fungal spores in the soil sample collection. The proposed project will build on that earlier work, specifically looking for evidence of intracellular *Burkholderia* species in our collection of soil samples. Further fresh samples will be obtained from northern WA during the course of the project to ensure further Burkholderia-positive cultures and viable eukaryotic microbiota to analyse for intracellular bacteria. A positive result will strengthen evidence for a new mode of transmission of Burkholderia infection.

References:

Methods used:
- Selective culture using specialized solid and liquid media
- Targeted nucleic acid amplification by PCR and sequencing of amplicons
- Recovery of mycorrhizal fungi and amoebic cysts
- Electron microscopy techniques
- Fluorescent in-situ hybridization
Arbovirus Surveillance and Research Group

The Arbovirus Surveillance and Research Laboratory (ASRL) monitors activity of mosquito-borne viruses including the flaviviruses Murray Valley encephalitis virus (MVEV), Kunjin virus (KUNV), and the alphaviruses Ross River virus (RRV) and Barmah Forest virus (BFV) in Western Australia (WA). In addition, the program aims to detect incursions of medically important exotic mosquito-borne viruses, such as Japanese encephalitis virus, West Nile virus and chikungunya virus. These viruses have caused large outbreaks of potentially fatal disease in other countries, often including neighbouring countries in southeast Asia. Monitoring of mosquito fauna and arbovirus activity is undertaken at key locations, particularly in the southwest of WA and the Kimberley and Pilbara regions, but also other parts of the state on an opportunistic basis. Year-round flavivirus surveillance is conducted in northern WA using sentinel chicken flocks. Environmental conditions and predisposing factors, and the incidence of human disease (provided by the DOH) are also monitored. In addition, research initiatives aim to improve the speed/specificity/sensitivity of detection of viruses and their infections, to improve the detection of flaviviruses that are serologically cross-reactive and difficult to distinguish, and to research the epidemiology and ecology of arboviruses and mosquito vectors in WA.

PROJECTS

1. Development and application of ELISAs for detection of IgM and IgG anti-Ross River virus antibodies in marsupials.

With Dr Michael Lindsay (Mosquito Borne Disease Control Branch, Western Australian Department of Health, 9385 6001, Michael.Lindsay@health.wa.gov.au), Dr Abbey Bestall (Murdoch University, A.Bestall@murdoch.edu.au) and Dr Katherine Belov (University of Sydney, k.belov@usyd.edu.au)

Ross River virus (RRV, Togaviridae: Alphavirus) is enzootic in many parts of Western Australia, and regularly causes large outbreaks of human disease. However RRV is primarily a zoonotic virus transmitted between vector mosquitoes and animal hosts, particularly marsupials. Activity of RRV in WA is monitored by detection of the virus in field collected mosquitoes and surveillance of human cases of disease. Recent studies have also investigated the prevalence of antibodies to RRV in Western Grey Kangaroo populations in a RRV enzootic area. Substantial increases in the prevalence of antibodies to RRV were observed in kangaroo populations during outbreaks of human disease. Studies are also underway to investigate the value of monitoring seroprevalence of antibodies to RRV in kangaroo populations in WA as an early warning of epizootic activity of the virus. The assays that are currently used to detect RRV-specific antibodies in kangaroo sera (serum neutralisation assays and RRV epitope-blocking ELISA) do not distinguish between recent and past infection, primarily because serum samples are single specimens in which rising antibody titres cannot be detected. For this reason, our laboratory is keen to develop ELISAs to detect IgM and IgG anti-RRV antibodies in marsupials. The assays will be developed using sheep anti-possum IgM and anti-possum IgG sera (that cross-reacts with other marsupial species) prepared by collaborative researchers at Macquarie University and the University of Sydney. Appropriate control sera will also need to be obtained. These new assays will be compared in terms of sensitivity and specificity with the standard techniques of neutralisation and RRV epitope blocking ELISA. The optimised ELISAs will be used to test a panel of kangaroo sera from different locations in WA prior to, during and following outbreaks of RRV disease in humans, to investigate the potential use of these assays for early detection of epizootic RRV activity in these areas.
2. Is the infection rate and titre of Ross River and Barmah Forest viruses in vector mosquitoes informative?

*With Dr Michael Lindsay (Mosquito Borne Disease Control Branch, Western Australian Department of Health, 9385 6001, Michael.Lindsay@health.wa.gov.au)*

RRV and BFV are two medically important mosquito-borne alphaviruses that cause outbreaks of debilitating arthritis, rash and fever in people in WA. The ASRL has monitored activity of these viruses in mosquitoes along the Swan Coastal Plain in the southwest of WA since 1987. A large number of mosquito pool homogenates have been confirmed to be infected with RRV and/or BFV since surveillance of these viruses in mosquitoes commenced. Although the minimum infection rate (MIR) of RRV and BFV in mosquito populations can be extremely high preceding and during outbreaks of disease, the infection rate is variable. Preliminary analyses in the early 1990s showed that high MIRs were often linked to large outbreaks of RRV disease, yet not always, and no detailed analysis has been undertaken to formally investigate the relationship between infection rate in the mosquito population and the likelihood of outbreaks of disease. In addition, determination of the titre of virus in mosquito pools has been shown to enhance mosquito-based arbovirus surveillance programs elsewhere. This project aims to investigate whether MIRs in mosquito populations and mosquito abundance are of predictive value in relation to outbreaks of RRV and BFV disease in WA, and to investigate the benefit of determination of virus titre in infected mosquito pools, particularly pertaining to potential vector incrimination. MIRs in mosquito populations in the Swan Coastal Plain will be determined using several published methods and compared to the number of human cases of disease and mosquito abundance. Virus titre will be determined by virus titration using TCID<sub>50</sub>s and tissue culture enzyme immunoassays (TCEIA). Given that this method can be cumbersome, time consuming and not practical in the long term, real-time reverse transcription (RT)-PCR<sub>s</sub> specific for RRV and BFV will also be developed to rapidly quantify RRV and BFV in mosquito pools, and the relationship between titration using TCID<sub>50</sub>/TCEIA and quantification of RRV and BFV RNA by real-time RT-PCR will be analysed. It is envisaged that these results will enhance the ability of the ASRL to predict future outbreaks of RRV and BFV disease and improve understanding of vector mosquito species in WA.
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 colonizes 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 organisms.

Prospective Honours students with a background in Molecular Biology, Biochemistry, and Microbiology are particularly encouraged to apply. Students will be exposed to a range of techniques including DNA sequencing, DNA cloning, cell culture, RT-PCR, protein analysis, and FACS analysis.

**PROJECTS**

1. **Endotoxin of Neisseria meningitidis**

This work is being conducted in collaboration with Professor David Stephens (Emory University, Atlanta, USA) and Dr Russell Carlson (Complex Carbohydrate Research Center, Athens, USA).

*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.

AIMS:

1. To epitope-tag known LOS biosynthesis enzymes and assess their localization within the bacterial cell by cell fractionation and Western Immunoblot.

2. Bacterial two hybrid system for detection of protein::protein interactions

2. **Sigma factors in Neisseria gonorrhoeae**

   *This work is being performed in collaboration with Professor John Davies (Bacterial Pathogenesis Program, Department of Microbiology, Monash University, Victoria) and Dr Paul Rigby (BIAF, UWA).*

Bacterial sigma factors are essential components of the RNA polymerase holoenzyme and determine promoter selectivity and specificity. Analysis of the *Neisseria gonorrhoeae* genome indicates that apart from the *rpoD* and *rpoH* genes there was only one other gene that encodes a potentially functional sigma factor. This gene, *ecf*, appears to encode a member of the extracytoplasmic function (ECF) alternative sigma factor family. Microarray analysis suggested that 8 genes (including *ecf*) were up-regulated when Ecfs levels are increased. Five of the Ecfs-regulated genes are clustered with *ecf* on the genome, and appear to form a single transcriptional unit. The first two open reading frames in this cluster, NGO1947 and NGO1948, appear to encode putative anti-sigma factors which regulate the amount of free Ecfs factor in the cytoplasm. To examine their direct role in regulating Ecfs, we will clone each gene and add epitope tags for protein purification and the generation of monoclonal anti-sera. These epitope tagged proteins will be over-expressed in *N. gonorrhoeae* and the location of the proteins determined by cell fractionation and Western immunoblot. To examine the interaction of the proteins with one another, the genes will be cloned into a bacterial protein two-hybrid system for analysis of protein::protein interactions.

AIMS:

1. To epitope-tag Ecfs, NGO1947 and NGO1948 and assess their localization within the bacterial cell by cell fractionation and Western Immunoblot.

2. Protein purification for anti-sera production.

3. **Cellular biology of the human nasopharynx**

   *This work is being performed in collaboration with Professor David Stephens (Emory University), Dr Steve Webb (Royal Perth Hospital), Dr Bastiaan DeBoer (PathWest) and Dr Paul Rigby (BIAF, UWA).*

*Neisseria meningitidis* naturally inhabits the nasopharynx of humans, and in some instances, causes invasive infections culminating in rapidly fatal sepsis. Early studies showed that meningococci bound to non-ciliated epithelial cells in the nasopharyngeal organ culture model (NPOC). Upon attachment by meningococci, microvilli on the surface of these cells became elongated, eventually forming lamellipodia which engulf the bacterium as it is internalized. Transformed or primary epithelial cell cultures have provided the simplest model to analyze bacterial adherence and invasion, and has allowed for the identification of a number of neisserial adhesins (i.e. pili, Opa, Opc) and additional putative virulence determinants which affect bacterial adherence and invasion into host cells (i.e. lipooligosaccharide [LOS], capsule, PorB). To date, this data has been obtained using transformed cell lines growing *in vitro*, and attempts to translate these observations to the original NPOC model have yet to be attempted.

AIMS:


2. Examine the co-localisation of meningococci with known cell types in human nasopharyngeal epithelium.

3. Examine the role of lipopolysaccharide in attachment of meningococci to nasopharyngeal epithelium.
Deep sea sulphate-reducers

**Supervisors:** Dr. Anna Kaksonen and Dr. David Sutton
Email: anna.kaksonen@csiro.au   Tel. 9333 6253

**Description:** The subsurface biomass (intraterrestrial organisms) is recognized as a major fraction of planetary biomass. Within many deep biosphere environments, sulphate-reducing bacteria (SRB) are among the most numerous and may be responsible for the formation of many sulphide ore deposits in subsurface environments. A number of previously unexplored deep subseafloor sediments were retrieved from the Nankai Trough seismogenic zone off the southwest coast of Japan. Sulfate-reducing bacteria (SRB) were enriched from the sediments and community profiling with denaturing gradient gel electrophoresis (DGGE) showed that the cultures harbor novel SRB. The proposed research will involve isolation of SRB from the mixed cultures and their physiological and molecular characterization. Moreover, the diversity of SRB in the deep sea sediments will be studied with clone libraries. Marine halophilic sulphate-reducers could potentially be used for purifying saline sulfate-containing wasters such as acid mine drainage or waters from acid sulfate soils.

**Aims:**
1. To isolate and characterize SRB from mixed cultures enriched from deep sea sediments
2. To study the diversity of SRB in deep sea sediments using dissimilatory sulphite reductase gene (dsrAB) clone libraries.

**Techniques to be used include:** Anaerobic culture techniques, DNA extraction, PCR, Clone libraries, DNA sequencing and phylogenetic analysis

**References:**

**Method development for detecting and enumerating pathogenic free living *Naegleria fowleri***

**Supervisors:** Dr. Geoffrey Puzon and Dr. David Sutton
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**Description:** *Naegleria* are free living amoebae readily present in the environment which feed on bacteria. *Naegleria* are thermophilic organisms able to thrive at elevated temperatures, < 40 °C, enabling them to colonize engineered environments, i.e. swimming pools, thermal ponds, and water distribution pipelines. Of the over 30 known species of *Naegleria* only *N. fowleri* is pathogenic. *N. fowleri* is the causative agent of the highly fatal disease primary amoebic meningoencephalitis (PAM). Studies on *N. fowleri* have focused on its detection in natural and engineered environments. Current detection methods for *N. fowleri* rely on slow culture based techniques. Molecular methods have been developed to detect *N. fowleri*, but lack the ability to distinguish viable from non-viable cells. The proposed research will involve development of novel molecular methods to
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differentiate viable \( N. fowleri \) from non-viable \( N. fowleri \) cells. The new developed methods will then be applied to assess the efficacy of novel disinfection methods, i.e. UV-disinfection, on \( Naegleria \).

**Aims:**
1. To develop and modify molecular methods for specific detection of viable \( N. fowleri \) cells.
2. To assay the efficacy of UV disinfection on \( Naegleria \) spp.

**Techniques to be used include:** Tissue culture techniques, DNA extraction, PCR, Flow cytometry, UV disinfection.

**References:**

Explore challenges to introduce biological P removal to existing wastewater treatment plants in Western Australia

**Supervisors:** Dr. Maneesha Ginige and Dr. David Sutton

**Email:** maneesha.ginige@csiro.au  Tel. 9333 6130

**Description:** Enhanced biological phosphorus removal (EBPR) in the activated sludge process promotes the removal of phosphorus from wastewater without the need for chemical precipitants. EBPR has been shown to facilitate the removal of P in excess of the 1-2%. The group of microorganisms that are responsible for EBPR are known as the polyphosphate accumulating organisms (PAOs). These organisms are able to store phosphate as intracellular polyphosphate, leading to phosphorus removal from the bulk liquid phase through the uptake by PAO cells. Under anaerobic conditions, this group of microorganisms have the competitive advantage over most other microorganisms, due to their ability to take up volatile fatty acids (VFAs) and to store them intracellularly as carbon polymers. Aerobically, the stored carbon is utilised for energy production and biomass growth. These organisms may therefore proliferate in activated sludge systems experiencing alternating anaerobic and aerobic conditions.

**Aims:**
1. To introduce EBPR to existing treatment plants, addressing some of the specific research questions below will be of great significance.
2. Are PAOs present in treatment plants currently not operated for EBPR and if so in which proportion?
3. How significant is the GAO population compared to PAOs that could prevent the introduction of EBPR?
4. How much of P could be removed biologically by introducing an additional anaerobic zone to existing BNR plants?

**Techniques to be used include:** Fluorescent in situ hybridisation (FISH), Quantification of bacteria using FISH and confocal laser scanning microscopy, Sequencing batch reactor operation and cyclic studies.

**References:**
Dendritic Cell Biology

Research in my laboratory involves the regulation of dendritic cells in humans. Research has centred on how cells die and are replaced and the influence of inflammatory proteases on cell migration.

PROJECTS

1. To determine whether lineage negative DC (plasmacytoid and myeloid DC) undergo CD95-mediated apoptosis as has been found for monocyte-derived DC.

Dendritic cells (DC) are potent activators of primary immune responses during their interaction with naïve T cells in secondary lymphoid organs. Activation of DC and their subsequent migration from non-lymphoid tissues to regional lymph nodes have been shown to be early steps during inflammatory processes and crucial events in the generation of cell-mediated immune responses against various pathogens. DC have been isolated from adherent blood mononuclear cells which have been cultured for seven days with granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 which give rise to cells with the phenotypic and functional properties of DC (Monocyte-derived DC or MoDC). CD95 (Fas/APO-1) and CD40 are members of a family of cell surface proteins that include the two TNF receptors, the nerve growth factor receptor, CD27, CD30, CD40, OX40, 4-1BB and the TNFR related protein (LT receptor).

Incubation of Mo-DC expressing CD95 with either agonistic CD95-specific antibodies or CD95 ligand (CD95L) leads to the death of the cells by apoptosis. CD40 ligand (CD40L, T-BAm, TRAP, or gp39) is a transmembrane protein of 32-39 kDa that is expressed on the surface of T cells and mast cells and has homology to TNF and other members of the TNF ligand family. Cross linking CD40 with either CD40L or anti-CD40 mAbs rescues Mo-DC from apoptotic death. Since Mo-DC are 7-day-cultured immature cells it is important to compare these results with immature DC obtained by direct immunodepletion from blood (lineage negative DC). Both plasmacytoid and myeloid DC will be prepared by immunodepletion. Many detectors of apoptotic death stain dead or dying cells, which may be washed away after staining. The cell-permeant nucleic acid stain LDS 751 has been used to discriminate intact nucleated cells from nonnucleated cells and cells with damaged nuclei, as well as to differentiate apoptotic cells from nonapoptotic cells. It is a vital, nucleic acid stain and can be added to living cells. There is no washing process and dead cells may be stained with propidium iodide and the results assayed by flow cytometry. It is proposed to use this novel method to measure apoptosis and compare with other methods of measuring cell death.

2. Enhanced survivability of dendritic cells: potential role of protease-activated receptors

The failure of host antigen presenting cells (APC) to present tumour antigen to the immune system is a means by which tumours escape detection. However, APC taken from cancer patients may be loaded in vitro with tumour antigens prepared from host tumour cells. These APC may then present these antigens, upon injection back into the patient, to host T cells. These T cells, once activated can recognize and destroy the tumour. It has also been shown that human blood-derived immature DCs are potent antitumour cytotoxic cells capable of inducing selective apoptotic death in a variety of human cancer cell lines both in vitro and in vivo. The generation of large numbers of APC (DC) for scientific study in recent years has led to the utilisation of these DC in immunotherapy. However, DC after injection into patients, do not survive and migrate to the tumour site and many die at the site of injection. Koppi et al., 1997 have shown that Mo-DC easily undergo programmed cell death, but also that DC can be protected by the addition of cytokines or by blocking receptors for initiating apoptosis. It is also known that after stimulation with antigen, DC upregulate chemokine receptors which aid in their movement. Dead and dying DC are not functional and so cannot be stimulated with antigen to upregulate maturation or chemokine markers, secrete chemokines, or release the cytokines that initiate and regulate responses. Enhancement of the survival and migration of DC to the tumour site is crucial in immunotherapy and it is important to discover suitable adjuvants which can bring this about. Our preliminary data have shown us that PAR on DC may be activated by PAR agonist peptides with consequent improvement in DC viability and induction of cluster formation. We will discover what this/these factor(s) are by HPLC and mass spectrometry and use them directly on DC to improve DC survival and migration.
Helicobacter Research Laboratory and Ondek

Our group is interested in the bacterium, *Helicobacter pylori*, a ubiquitous gastrointestinal pathogen which infects more than half the population of the world and is the aetiologic agent of gastritis and peptic ulcers. Generally, research has focused on the eradication of this organism. However, Ondek Pty Ltd. (Prof. Barry Marshall’s vaccine development company) is taking the novel approach of using this bacterium as a vaccine delivery system. Ondek Pty Ltd. aims to use genetically modified *H. pylori* to present parts of other pathogens to the immune system. It is anticipated that infection of the host with these genetically modified *H. pylori* will result in immunization against these other pathogens. Specifically, Ondek Pty Ltd. is aiming to produce an inexpensive, stable and easily administered vaccine against influenza, which would greatly benefit both developed and developing countries. Ondek is based in the new state of the art Marshall Centre for Infectious Diseases. Prospective Honours students with a background in Molecular Biology, Microbiology, Biochemistry, Genetics or Immunology are encouraged to apply. Students will be involved in genetic engineering of *H. pylori*, and the evaluation of immune responses to these genetically modified bacteria. Students will be exposed to a number of techniques including DNA cloning, DNA sequencing, bacterial transformation, protein analysis, immunoassays, confocal microscopy, FACS analysis and animal infection models. Further projects may become available and will be distributed later: please contact Dr Benghezal or Dr Alma Fulurija (afulurija@ondek.com) to discuss.

PROJECTS

1. Systems for the Presentation of antigens by *Helicobacter pylori* for Vaccine Development
   
   With Assoc Prof Mohammed Benghezal (mbenghezal@ondek.com)

The development of live attenuated vaccines requires a thorough understanding of the host-pathogen interaction and the availability of tools to engineer safe genetically modified organisms (GMOs). Molecular genetics of *H. pylori* will be used to present proteins of pathogens to the immune system, in order to elicit a specific immune response. Strategies for presenting antigens include cell surface display, secretion or cytoplasmic expression. Aims include: fusing antigens to various carrier proteins or macromolecules, examining their presentation *in vitro*, and identifying which of these result in an immune response in mice.
Comparative genomics of *Leishmania* species to determine the parasite genetic determinants of human disease.

Leishmaniasis is a neglected disease which afflicts millions of people each year with a broad spectrum of disease ranging from self healing cutaneous lesions to fatal systemic infection\(^1\). The *Leishmania* species involved is the main factor that determines what form of the disease the patient gets. There are no vaccines or prophylaxis and the drugs used are both toxic and difficult to administer\(^2\). Considered in the category of severely neglected diseases, there is an urgent need to identify new drug targets and vaccine candidates. A major initiative for this revolves around the recent studies on comparative genomics. To date, three species of *leishmania* (*L. major*, *L. braziliensis* and *Leishmania infantum*) have been completely sequenced, representing the most diverse disease phenotypes\(^3,4\). Comparative analysis of the genome data from these sequencing projects revealed a surprisingly small number of genes restricted to each of the different species\(^4\). Newly developed tools and advances in sequencing technology are allowing researchers to examine the genomes of these pathogens in ever greater detail to help unravel the causal mechanisms of diseases. The recent discovery of an Australian species of this pathogen that while capable of infecting human cells does not have the ability to cause disease has provided an ideal comparative model and potential vaccine candidate. There are two projects will examine the parasite factors involved in human disease with the aim of utilizing the findings to develop potential disease interventions. The first project will sequence and perform comparative analysis of clinical isolates from Brazil that cause well defined phenotypes in humans will identify specific parasite factors involved in modulating the host’s immune response. The second project will utilize the sequence of the indigenous Australian *Leishmania* species to improve our understanding of factors critical for establishing human infections\(^5\). It is the closest relative to the human pathogens and as such will represent an ideal comparator to help identify parasite genes responsible for the adaptation to survival in humans. The inability to induce pathology in humans makes this species an ideal candidate for the development of a safe attenuated vaccine. In addition, modified with the insertion of immunogenic genes, it will provide a possible safe attenuated vaccine candidate.

**Project Aims and Methods**

Both projects will initially start with a bioinformatic analysis of sequence data to identify and characterise genetic differences between different species and isolates of *Leishmania*. *In silico* predictions will be experimentally verified using rtPCR with the most promising candidates carried through to transfection assays to define specific functional phenotypes\(^5\). Genes associated with virulence, persistence and drug resistance will be identified using *in-vitro* methods in human cell lines. The projects will involve extensive bioinformatic analysis using tools to visualize, annotate and compare the assembled sequences, human and pathogen cell culture and a range of molecular biological techniques.

**REFERENCES**

1 WHO Leishmania recorded figures for prevalence and incidence, Available at http://www.who.int/tdr/diseases/default.htm.
1. The molecular epidemiology of *Clostridium difficile* infection in children

**Supervisors:**
Professor Tom Riley (9346-3690, Professor Barbara Chang (barbara.chang@uwa.edu.au) and Dr Lyn Waring (Microbiology, PMH, Lyn.Waring@health.wa.gov.au))

**Background:** There has been great concern world-wide following the recent emergence in Canada, the USA, and now Europe, of a highly virulent strain of *C. difficile* (PCR ribotype 027). Rates of detection of *C. difficile* have risen dramatically, *C. difficile* disease has become more severe, with an attributable mortality of >10% in those aged >60 years. In Quebec Province, Canada, in 2004, there were over 7000 cases with over 1000 deaths caused by *C. difficile* 027. In the UK recently, there have been several highly publicised outbreaks with over 50,000 cases in 2005 and over 2,000 deaths. On average each case of non-epidemic *C. difficile* infection (CDI) results in an extra 18 days in hospital for a cost of $1.25 million per annum for a typical Australian hospital. The majority of patients with CDI have been exposed to antimicrobials that reduce ‘colonisation resistance’ of the large intestine allowing subsequent infection. Acquisition of *C. difficile* is facilitated by its ability to form resistant spores that remain viable in the hospital for long periods. Toxigenic *C. difficile* usually produce two toxins, toxin A and toxin B that are thought of as the major virulence factors. 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, 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 is no evidence that the epidemic strain of *C. difficile* is established in Australia, however, the first case of ribotype 027 infection has recently been reported. One area of *C. difficile* research that has largely been ignored is the role of *C. difficile* in infections of young (<2 years of age) and older children. *C. difficile* colonises/infects most young animals and is known to cause disease in some, such as piglets. Outbreaks of CDI in haematology/oncology units of children’s hospitals have been recorded. Little is known of the epidemiology of CDI at Princess Margaret Hospital (PMH), the main paediatric teaching hospital in Perth.

**Aims:**
1) To determine the prevalence of *C. difficile* in children in haematology/oncology patients at PMH.
2) To determine the molecular epidemiology of CDI in haematology/oncology patients at PMH.
3) To determine the extent of environmental contamination at PMH by spores produced by *C. difficile*.

**References:**

1. Antimicrobial and natural products research  
*Dr Kate Hammer (Katherine.hammer@uwa.edu.au), Dr Kerry Carson (Kerry.carson@uwa.edu.au) and Dr Christine Carson (Christine.carson@uwa.edu.au)*

Our group is located within the PathWest Laboratory Medicine building at the Queen Elizabeth II Medical Centre and we conduct largely applied research. This covers a wide range of areas, with a broad focus on antimicrobial agents and some specific micro-organisms. As such a variety of projects is always available covering any aspect of clinical microbiology. This laboratory has a long history of projects investigating the antimicrobial activity of antimicrobials, natural products and other compounds. The major natural product investigated so far is tea tree oil, the essential oil obtained from the Australian native plant Melaleuca alternifolia. This essential oil has been the subject of research by the group for over 10 years. The group has investigated other natural products such as essential oils from other native Australian plants and the honey derived from Australian native bees. We have been contracted by several pharmaceutical and biotechnology companies to investigate new antimicrobials.

**Current research areas:**  
Project supervisors: Dr Kate Hammer and Dr Kerry Carson

1) **Antimicrobial activity of essential oils**  
This research focuses on investigating the spectrum of antibacterial and antifungal activity, and mechanisms of action of a range of essential oils and plant extracts.

2) **Effects of tea tree oil on susceptibility to other antimicrobial agents**  
Recent evidence suggests that exposure to tea tree oil may alter subsequent susceptibility to antibiotics (McMahon et al., 2007). If confirmed, the underlying mechanisms require investigation.

3) **Microbial stress responses to tea tree oil**  
It is possible that microbes mount stress responses to sub-inhibitory levels of tea tree oil as a way of compensating for any sublethal injury resulting from treatment with the oil.

4) **Resistance to essential oils**  
Essential oil resistance has not been demonstrated in the literature and may be due to the multi-component nature of essential oils and that the membrane is a primary site of antimicrobial action. Whether significant tolerance can be induced remains to be seen.

5) **Interference of essential oil terpenes with microbial quorum sensing**  
Several essential oils, including tea tree oil, inhibit the formation of biofilm, which is controlled by the cell-to-cell signalling known as quorum sensing. Furthermore, the essential oil component cinnamaldehyde has been demonstrated to block quorum sensing. A systematic investigation of different chemical classes of essential oil components (terpenes) may reveal further compounds that interfere with quorum sensing and may have potential as anti-biofilm agents.

**References**
Bacterial Pathogenesis Group

The general aim of our research is to understand how bacterial virulence genes and pathogenicity islands act to cause disease in humans. More specifically, our research is focused on understanding the pathogenesis of diarrhoeal diseases caused by Enterotoxigenic *E. coli* (ETEC) and *Shigella* spp. Both of these organisms are transmitted via the faecal-oral route and cause intestinal infections that lead to severe, life-threatening diarrhoea. It has been estimated by the WHO that these two bacteria are responsible for the deaths of up to two million people every year. However, despite the heavy toll on human life, there are no broadly effective vaccines available to prevent such infections and our understanding of the pathogenesis of these diseases is incomplete. Projects in these areas of research will involve the use of standard microbiological techniques, cell culture and a variety of molecular techniques including cloning, mutagenesis, PCR, sequencing and proteomics.

**PROJECTS**

1. **Rns-regulated virulence genes in ETEC**
   The primary virulence determinants in ETEC are long, rod-like surface organelles, called pili (fimbriae), which mediate bacterial attachment to intestinal epithelial cells and therefore allow the bacterium to colonise the host. Once it has established a foot-hold in the intestine, ETEC secretes enterotoxins that cause massive water loss, leading to death of the host if not treated. The expression of a type of ETEC pilus termed CS2 is directly controlled by a transcriptional regulator called Rns. However, work in our laboratory suggests that Rns is a more general regulator of virulence genes in ETEC. This work contradicts the current dogma that Rns only regulates the expression of pili. As well as regulating the expression of known virulence determinants, we have found that Rns also regulates the expression of two proteins of unknown identity. The aim of one project in our lab is to identify these proteins and determine their roles in virulence. This will involve determining the amino acid sequence of each protein by proteomic analysis and applying this information to the cloning and characterisation of the genes that encode them. This project has the potential to identify ETEC virulence genes that are entirely novel and therefore to increase our understanding of how ETEC causes disease.

2. **Receptor binding activity of ETEC pili**
   (in collaboration with Prof. Charles Bond),
   CS2 pili belong to a family of closely related pili, including CS1, CFA/I and others, which are expressed exclusively by ETEC. CS2 pili consist of 2 distinct protein subunits (pilins) termed CotA and CotD. The two pilins are assembled into a pilus by two assembly proteins; a periplasmic chaperone termed CotB and an outer membrane transporter termed CotC (1). The shaft of the pilus is comprised of the major pilin, CotA, while the minor pilin, CotD, is only found at the pilus tip and mediates the adherence of the pilus to erythrocytes (2). This binding activity has been correlated with binding to intestinal cells, presumably because erythrocytes and intestinal cells carry identical or structurally similar receptor for CS2. Although receptor binding requires CotD, our preliminary data suggest that both CotA and CotD contribute amino acid residues to a combined receptor-binding pocket in a manner analogous to that of the light and heavy chains of immunoglobulins, which both contribute to antigen binding.
This is a novel paradigm in pilus-mediated attachment and the aim of this project is to further test this hypothesis. Specifically, CotD protein will be purified and its binding activity will be compared to that of the intact pilus where it naturally interacts with CotA. This will involve (i) constructing a hexahistidine-tagged CotD fusion protein that can be easily purified by affinity chromatography and (ii) developing ELISA-type assays to compare the binding activities of purified CotD and pili. Differences in receptor binding or specificity would confirm the hypothesis. The second aim of this project is to purify pili in a form that is suitable for crystallisation. This will be the first step in determining the structure of the pilus. Subsequent structural analyses, beyond the scope of this Honours project, will provide an insight into how CotA and CotD interact at the pilus tip and how this determines receptor binding.

3. Lateral transfer and maintenance of the *she* pathogenicity island in *Shigella flexneri*

*S. flexneri* is a Gram-negative bacterial pathogen of the intestine that causes bacillary dysentery in humans. Its genome contains a large pathogenicity island termed the *she* PAI, which carries virulence genes encoding enterotoxins and modulators of inflammation (3). The PAI is a selfish, laterally transferred genetic element that integrates into and excises from the chromosome in much the same way as a temperate bacteriophage (4). When it is not integrated into the chromosome, the PAI exist as a transient, extrachromosomal, circular DNA molecule resembling a plasmid. However, the PAI lacks genes required for autonomous replication and so, unless it integrates back into the chromosome, will be lost in dividing daughter cells. The mechanisms by which the PAI is laterally transferred and stably maintained in the chromosome are not known but these are important processes that have contributed to the evolution of virulence in *S. flexneri*. In this project we intend to study whether the PAI is mobilised to new bacterial hosts by helper plasmids which supply conjugative transfer functions or by phages which specifically package the PAI circle for transfer by an unusual type of specialised transduction. In addition, this project will test the hypothesis that two PAI genes are essential for stable maintenance of the PAI in the chromosome. Specifically, we will test the hypothesis that these two genes allow the PAI to hold the bacterial cell hostage and ensure the PAI’s survival by poisoning bacterial cells in which the PAI has spontaneously excised and has been lost during cell division. This will involve cloning the two genes to test their activities and inactivating them in *S. flexneri* to test their hypothetical roles in maintenance of the PAI.

Literature cited

Murine cytomegalovirus (MCMV) is a mouse-specific herpesvirus that is commonly used as a model for human cytomegalovirus 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 and tissue tropisms of the virus. Several genetically different strains of MCMV have been isolated from a single mouse, and we are interested in the relationship between these different strains and the resulting disease in the host animal. 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. The Cytomegalovirus Research Group is located in state of the art laboratories within the new Marshall Centre for Infectious Disease Research and Training in the Discipline of Microbiology and Immunology.

Cytomegalovirus Research Group

Projects

1. Requirements for MCMV endothelial cell tropism.
   Dr Alec Redwood and Dr Lee Smith

   MCMV replicates in most cells in the body, however its replication in host endothelial cells is of particular interest because these cells play a role in the transmission of the virus to macrophages, which then enable the virus to spread to distal sites. In addition, infection of endothelial cells is likely to play a role in the development of atherosclerosis, which has been associated with human cytomegalovirus infection. We have recently identified two strains of MCMV that demonstrate different capacities for endothelial cell replication. One strain C4C replicates to high titres in endothelial cells and the other, C4B replicates poorly in these cells. This project will investigate the causes of poor endothelial cell replication by C4B, specifically the role of apoptosis, cell entry and cell-to-cell spread in this defect. It is anticipated that these viruses will be used to develop models of atherosclerosis in mice. As such this project would suit a student interested in molecular biology, virology and basic cellular biology and will involve cell culture, molecular biology and flow cytometry.

2. Novel murine cytomegalovirus genes of unknown function.
   Dr Lee Smith and Dr Alec Redwood

   We have recently sequenced the genomes of 4 strains of MCMV, one laboratory strain and 3 strains recently isolated from wild-trapped mice. A comparative genomics analysis of these genomes has identified several putative novel genes which are highly conserved between all viral strains, yet which thus far remained uncharacterised. Quantitative real-time PCR analysis has shown that at least 4 of these putative genes are transcribed at early time points (4-10 hours) post-infection, and we wish to further characterise these new genes to elucidate their function. This project will involve gene cloning, protein analysis, cell imaging and quantitative real-time PCR, as such would suit a student interested in virology, molecular biology or both.
3. Determinant of MCMV dissemination to the salivary gland
Dr Alec Redwood and Dr Lee Smith

MCMV replicates in most organs in the body, however its replication in the salivary gland is of particular importance as the virus transmits to new hosts via the saliva. Dissemination to the salivary gland is believed to take place inside host macrophages. Once in the salivary gland, the virus can persist from many weeks, long after the virus is cleared from most other organs. Length of persistence at this site is likely to influence the period of time that an individual remains infective. The mechanism by which cytomegaloviruses such as MCMV and HCMV persist in the salivary gland remain unknown but are believed to be linked to the ability of the virus to evade host immune responses.

We have two strains of MCMV that exhibit differences in their capacity to persist in the salivary gland. This study will address the mechanisms of salivary gland persistence by comparative studies of the immune response, in the salivary gland, of mice infected with the two different strains of MCMV. This project would suit a student interested in immunology and virology and will involve such techniques as flow cytometry, tissue culture, viral isolation and real time PCR.

4. The effect of MCMV on pregnancy.
Dr Megan Lloyd, Dr Lee Smith, Professor Geoff Shellam

Human cytomegalovirus (HCMV) has replaced rubella virus as the most important viral infection of the fetus in utero, and is responsible for significant morbidity and mortality worldwide. Because of the strict species specificity of HCMV, experimental studies of CMV infection employ the mouse model using murine CMV (MCMV). However, although most characteristics of MCMV-induced disease mimic HCMV infection, it is generally thought that MCMV does not readily cross the placenta and infect fetal mice. However, preliminary data obtained in our laboratory suggests that this is not the case, and that fetal infection with MCMV is possible.

This project therefore seeks to define the effect of both laboratory and wild-derived MCMV strains on mouse pregnancy by investigating both fetal and maternal health. Female mice at different gestational stages will be infected with different MCMV strains (either one strain, or a mixture of several strains) and the effect of this infection on fetal and maternal health will be determined by measuring fetal and placental weights and looking for the presence of MCMV in fetal and maternal tissues. Virus detection will be carried out using a variety of methods such as quantitative PCR, in-situ hybridization and ELISA. By characterising the effect of these strains on pregnancy, we will be better able to model congenital HCMV infection. This would significantly contribute to the understanding of cytomegalovirus disease.

5. Infection and disease caused by cytomegalovirus in breast milk
Dr. Megan Lloyd, Dr. Lee Smith and Professor Geoff Shellam

Cytomegaloviruses(CMV) are herpesviruses with a large DNA genome. They infect humans and a variety of animal species and cause diseases which are often severe in those whose immune systems are immature or suppressed. Human CMV is the leading viral infection of the foetus following transplacental transmission from the infected mother. It may also cause disease in the newborn following transfer to the suckling child in breast milk, causing mental retardation, hearing loss and damage to other tissues.

This project uses a mouse model and the closely related mouse CMV(MCMV) to investigate infection and disease in suckling pups due to MCMV acquired in breast milk from a virus infected mother. Mothers will be infected acutely at the time of birth of the pups or 8 weeks prior to pregnancy as a model for latent infection and reactivation during pregnancy. qPCR will be used to measure MCMV in breast milk and tissues of the pups at various times, and tissue sections will be taken to assess disease development in the brain, eyes and viscera. Mental retardation and hearing loss will be studied using behavioural and auditory tests.

The project also investigates how the level of MCMV in breast milk may be controlled by the innate immune system and by the ability of MCMV to replicate in endothelial cells. Finally, since cytomegalovirus usually establishes mixed infections with different strains, the possibility that passage through the breast and breast milk may select for MCMV strains with distinct properties in an otherwise multiply infected mouse will be studied. The project offers training in a very well established lab in both in vivo studies , virus handling and assay, qPCR, disease assessment and studies of innate immunity.
Inflammation and Infectious Diseases Group

Our group is interested in inflammatory and atopic lung diseases such as asthma and allergic rhinitis as well as respiratory and prostate infections. Our long-term aim is to elucidate the immunomodulatory effects of the allergens and pathogen associated molecular patterns responsible for these diseases and to develop novel therapeutic strategies for their intervention. We have isolated and characterised several house dust mite, pollen, and cockroach allergens. Prospective Honours students with a background in Immunology, Microbiology, Molecular Biology, Biochemistry or Molecular Genetics are particularly encouraged to apply. Students will be exposed to a range of techniques including DNA cloning gone array, cell culture, transfection assays, cytokine ELISA, RAST and RAST-inhibition assays, RT- and Q-PCR, protein expression and analysis, bacterial characterisation, enzymology, confocal and epifluorescence microscopy, immunohistochemistry and FACS analysis.

PROJECTS

1. Anti-Microbial Peptides and Pro-Phenyloxidase Activity in the House dust Mite
   With Dr Leslie Mathaba (lmathaba@cyllene.uwa.edu.au)

   Invertebrates produce a variety of cationic antimicrobial peptides (e.g., defensins, cecropins and attacins) and enzymes involved in melanisation in response to potential pathogens. Most of the work has been performed in insects and little information exists with regard to their presence in other arthropods such as arachnids (spiders and mites). Similarly, the enzyme pro-phenyloxidase is involved in producing melanin which results from the production of quinones and plays an important antibacterial role. Whether dust mites, which are associated with asthma, produce anti-microbial peptides or possess the pro-phenyloxidase system is unknown. Our research on dust mites, has resulted in the isolation of 2-3 bacteriolytic enzymes, which could play a role in defending mites against bacterial pathogens. Students will undertake an analysis of such antimicrobial products in mites using homology and enzymatic based approaches. Our preliminary studies have revealed the existence of such peptides using a mite cDNA library and primers based on the amino acid sequences of androctonin and gomesin, hairpin-like beta sheet structures from Androsctonus australis and Acanthoscurria gomesiana in a low stringency PCR analysis.

2. Modulatory Effects of Rye Grass Pollen Allergens on Respiratory Epithelial Cell Function
   With Dr Martha Ludwig and Professor Alice Vrielink (martha.ludwig@uwa.edu.au)

   Aeroallergens are considered to be the most common cause of allergic disease and are derived from a variety of complex particulate sources present in the environment. They include pollens, fungal spores, insect and mite faeces, animal danders and dusts, and exposure may be perennial or seasonal. Allergens from the pollens of several grass species have been described at the molecular level and include species belonging to the clinically important subfamilies Pooidae and Panicoideae. There is significant sequence similarity and, hence, immunological cross-reactivity between allergens from botanically related pollens which has made it possible to group allergens from related species together. This has resulted in the description of more than 13 distinct groups of proteins with diverse biochemical properties. To cause disease, an allergen must make contact with the respiratory mucosa and, in previous studies, we have shown that respiratory epithelium responds to exposure to allergens in this way by producing a range of pro-inflammatory mediators that are able to contribute to allergic disease. However, whether pollen allergens do this is unclear but recent exciting preliminary data from our laboratories suggest that they do. These novel findings indicate that further studies are warranted and in this project, students will continue and extend these studies. In doing so, students will be exposed to state-of-the-art cloning and protein expression techniques, gene array studies, cytokine measurements and possible proteins structure determination.
3. Cloning, expression and characterisation of mite proteases and bacteriolytic enzymes.  
*With Dr Leslie Mathaba (lmathaba@cyllene.uwa.edu.au)*

Domestic mites are medically and economically important pests responsible for the development of allergic diseases and for the destruction of stored grain and seed resulting in poor yields. Our laboratory has been involved in the isolation and characterisation of mite allergens. Recently, we isolated a new cysteine protease from the mite *Dermatophagoides pteronyssinus* designated Der p 9/LM-1. Der p 9/LM-1 has been partially characterised and shown to be a very important allergen recognised by over 70% of mite allergic individuals. We also observed that mites produced enzymes possessing bacteriolytic activity. Bacteriolytic enzymes have never been reported in mites and this was exciting for us given that some bacteriolytic enzymes have been implicated in allergies. We have isolated and sequenced a cDNA encoding a 14 kDa bacteriolytic enzyme and the sequence data showed homology with bacterial rather than eukaryotic proteins suggesting that it could be of bacterial origin. Expression of these cDNA is essential to enable a detailed physicochemical and immunochemical characterisation of Der p 9/LM-1 and the 14 kDa bacteriolytic enzyme. In addition, we have shown that these proteins are produced by other mites (e.g., *Blomia tropicalis*). Using the PCR and other techniques the genes/cDNA coding for these proteins in other mites will be isolated for comparison with the *D. pteronyssinus* cDNA sequences.

4. Protection from Complement-Mediated Damage on Respiratory Epithelium.  
*With Dr Asokananthan (asoknithi@yahoo.com)*

Complement comprises groups of proteins that play an important role in both innate and adaptive immunity. They act as opsonins to facilitate phagocytosis and cause lysis of cells via the membrane attack complex (MAC). So potent are they that a number of regulatory proteins exist to moderate their activities once activated. In this regard, CD55 and CD59 are important because they accelerate the decay of membrane bound convertases and inhibit the MAC activity. We have recently shown the upregulation of the genes encoding these proteins by pro-inflammatory stimuli. These are novel findings in the context of the lung and we wish to further explore the biology of these proteins. In addition, these proteins exist as isoforms which are produced by alternate splicing mechanisms and may be secreted. In this study, students will determine whether their secretion is modulated by pro-inflammatory stimuli and whether different isoforms are differentially modulated. Stimuli will include microbial pathogen associated molecular patterns, pro-inflammatory proteases and allergens. In doing so, students will be exposed to cell physiology techniques, ELISA, real time PCR and protein characterisation studies. Findings from such studies may help us understand the mechanisms involved in protecting the lung from pro-inflammatory insults.
Our group is interested in using an understanding of the diversity and roles of microbes in natural (particularly marine) systems to identify potential applications, particularly in the area of disease control and industrial applications. Our focus is on microbes with the potential to act as biological or probiotic control agents against bacterial agents of human and aquaculture disease, and on natural products (of microbial or marine invertebrate origin) that have antimicrobial activities which may have application in clinical and industrial settings. We also study marine microbial diversity and its interactions with host organisms (from mutualistic symbiosis to disease), as study of these is central to detecting interactions mediated by chemicals with potential commercial applications. To this end we have developed appropriate isolation, identification and bioassay techniques for use in an established model disease situation. Our long-term aim is to develop biological control agents and antimicrobials for use in disease prevention and control strategies and in control of biofouling. Prospective Honours students with a background in Microbiology and Molecular Biology, Biochemistry or Chemistry are particularly encouraged to apply. Students will be exposed to a range of microbiology and molecular biology techniques.

PROJECTS

1. Quorum sensing inhibitors – an exciting new weapon in bacterial disease control?

Aims of the project are to seek quorum sensing inhibitors of *Vibrio harveyi* and investigate their nature and modes of action, and to assess their potential application in bacterial disease control.

Quorum sensing is a bacterial cell communication process essential in many disease-associated processes including biofilm formation, virulence mechanisms or avoidance of host immune responses, and is mediated by ‘autoinducer’ molecules via pathways which have been extensively studied. Quorum sensing inhibitors (termed ‘quorum quenching compounds’) offer exciting applications in control of bacterial disease, as there is a growing body of evidence that such chemicals can reduce virulence in some pathogenic bacteria. As quorum quenching compounds only inhibit communication and do not kill bacteria, development of bacterial resistance, as occurs with antibiotics, may be reduced or not occur.

A model system for detecting and investigating quorum quenching compounds, their mode of action and effect on virulence has been developed in Microbiology at UWA. The model is based on *Vibrio harveyi*, a bioluminescent bacterium and devastating aquaculture pathogen causing ‘luminous vibriosis’ of cultured finfish, crustaceans and molluscs. Bioluminescence in *V. harveyi* is a quorum sensing process which appears to be related to disease-causing ability, as mutants in quorum sensing genes lose virulence. The *V. harveyi* isolate used in our system is highly pathogenic to the crustacean *Artemia*, the disease host used in the established model.

Quorum quenching compounds are produced by a number of bacteria (e.g. some *Bacillus* spp.) and certain plants and algae. Secondary metabolites of the marine alga *Delisea pulchra* have been reported to both inhibit bioluminescence of *V. harveyi* and reduce its virulence to prawns. In recent studies we have found strong evidence that other marine organisms, including sponges, also contain compounds with quorum quenching activity against *V. harveyi*. The production of such compounds as part of evolved defense mechanisms in marine organisms is not surprising, as they live permanently bathed in a medium (seawater) containing very large bacterial populations, typically in excess of 10^6/ml.

In this project quorum quenching compounds which inhibit *V. harveyi* quorum sensing will be sought from a diversity of marine bacteria. Active compounds will be partially or fully purified, and the bacteria producing them will be identified using molecular methods. The extracted compounds and the bacteria that produce them will be tested for their ability to inhibit quorum sensing processes (eg bioluminescence, biofilm formation, virulence factor expression) and to protect *Artemia* from infection by *V. harveyi*. The discovery of these types of compounds may well provide new opportunities for drug development as well as enhancing knowledge of defense mechanisms in marine organisms. Techniques to be used include DGGE, PCR and sequencing, screening of natural products for inhibition of *Vibrio harveyi* or its quorum sensing system; chemical extraction
and purification of bioactive compounds; bioassays (bioluminescence, biofilm, virulence factors); and *Artemia* bioassays for assessment of control of disease caused by *Vibrio harveyi*.

**References:**
Hepatitis C Virus Research Group

Hepatitis C virus (HCV) is a major health problem in Australia with over 200,000 people currently infected, around 9,000 new cases annually and in 1998 estimated treatment and care costs were in excess of 40 million dollars. Primary HCV infection is only resolved by about 20% of individuals with the remaining 80% developing varying severity chronic infection that may persist for decades if untreated. During the chronic stage of disease, HCV can cause progressive liver damage and is the commonest cause of liver transplantation in all developed countries. It is also associated with increased risk of hepatocellular carcinoma. HCV is currently treated with pegylated interferon-α (PEG-IFN) and ribavirin to increase the response rates to between 50 and 80% depending on the viral genotype. Despite current advances, many patients are still refractory to treatment; the reasons for this are not completely understood. Some patients are able to carry HCV at significant viral loads with little or no associated pathology, whilst others with very low viral loads may have substantial liver damage. This dichotomy is not without probable cause as the immune responses that may assist with virus eradication may also play a role in the destruction of liver tissue in kind of double edged sword. For untreated or treatment failure patients progression to end stage liver disease is the commonest outcome. With poor prognosis liver transplant is the only treatment option. Transplant patients are immune suppressed after transplant to aid organ engraftment. Unfortunately the immune suppression also allows HCV still within the patient’s blood to infect the new organ and replicate unchecked by the immune system. This can cause serious damage to the new organ and rejection. Current therapy for HCV infection is contraindicated in transplantation due to immune suppression and thus there are no therapies available to reduce the viral burden. With HBV, anti HBV immunoglobulin is used and maintains viral load at a low level improving transplant survival and minimising damage. In HCV infection this is not yet available, largely due to the lack of suitable immunoglobulin that is in turn related to our inability to generate good antibodies against HCV. Despite the apparent lack of B cell mediated immunity directed at HCV in infected patients, there are infected individuals that have strong antibody responses to the virus and maintain low or undetectable viral loads. This indicates that in these patients virus replication may be suppressed by circulating antibody. These patients are a primary target for cloning B cells producing infection neutralizing antibodies. Production of monoclonal antibodies (MAbs) is not new and was first described in 1975 by Kohler and Milstein and is now commonplace in both mouse and rat systems. The production of human antibodies is also possible with the availability of suitable human myeloma fusion partners. These systems are now quite efficient but in the case of HCV to date, there have been some inherent problems associated with the production of effective (infection-neutralizing) monoclonal antibodies.

Monoclonal antibody production involves several critical steps. These are 1) vaccination or infection of the target organism from which the B cells will be cloned; 2) obtaining B cells from the organism (usually spleen or lymph nodes used) and fusion with an immortalised cell line to immortalise the B cells and 3) screening of cloned B cells for the production of antibody directed to the target antigens or organisms. In the case of the hepatitis C virus all of these steps are problematic and prohibitive to the production of infection neutralizing antibodies. Firstly HCV cannot be produced in small animal models or grown in culture preventing the whole virus being used to vaccinate or infect mice or rats, the usual animals used to produce monoclonal antibodies. Secondly, most HCV antigens have been expressed in E. coli often resulting in loss of native protein conformation and glycosylation, both of which are particularly important for the HCV surface glycoproteins E1 and E2. Some researchers have thus attempted to express HCV proteins in cell culture systems to promote native protein structure. Unfortunately only small
amounts of antigens are available from these systems making their use as immunogens problematic. Also, without a cell culture system or small animal model for HCV infection, identification of infection neutralizing antibodies is near impossible. Most monoclonal antibodies produced and characterised to date against HCV recognise linear unglycosylated epitopes, a consequence of screening with bacterially expressed and denatured antigens, often in a Western blot format.

We have developed one of the few HCV culture replicative models for HCV and are using this model to screen candidate antibodies produced from a human hybridoma line. We also have access to a more efficient myeloma fusion partner that we will use to generate more antibody lines. Our long term goal is to use our model system to study antibody and drug inhibitors of HCV infection with an end goal to developing therapeutic agents to control HCV infection. Our projects are concerned with advancing our antiviral antibody projects in terms of identification of the antibody targets and developing new assays to identify cross reactivity with other HCV genotypes. We hope that this will identify new therapeutic agents to combat this serious infectious disease.

It is likely that we will be able to take only Mid-year Honours entrants in 2010. For further discussion of projects please contact Dr Watson.
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 in the left hand menu bar of StudentConnect.

**Applications will open online on Wednesday 7 October and close on Tuesday 8 December.**

**Non-UWA Applicants**

If you have not previously been enrolled at UWA, you apply through one of the following centres, depending on your circumstances.

**Applications close on Friday 18th December 2009**

**Domestic Students**

Australian citizens, permanent residents and/or holders of a humanitarian visa or New Zealand citizens apply through the UWA Admissions Centre.

**International Students**

International Students apply through the UWA International Centre.

**All applicants need to complete the BBCS Honours Preference Form.**
## Personal Details

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**Former Family Name (if applicable)**

Please attach evidence of change of name to application

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## Enrolment

Are you currently enrolled or have you previously enrolled as a student of The University of Western Australia?

YES [ ] NO [ ] If YES, state student number: [ ] Year last attended:

## Course Information

1. **Application for commencement:** (Please tick)
   - Note: Applications are only permitted for the next available intake.
   - Start of Year (February commencement) [ ]
   - Mid-Year (July commencement) [ ]

2. Please list all the UWA Honours degree courses that you intend to apply for in order of preference:
   - [Please submit separate School / Faculty Approval Form for each course]

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<th>Preference</th>
<th>Course Title (eg. Bachelor of Science Honours)</th>
<th>Course Code (eg. 5011H)</th>
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<th>Major/Programme Code (eg. MJ-GRMAN)</th>
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3. **Are you applying for Joint or Cognate Honours within your degree course?**
   - YES [ ] NO [ ]
   - If YES, list combination (eg Anthropology and Mathematics)
   - Note that you must have discussed this combination with both Schools responsible for the programme

## Secondary School Qualifications

Please attach correctly certified copies of your results (not necessary for WA TEE results from 1976 onwards)

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## Office Use Only

- ENTERED ON CALLISTA _________________
- OFFER [ ] NO OFFER [ ] NOTIFICATION SENT ________________
- SENT TO FACULTY _________________
- CONDITIONAL OFFER [ ] CONDITION SATISFIED; LIFTED ON CALLISTA
### Post-secondary & Tertiary Qualifications

Please provide details of ALL study you have undertaken at a tertiary institution, and attach correctly certified copies of your results. Official Academic Transcripts are required, NOT statements of examination results (UWA results are not required).

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<th>Years Undertaken</th>
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### Personal Statistical Details

You must attach proof of citizenship/permanent residence status to your application. Acceptable documents include: original or certified copy of an Australian or New Zealand Birth Certificate; ID page of your passport (and relevant visa pages, if you are an Australian PR).

1. Are you of Aboriginal or Torres Strait Islander origin?  
   - YES  
   - NO

2. What is your Citizenship or Residency Status?  
   - Australian Citizen
   - New Zealand Citizen  
   - Possess a permanent residency visa  
   - Possess a temporary entry visa  
   - Other

3. In what country were you born? (if not born in Australia)  

4. Year of arrival in Australia (if applicable)

5. Do you speak a language other than English at your permanent home residence?  
   - YES  
   - NO

   If yes, what is the other language?

### Admission Statistical Details

Please attach to the application original or certified copies of documentation to support Admission Statistical Details

1. Entry Qualifications - What is your highest educational attainment?  
   - Completed Higher Education postgraduate level course
   - Completed Higher Education bachelor level course
   - Completed Higher Education sub-degree level course (e.g. diploma)
   - Incomplete Higher Education course
   - Completed TAFE award course
   - Completed final year of secondary education course at school or TAFE
   - Completed other qualification or certificate of attainment or competence
   - No prior educational attainment

2. In what year did you achieve your highest educational attainment?

### Declaration

All applicants must read, sign, and date the declaration below

I declare that I have read and understood the Information Sheet for Domestic (External) Honours Applicants. I declare that the information provided by me in connection with this application is true and complete. I understand that UWA reserves the right to vary or reverse any decision regarding admission or enrolment made on the basis of incorrect or incomplete information provided by me, and that any such act on my part will be placed on record and will form part of confidential information forwarded to selectors in assessing any subsequent applications. I authorise UWA to obtain results and records from any examining body or educational institution, and to disclose information to the Australian Vic-Chancellors’ Committee and its member institutions.

I understand that the University’s authority to collect the information on this form is given by the Higher Education Support Act 2003; that the information is collected to allow the University to properly administer its course programmes; that the information may be shared for these purposes between the Australian Taxation Office and the Department of Education, Science and Training; and that the information may not otherwise be disclosed without my consent, unless authorised or required by law.

APPLICANT’S SIGNATURE: _______________________________  DATE: __________________________
Honours or GradDipSci in 2010

PROJECT PREFERENCE FORM

The purpose of this form is to ascertain your interest in our Honours/GradDipSci courses. It is appreciated that students may be exploring Honours/GradDipSci in more than one discipline. Phone the BBCS School Office (64884402) to be referred to the appropriate Coordinator to discuss any questions you may have.

Please return form to BBCS School Office by Wed 8th Dec 2009

I am interested in Honours/GradDipSci in 2010 within the Discipline of:

- [ ] Biochemistry & Molecular Biology
- [ ] Microbiology & Immunology
- [ ] Chemistry
- [ ] Physiology

Note: You need to fill out a separate form for each Discipline if you are considering projects in more than one. Include projects for any Programme (e.g. Genetics, Green Chemistry, Biomedical Science etc) that will be located within one of the above Disciplines.

I am considering mid-year entry to Honours in 2010

I am considering deferring Honours until 2011

I will [ ] will not [ ] be available for interview during the week 14 December - 18 December 2009

1. CONTACT DETAILS

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

Address(es) (during period November/December 2009 – January 2010):

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

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

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

2. PROJECT PREFERENCES

In order of preference:

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If there are any points you would like us to take into consideration please note them below:

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

The Faculty’s End-on Honours on-line application form must be completed by December 8th 2009. Prospective candidates will be interviewed 14 December - 18 December 2009, although other arrangements can be made if candidates are unavailable. Those students who have submitted this project preference form and who are eligible to enrol in the course will be emailed a confirmation of eligibility as soon as exam results are known [approximately 21 December], and allocation of projects will be advised as soon as possible after this. Student Administration will send you an Authority to Enrol letter in January 2010.