

# Burnet Institute

## 2008 Honours Projects - Laboratories Only

### **Project Title: Inhibition of cytokine signalling by HIV-1**

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### **Description:**

HIV-infects cells of the macrophage lineage resulting in defects in macrophage function<sup>1,2</sup>. We recently examined GM-CSF receptor signalling in HIV-infected monocyte-derived macrophages (MDM) since this growth factor is important in stimulating or maintaining immune functions of macrophages. It was found that HIV inhibits GM-CSF induced STAT5A activation without affecting expression of the signalling proteins within this pathway<sup>3</sup>.

One potential mechanism for this inhibition is induction by HIV-1 of the negative regulators of STAT activation, the SOCS proteins. These consist of a family of 8 members (SOCS1-7 and CIS) which regulate cytokine signalling. Our recent data show that inhibition is associated with increased expression at both the protein and mRNA level of CIS in HIV-1-infected cultures<sup>4</sup>. As SOCS proteins can potentially downmodulate signalling from a wide variety of cytokines, this novel observation suggests that HIV-1-infected macrophages may suppress cytokine responses in tissues. It is not clear whether the inhibition of GM-CSF signal transduction is a direct effect on the HIV-infected cell or due to inhibitory cytokine production affecting bystander cells nor whether inhibition is restricted to GM-CSF signalling or applies to other cytokines which activate STAT5A, such as IL2. To help answer these questions, our laboratory has established techniques to analyse cells infected with HIV-1 expressing enhanced GFP so that infected cells can be easily distinguished from uninfected, bystander cells within the culture.

### **Hypothesis:**

That HIV infection of macrophages inhibits cytokine signalling via expression of CIS.

### **Aims:**

1. To develop a flow cytometry assay for GM-CSF and IL2 signal transduction and determine whether HIV-infection of monocyte-derived macrophages inhibits cytokine signalling in bystander cells.
2. To use nucleofection technology to inhibit CIS expression in macrophages and determine its effects on HIV-induced inhibition of GM-CSF signalling.
3. To use the above established techniques to compare cytokine signalling in peripheral blood mononuclear cells from HIV-infected patients and control subjects.

### **References:**

1. Chan, H. T., K. Kedzierska, J. O'Mullane, S. . Crowe, and A. Jaworowski (2001) *Immunol Cell Biol* 79:429-35.
2. Kedzierska, K., P. Ellery, J. Mak, S. R. Lewin, S. . Crowe, and A. Jaworowski (2002) *J Immunol* 168:2895-903.
3. Warby, T., Crowe, SM. and A. Jaworowski (2003) *J. Virol.* 77:12630-38
4. Anjana Chakravorty (2005) unpublished data.

## **Project Title: Analysis of single cell signal transduction in HIV patient samples**

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**Background:** Our previous research using *in vitro* models has shown that HIV-1 infection inhibits signal transduction downstream of several receptors expressed on monocytes/macrophages<sup>1,2</sup>. In particular, activation of the tyrosine kinase Syk by Fc $\gamma$  receptors (CD16 and CD64) is impaired because of decreased synthesis of a key signalling protein FcR $\gamma$ . This potentially leads to decreased phagocytosis (via CD64) and antibody-dependent cellular cytotoxicity (via CD16) by monocytes and natural killer cells respectively, processes which both require FcR $\gamma$ , and hence impaired innate immune responses. Although defects in phagocytosis and ADCC have been reported in AIDS patients, the underlying mechanisms are not known.

Recent advances have allowed signal transduction events, such as the activation of Syk, to be measured at the single cell level by flow cytometry. In this project, Syk activation by selected immune receptors will be measured in cells obtained from HIV-1-infected patients to determine whether defective signalling occurs *in vivo*.

**Hypothesis:** That HIV-1 decreases FcR $\gamma$  dependent activation of Syk in NK cells and monocytes obtained from HIV-1-infected patients leading to defective ADCC and phagocytosis respectively.

**Overall goal.** To determine the underlying causes of innate immune deficiency in HIV-1 patients.

### **Aims:**

1. To establish an *ex vivo* flow cytometric assay for Syk activation (a) in NK cells following CD16 cross-linking and (b) in monocytes following CD64 cross-linking.
2. To measure CD16 and CD64 signal transduction in a cohort of HIV-1 infected individuals and in uninfected control subjects.
3. To correlate signal transduction efficiency with functional measures of NK cell and monocyte function.

### **References:**

1. Warby, T., Crowe, S.M., Jaworowski, A. (2003) "HIV-1 Infection inhibits GM-CSF induced activation of STAT5A in human monocyte-derived macrophages (MDM)." *J. Virol.* 77, 12630-12638.
2. Kedzierska, K., Ellery, P., Mak, J., Lewin, S., Crowe, S.M. and Jaworowski, A. (2002) "HIV-1 downmodulates gamma signalling chain of Fc $\gamma$  receptor in human macrophages: a possible mechanism for inhibition of phagocytosis." *J. Immunol.* 68, 2895-2903
3. Leeansyah, E., Wines, B.D., Crowe, S.M. and Jaworowski, A. (2007) "The mechanism underlying defective Fc $\gamma$  receptor mediated phagocytosis by HIV-1 infected human monocyte-derived macrophages." *J. Immunol.* 178(2):1096-104.

**Project Title: A Cytomegalovirus analogue of IL-10**

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**Background:** HCMV is a ubiquitous herpes virus which is a leading cause of mortality in immunocompromised individuals such as HIV patients. Our collaborators in Sydney have recently discovered that human cytomegalovirus (HCMV) encodes a homologue of IL10, termed LA-cmvIL10, which is expressed during latent infection<sup>1</sup>. HCMV infection is associated with increased susceptibility to opportunistic infections however the role of LA cmvIL10 in this process is not understood. Since IL10 is a potent immunomodulatory cytokine which inhibits Th1 responses of mononuclear cells required to control many opportunistic infections, a role for the HCMV homologue is suggested.

**Hypothesis:** LA-cmvIL10 impairs monocyte responses to opportunistic infection.

**Overall goal.** To define the role of LA cmvIL10 in increased mortality in HIV-1 infected individuals.

**Aims:**

1. To determine the effect of LA-cmvIL10 on phagocytosis of opsonised targets by
  - a. Monocytes
  - b. Monocyte-derived macrophages
2. To determine the effect of LA-cmvIL10 on the phenotype and function of monocyte subsets.
3. To use microarray analysis to determine whether LA-cmvIL10 alters the immunological response of macrophages following phagocytosis.

**References:**

1. Jenkins C, Abendroth A, Slobedman B. (2004) "A novel viral transcript with homology to human interleukin-10 is expressed during latent human cytomegalovirus infection". *J Virol.* 78(3):1440-7.

## **Project Title: Population genomics of malaria parasites**

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A child dies from malaria every 30 seconds. This intolerable burden of malaria is partly due to the extensive genetic diversity among malaria parasites. More information about the natural diversity and fluctuations of malaria parasite populations is needed to understand the evolution and spread of drug resistance, to design and implement the most appropriate vaccines and, to define the relationship of parasite diversity with malaria epidemiology. My research uses close ties with field researchers in Papua New Guinea (PNG), Africa, Asia and Latin America; and a combination of **epidemiology, population genomics and bioinformatics** to understand the diversity and evolution of the major human malaria parasites *Plasmodium falciparum* and *P. vivax*. Projects will be based at the Burnet Institute laboratories in Melbourne, and include:

### **(1) Molecular epidemiology of malaria in PNG**

This project will examine the population biology of *P. falciparum* and *P. vivax* in PNG where malaria transmission is intense, year round. Parasite diversity will be investigated at the individual, local and regional level by genotyping genome-wide microsatellite and SNP markers. The population genomic data will be examined in relation to the epidemiology of malaria. The data will be compared to that from other malaria endemic areas around the world.

### **(2) Population genomics of the immune evasion (*var*) genes of *P. falciparum* in PNG**

The highly polymorphic *var* multigene family encode the major variant surface antigen, PfEMP1. Each malaria parasite has up to 60 *var* genes that are switched on or off to change the antigenic properties of the parasite infected red blood cell and allowing immune evasion. Understanding the diversity of the *var* genes will provide insights into the transmission of malaria and may lead to a PfEMP1-based vaccine. We have developed molecular and bioinformatic tools to investigate the diversity and evolution of the *var* genes on a large scale. See Barry et al. (2007) *PLoS Pathogens* 3(3): e34 doi:10.1371/journal.ppat.0030034. This project will utilise these tools to study *var* gene diversity in different parasite populations of PNG. Experimental techniques to be used include PCR, cloning and sequencing. Data analysis involves a combination of bioinformatics and population genetics. The data will be compared to that from other malaria endemic areas around the world.

### **(3) Population genomics of malaria vaccine candidates**

Several surface antigen molecules are being developed as malaria vaccine candidates and are undergoing clinical trials. This project will investigate the diversity and evolution of key malaria vaccine candidates so that vaccine formulations and clinical trials can be better designed. The project will involve both the collection of data in the laboratory and the utilisation of data already available from the GenBank database. Experimental techniques to be used include PCR, cloning and sequencing. Data will be analysed using bioinformatic techniques specific to the analysis of DNA sequences. Population genomic analysis will be done using standard techniques.

## **Project Title: “Investigating tissue levels of apoptosis and mitochondrial DNA for monitoring and predicting HIV disease progression.”**

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Our group is investigating the complications of HIV infection and its treatments. We have developed novel assays for quantifying the cellular changes associated with antiretroviral therapy, but results may also be influenced by HIV disease progression. For example, we have developed ligation mediated PCR (LM-PCR) for quantifying apoptosis. In patients on effective HIV therapy, we have shown increases in PBMC LM-PCR results with clinically significant drug toxicities. Preliminary results also demonstrate increasing PBMC apoptosis with a falling CD4 cell count in untreated HIV patients. We use real time PCR to quantify mitochondrial DNA (mtDNA) and have demonstrated mtDNA depletion in tissues from patients exposed to particular antiretroviral agents. Whether mtDNA depletion occurs with HIV disease progression remains controversial. Optimising the clinical use of LM-PCR and mtDNA assays requires understanding how HIV disease progression influences results.

**Aim:** The aim of this project is to refine our understanding of how apoptosis and mtDNA in peripheral blood and adipose tissue are influenced by HIV disease progression, and to establish whether these assays may have utility in *predicting* disease progression.

### **Research Plan**

Peripheral blood samples will be collected from a cohort of Melbourne HIV patients who are not on antiretroviral therapy. Samples from patients with a range of CD4 cell counts will be used to address a number of questions

1. Both PBMCs and CD4 cells will be extracted from each sample, allowing examination of whether LMPCR and mtDNA results are similar in both tissues (a critical question for optimising the use of these tests on peripheral blood)
2. LM-PCR results will be compared with CD4 cell counts to further investigate our finding that patients with a lower CD4 cell count have higher levels of peripheral blood apoptosis. We will also assess whether mtDNA in CD4 cells is lower in those with lower CD4 cell counts, as has been previously suggested.
3. Using serial CD4 cell counts from patients' files, we will examine whether elevated LM-PCR results are associated with more rapid rates of CD4 cell loss: clarifying whether LM-PCR has a role in *predicting* disease progression. A sub-set of patients who commence antiretroviral therapy will provide samples before starting treatment and during “on treatment” monitoring visits. This will allow assessment of how PBMC / CD4 cell apoptosis and mtDNA change with control of HIV replication, and whether changes are reflective of the subsequent speed and degree of CD4 cell recovery.

We have shown that changes in mtDNA with antiretroviral therapy are tissue specific, and differ between adipose tissue and PBMCs. We have collected adipose tissue samples from SIV infected macaques at all stages of SIV disease (n=35) as well as control animals (n=6). These samples provide a unique opportunity to examine changes in LM-PCR results and mtDNA occurring in adipose tissue with retroviral disease progression.

**Summary:** This project will provide the student with opportunities to work with clinical specimens and to contribute to optimizing the use and interpretation of novel assays that show promise in both the clinical and research settings. The use of animal samples will allow extension of this work to areas less accessible for clinical studies. The student will learn a range of laboratory techniques, ranging from processing patient samples and isolation of PBMCs and CD4 cells, through to DNA purification and manipulation, electrophoresis as an analytical tool, and ligation mediated and real time PCR. The student will also gain a basic understanding of relevant statistical methods and experience in presenting and publishing their results.

## **Project Title: Investigation of human heterotypic immune responses to avian influenza**

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Avian influenza continues to infect birds and humans throughout the world, and there is no vaccine yet available. Immunity to infection from influenza virus involves both antibody responses, which are able to neutralize viral infectivity, and T cell responses which are able to kill influenza infected cells and inhibit viral replication. While antibody responses tend to be very subtype and strain specific, T cell responses are often directed to more conserved epitopes of the virus, and so may cross react to viruses of different subtypes (heterotypic responses). The development of heterotypic immune responses by infection or vaccination may modulate the morbidity or mortality experienced on exposure to the highly pathogenic avian strains of influenza virus.

Our group has previously demonstrated that some humans previously infected with the currently circulating human strains of influenza (H1 and H3 subtypes) have cross-reactive T cell responses that are also able to recognise epitopes on the avian H5 influenza haemagglutinin. These responses were more prevalent in individuals recently vaccinated with the current human influenza vaccine. This project aims to investigate further the nature of these heterotypic cellular immune responses, and identify the phenotype and function of the responding cells. It will also aim to determine if the development of cross-reactive responses from vaccination or natural infection can modulate the severity or symptoms experienced after re-vaccination or re-infection with the human influenza virus. This will be done using blood samples from a cohort of human volunteers followed before, during and after either vaccination or infection during the 2008 influenza season.

This project will involve a range of immunological techniques, including ELISAs for assessing antibody responses, ELISPOT assays for the production of IFN- $\gamma$  and IL-2 by T cells, flow cytometry for assessment of responding cell phenotypes, and cytokine array assays. All of these assay systems are currently established in our laboratory. The student will be fully trained in all assays and in the safe handling of potentially infectious human blood, and this project does not involve the use of live avian influenza virus.

This project would be of interest to a student who would like to do a project of direct relevance to the understanding of human infection with influenza virus, which may aid in the design of new vaccines for avian influenza.

## **Project Title: B cell responses in hepatitis C infection**

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Hepatitis C virus results in chronic infection in the majority of individuals infected, and can lead to chronic liver disease, cirrhosis and hepatocellular carcinoma. Our group is interested in studying various aspects of the immune response to hepatitis C virus to understand why some people are able to quickly clear the virus, and others go on to lifelong infection.

One aspect of hepatitis C infection that is not well characterised is the B cell and antibody response to the virus. It is clear there is some dysfunction in the development of HCV-specific B cell responses and in the generation of effective neutralising antibody responses. Antibody responses in HCV infection tend to be delayed, and of relatively low affinity with little isotype switching or affinity maturation. In addition some individuals display evidence of autoimmunity caused by non-specific activation of self reactive B cells, leading to disorders of antibody production such as mixed cryoglobulinaemia and glomerulonephritis.

We would like to investigate B cell function in HCV in more detail by studying the maturation of B cells and production of memory B cell responses to HCV antigens. We have developed a memory B cell ELISPOT assay and will assess the levels and specificity of memory B cell responses in a cohort of individuals recently infected with HCV, compared to those chronically infected.

This project will involve assays of cellular immune function such as cytokine production by bioplex array and ELISPOT. We will also sort and culture B cells from HCV infected individuals and assess their phenotype and function. We will also assess the effect of the addition of exogenous cytokines such as IL-10, known to be increased in chronic HCV, on B cell function.

This project will be of interest to students wishing to work directly with human cells in an important human infectious disease.

Full training will be provided on working with samples containing infectious human pathogens.

## **Project Title: Novel Drug Resistance Mutations in the HIV-1 Reverse Transcriptase**

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Human immunodeficiency virus type 1 (HIV-1) is a lentivirus belonging to the Retroviridae family. HIV-1 has infected over 40 million individuals worldwide and is the causative agent of acquired immune deficiency syndrome (AIDS). The hallmark of retroviruses is the capacity to convert the single-stranded viral RNA genome into a double-stranded proviral DNA precursor. This process is catalysed by the HIV-1 reverse transcriptase (RT), which exists as a heterodimer comprising two subunits of 66 (p66) and 51 (p51) kDa in size. The RT is critical for HIV-1 replication and has become a successful target for drug therapy. The Molecular Interactions Group is interested in protein:protein interactions that are important for HIV-1 replication and the study of the mechanism of action of anti-HIV-1 drugs and how the virus mutates to become resistant to these agents. One of our research areas is focused on the HIV-1 RT and the role of mutations in the RT that appear in HIV-1 isolates from patients that have received antiretroviral therapy. These mutations include changes that appear in a region of the RT (beyond codon 320), which is normally ignored in drug resistance genotyping assays. The aim of this study is to determine the role of these mutations in conferring resistance to RT inhibitors, their effects on viral fitness, and the impact of mutations that confer drug resistance on the interaction between the two subunits of the RT. The types of techniques that may be performed in this project include cell culture, cloning, PCR, site directed mutagenesis, SDS-PAGE, Western blots, fitness assays, HIV-1 propagation and phenotypic drug susceptibility assays.

**Web site:** <http://www.burnet.edu.au/researchandprograms/virology/teams/mig>

## **Project Title: Living on the edge: Injecting drug use and bloodborne virus infection risk in outer suburban Melbourne**

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### **Description:**

Little is known about injecting drug users (IDUs) in outer Melbourne, including prevalence of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) in the population. An annual national survey of attendees of needle and syringe programs (NSPs) produces output for Victoria, but the survey is only conducted through primary NSPs, which are overwhelmingly located in inner Melbourne. Melbourne covers a large and diverse region, and it is plausible that IDUs in outer suburbs are not well represented by the national survey data. This study will produce a snapshot of outer urban IDUs' socio-demographic characteristics and BBV risk behaviours, measure blood-borne virus (BBV) prevalence, test associations between prevalence and risk behaviours, and make comparisons between the participants and IDUs recruited at inner-urban NSPs.

### **Methods:**

IDUs resident in Melbourne's outer western, northern and southern suburbs will be recruited through secondary NSPs and mobile primary NSPs and snowballing from there. Face-to-face interviews will be conducted using a version of the national NSP survey questionnaire, and finger-prick blood samples will be collected for testing for antibodies to HCV and HIV. Qualitative data may also be collected and analysed for themes relating to access to injecting equipment and other services. Quantitative results will be compared with those from a previous Burnet Institute survey of IDUs resident in the Eastern Metropolitan Region, as well as with National Needle and Syringe Program Survey data.

### **Outcomes:**

The results of this study will be a unique set of epidemiological data specifically about outer metropolitan IDUs, one which will be of interest to all individuals and organizations working to reducedrug-related harm in Victoria. Analysis will enable the provision of guidance on how to improve outersuburban IDUs' access to services and reduce their BBV infection risks.

**Project Title: You say, I say, hearsay; the congruence of personal and third-party data supplied by members of social networks**

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Social network analysis is a powerful set of tools for understanding the structure of interactions between people and the effects of those structures on important human phenomena. Many public health problems, including infectious diseases such as HIV and the hepatitis viruses, are transmitted through social relationships. Thus, the structure of those relationships has a powerful effect on disease progression through the community.

Burnet Institute researchers have conducted two social network studies over the past seven years, both focused on the influence of networks on the distribution and transmission of the hepatitis C virus between people who inject illicit drugs. Participants are interviewed and bled and asked to introduce their injecting partners to us for interview, leading to the development of complex maps of injecting and sexual relationships.

In this honours project, we aim to investigate the differences between what person A says about person B (he has had X injecting partners and Y sexual partners in the past year) and what B says (X injecting partners but Z sexual partners, for example). We wish to measure the accuracy of hearsay evidence in our social networks, and use those results to improve the predictive ability of egocentric social network models.

**TITLE: UNDERSTANDING MECHANISMS OF REGULATING BLOOD CLOTS.**  
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**Hypotheses:** Tetraspanins form lateral associations with cognate binding partners including integrins and collagen GPVI receptors in platelets to regulate signaling, trafficking and compartmentalisation that influences platelet thrombus formation and stability *in vivo*. Platelets are small cells in the blood stream that play an important role in preventing excessive blood loss at sites of tissue injury by sticking together and forming a haemostatic plug. Excessive platelet clumping in diseased blood vessels can lead to blockages and cause thrombotic diseases such as heart attack and stroke, two of the biggest killers of humans in the western world. In this proposal, we will seek to understand the repertoire of cognate receptor relationships of tetraspanins in platelets coupled with cross-talk in signaling pathways and compartmentalisation and how this impacts on thrombosis and stroke in mouse models.

**Overall Objectives:** (1) To define cognate binding partners of tetraspanins in platelets, (2) to investigate mechanisms of signaling cross-talk, and (3) to examine the pathophysiology consequences of cognate tetraspanin interactions in mouse thrombosis and stroke models.

**Background Synopsis:** The research plan is an integrated, multidisciplinary approach involving a chief investigator and associate investigators with skills in protein chemistry, platelet biology, whole animal biology (thrombosis and stroke) and haemostasis.

- 1). In this aim, we will define the repertoire of binding partners of tetraspanins in platelets. Using several approaches including co-immunoprecipitation/western blot studies from resting and activated human and mouse platelets, SDS-PAGE with Coomassie blue or silver protein staining, a proteomics/mass spectrometry approach and analysis of platelets from various tetraspanin, immunoreceptor, and FcR  $\gamma$ -chain knockout mice for cell surface expression of binding partner. In addition, we will examine the functional consequences of tetraspanin:integrin interactions in platelet spreading.
- 2). In this aim, we will define mechanisms of signaling cross-talk between tetraspanins and cognate binding receptors by characterising several putative signaling molecule interactions with tetraspanins such as CD151 including SHP-2, ERK-1/2, and calmodulin proteins.
- 3). In this aim, we will examine the pathophysiological consequences of cognate tetraspanin interactions in murine platelets and mouse thrombosis and stroke models. In Aim 3.1, the biological importance of multimerisation of tetraspanin interactions in integrin  $\alpha_{IIb}\beta_3$ -mediated platelet interactions *in vitro* will be investigated using soluble EC2 domains of tetraspanins as competitive inhibitors. In Aim 3.2, we will elucidate the thromboregulatory role of tetraspanins in the ischaemic brain using a siliconised thread MCA occlusion mouse model.

TITLE: THE BIOLOGY OF IMMUNORECEPTORS IN INFECTION AND IMMUNITY.  
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**HYPOTHESIS:** The central hypothesis of this proposal is that Ig-ITIM superfamily members serve as recognition adhesive receptors for bacterial pathogens including *Staph. aureus* and modulate phagocytosis of *Staph. aureus* to regulate innate immunity *in vivo*.

**BACKGROUND:** Inflammatory and infectious diseases are increasingly representing major global public health challenges. Growing resistance to antibacterial therapy contributes to continued morbidity, mortality and unmet medical need. To address these challenges, our studies aim to elucidate some of the fundamental mechanisms of bacterial pathogen-host interactions and physical interface involving immunoreceptors. Immune cells are equipped with an array of immunoreceptors to detect pathogens and to initiate innate and adaptive immunity. Ig-ITIM superfamily members are expressed by immune cells including macrophages, dendritic cells, T cells, B cells and NK cells. These include PECAM-1 and CEACAM1, which are adhesive, endocytic and signalling receptors that play an important role in innate and adaptive immunity.

**AIMS:** To achieve our overall goals we will investigate the immunoreceptor: pathogen recognition, as well as the functional importance of Ig-ITIM superfamily members in infection and immunity, both *in vitro* and *in vivo*. The research plan is an integrated, multidisciplinary approach involving skills in protein chemistry, infectious diseases, immunology and whole animal biology.

**Aim 1.** To define mechanisms of immunoreceptor recognition of *S. aureus* bacteria *in vitro*? This study will principally examine *in vitro* bacterial pathogen recognition of different Ig-ITIM superfamily members. In this aim, we will define the physical interface between *S. aureus* bacteria (clinical isolates and laboratory strains) and immunoreceptor binding determinants using ELISA analysis. By using recombinant immunoreceptor Ig-domains and *S. aureus* mutants, we will map the respective binding sites on the Ig-Domains of immunoreceptors required for binding the bacteria.

**Aim 2.** To define mechanisms of action of immunoreceptors *in vitro*. We propose that Ig-ITIM superfamily members serve as recognition receptors arrayed on immune cells whose signalling responses are important in the modulation of endocytic/phagocytic responses. In this aim, we will investigate the response of wild-type versus PECAM-1 KO, CEACAM1KO and PECAM-1/CEACAM1 KO neutrophils in terms of production of pathogen killing (phagocytosis of macrophages±opsonisation), pathogen processing (inflammatory cytokine production), and pathogen presentation of Toll-like receptors.

**Aim 3.** To define the role that immunoreceptors play in the pathogenesis of *Staph. aureus* infection *in vivo*. In this aim, we will investigate the biological function of Ig-ITIM superfamily members, PECAM-1 and CEACAM1 as recognition receptors for bacterial pathogens *in vivo*, using a *Staph. aureus* mouse models. Parameters to be measured include survival, bacterial clearance in organs and blood, clinical chemistry to monitor organ failure, cellular immunology profiles and histology of organs.

## **Project title: Development of a panfungal real-time PCR for diagnosis of fungal infection in high-risk haematology patients**

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Invasive Fungal Infections (IFI) are very common in patients undergoing chemotherapy for haematological malignancies or post bone marrow transplantation. The high mortality rates are due mainly to the inability of conventional diagnostic tests (i.e. culture and biopsy) to make an early and accurate diagnosis. Until recently *Aspergillus* was the most common fungus causing invasive infection but changes in antifungal prophylaxis, chemotherapy regimens and transplant procedures has resulted in an emergence of more resistant fungi. Real-time PCR assays for the detection of *Aspergillus* have been developed but no panfungal real-time PCR assay for the detection of all fungi including *Aspergillus* is available to date.

The aims of this project are to assist in the development of a quantitative panfungal real-time PCR; evaluate its diagnostic accuracy (i.e. sensitivity, specificity) in a prospective screening study of serial blood, serum and bronchoalveolar lavage (BAL) samples from a cohort of patients undergoing bone marrow transplantation or chemotherapy for haematological malignancies, and compare it to other available diagnostic tests such as galactomannan ELISA. Fungal species identification will also be determined via cloning and sequencing of real time PCR products and comparison to genetic databases.

Techniques and skills expected to be used in this project include:

- DNA extraction from blood, BAL and serum
- Real-time PCR
- Cloning
- DNA sequencing
- PCR optimization and primer design
- ELISA

**Project title: Development of monoclonal antibodies specific for Fc $\gamma$ RII; a low affinity receptor for IgG**

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**Antibodies are one of the most important mediators of the immune system.** The interaction between immune complexes (antigen: antibody) and Fc receptors is critically important to normal immunity; required for the induction of effector function, release of inflammatory cytokines and the uptake of complexed antigens for subsequent processing and presentation of antigen and regulation of antibody responses.

The Fc receptors for IgG (Fc $\gamma$ R) are a diverse family of cell surface proteins that bind the Fc portion of immunoglobulin. The receptors consist of two or three extracellular Ig domains. Ligand binding is known to occur within the second Ig domain. Key to the detection of these receptors is having reagents that interact with the receptor with high affinity and specificity. Currently this laboratory has a bank of monoclonal antibodies that specifically bind to the second domain of Fc $\gamma$ RII.

**The aim of this project is to produce monoclonal antibodies to the first Ig domain of Fc $\gamma$ RII that can be used in a variety of cellular assays.** Currently the monoclonal antibodies that have been generated in laboratories elsewhere have very limited uses in such assays.

## **Project Title: Understanding how receptors for Immunoglobulin are organised on the cell membrane**

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**Fc receptors** for IgG (Fc $\gamma$ R) are a diverse family of cell surface proteins that bind the Fc portion of immunoglobulin. The aggregation of these receptors by immune complexes induces a number of important antibody dependant effector functions including antibody dependant cellular cytotoxicity, the uptake and destruction of pathogens, and also leads to the release of inflammatory cytokines. Traditionally, these receptors have thought to exist on the cell membrane in a monomeric state, associated non-covalently with signalling molecules which are necessary for receptor signalling function and stability. Using crystallographic analyses of Fc $\gamma$ RIIa<sub>1</sub>; a low affinity member of the Fc $\gamma$ R family; we found that non-covalent dimerization of the ligand binding chain itself may be possible.

This data was later confirmed using mutagenesis of the dimer interface and signalling assays<sup>2</sup>. This work was the first description of Fc receptor dimerization. Indeed it still remains that very little is known about the molecular organisation of this receptor family within the cell.

The aim of this project is to **examine how the organisation of the Fc receptors influences cell activation and ligand binding**. In particular we aim to; **(a)** further examine the biological significance of the Fc $\gamma$ RIIa dimer interface; **(b)** define the extent of homodimerization in the broader FcR family. The project will incorporate the use of **molecular biology, cellular transfection and protein analysis** to examine the importance of this region within the receptor.

1. Powell MS., Maxwell, KF., et. al. The Structure of the Human Lueukocyte Fc receptor, Fc $\gamma$ RIIa. *Nat. Struct. Biol.*, 1999, 6: 437-442.
2. Powell, MS., et.al. Alteration of the Fc $\gamma$ RIIa dimer interface affects receptor signaling but not ligand binding. *J. Immunol*, 2006, 104, 118-123.

## **Project title: Assembly and release of hepatitis C virus from infected cells**

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Hepatitis C virus (HCV) is a major cause of chronic hepatitis, which can lead to hepatocellular carcinoma and is currently the leading single indicator for liver transplantation in the developed world.

The threat posed to the health systems in developed and developing countries by HCV infection continues to grow at an alarming rate. In the most recent report of WHO on HCV prevalence, it has been estimated that 123 million individuals are infected with the virus worldwide. There are approximately 210,000 HCV-positive individuals in Australia, a figure which continues to grow. The current treatment for HCV is a combination of interferon and ribavirin that is only effective in about 50% of patients. Moreover, the current high-cost of treatment, the duration of treatment (24-48 weeks), associated adverse side effects and the emergence of resistant virus strains [4] means that novel antiviral agents are urgently required.

HCV (Family *Flaviviridae*, genus *Hepacivirus*) is a small enveloped virus with a positive-sense single stranded RNA genome of about 9.5 kb, that encodes a polyprotein of about 3000 amino acids. The HCV polyprotein is co- and post-translationally cleaved into 10 structural and non-structural proteins (NS). Until recently, due to lack of a robust cell culture system to propagate the virus *in vitro*, it was impossible to investigate the role of viral proteins in viral assembly and release. We have established the recently developed cell culture system to propagate HCV in our laboratory. This has provided us an opportunity to closely examine the late steps of virion formation in cultured cells. Our recent published data demonstrated that viral proteins play a critical role in the outcome of infection. To better understand the virus biology, we will further characterize the viral proteins that are required in the early steps of the viral assembly and trafficking of the viral particles from ER to the extracellular environment. We believe that this work will assist us to design novel antiviral drugs.

This project will offer the student a range of techniques related to molecular biology, including cloning, sequencing, site-directed mutagenesis, as well as a number of virus-specific techniques including cell culture and analysis of virus replication.

## **Project title: Identifying HCV specific regulatory T cell epitopes**

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**Aim:** To identify T cell epitopes which are recognised by HCV-specific regulatory T cells, so that these suppressive epitopes can be eliminated from future vaccine candidates.

**Background:** Hepatitis C virus (HCV) infects 3% of the world population. A proportion of these individuals will eventually develop cirrhosis, cancer and liver failure. There are currently very limited treatment options for HCV and no available vaccine. Chronic HCV infection is associated with a weak, narrow cell-mediated immune response that is characterized by a low frequency of HCV-specific effector T cells, and the reasons behind this are poorly understood.

Regulatory T cells (Treg) suppress a diverse range of immune responses, including anti-viral responses. We have found that although HCV specific IFN- $\gamma$  producing anti-viral effector T cells are few, HCV-specific CD25+ FOXP3+IFN- $\gamma$ - Treg are abundant. It is vital to determine which regions of the HCV proteins are recognised by Treg, so that these regions could be avoided in future vaccine design.

**Method:** The protocol to culture PBMC from HCV patients and identify HCV-specific Treg has been established in the laboratory. The patient's blood sample will be collected from the Liver Clinic of the Alfred Hospital, and a small amount of the blood will be sent to Red Cross Blood Bank Service for HLA typing.

Initially, we will use a set of individual 18-mer peptides derived from HCV proteins to stimulate the patients' PBMC culture, and later on depending on the results, we may use truncated or extended version of certain 18-mers.

**Reference:** Li S, Jones KL, Woollard DJ, Dromey J, Paukovics G, Plebanski M, and Gowans EJ. Defining target antigens for CD25+FOXP3+IFN- $\gamma$ - regulatory T cells in chronic hepatitis C virus infection. *Immunol Cell Biol* (2007, Epub ahead of printing).

## **Project title : The effect of HIV infection on matrix metalloproteinase (MMP) expression on monocytes and macrophage subsets.**

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### **Background**

Macrophages are long lived cellular reservoirs of HIV, particularly in tissues such as the brain. HIV can not be eradicated by the current highly active anti-retroviral treatment (HAART). Our laboratory studies the contribution of cells of the macrophage lineage to the persistence of HIV. A major focus of our research is monocyte subsets, their susceptibility to HIV infection and changes with infection, trafficking of monocytes across our blood brain barrier model and the effect of HIV on the monocyte/macrophage subsets found in tissues including the brain.

Matrix metalloproteinases (MMPs) are involved in a wide range of proteolytic events in both normal and pathogenic circumstances and are key to a wide range of biological processes including tissue remodelling and the mediation or release of biological factors. MMPs produced by monocyte activation can compromise the blood brain barrier integrity and influence monocyte transmigration into the brain.

Increased infiltration of immune-activated monocytes from the blood brain barrier is a characteristic of HIV associated dementia (HAD) (1). Elevated MMP production by monocytes can degrade ECM of the blood-brain barrier and may contribute to transendothelial migration HIV infected cells into the CSF and the development of HIV-related neurological damage(2). HIV-infected macrophages secrete pro-MMP-2 which is activated by exposure to MT1-MMP on neurons. MMP-2 processes SDF-1 into a highly neurotoxic protein. SDF-1 is a chemokine which is over-expressed by astrocytes during HIV infection.

MMPs play a role in neural apoptosis, leading to neurodegeneration (3).

**Significance** - to investigate the contribution of monocytes to viral reservoirs of HIV

### **Project description**

This project involves investigating the role of HIV infection on MMP expression by subsets of monocytes and by tissue macrophages.

### **Aims**

- (1) To characterise the MMPs expressed by monocyte subsets from HIV infected and non-infected individuals
- (2) Define the level of and types of MMPs expressed as monocytes mature into macrophages, comparing HIV infected with non-infected cells.
- (3) Investigate differences in MMP expression by monocytes as they migrate across the blood brain barrier and by macrophages in various tissues (+/- HIV infection)

### **Techniques**

This project will include a wide range of cellular and molecular techniques: isolation and culture of human monocytes and macrophages, HIV infection of cells, DNA and RNA isolation, PCR, flow cytometry, laser capture microscopy, western blot and zymography.

### **References**

Webster and Crowe. Matrix metalloproteinases, their production by monocytes and macrophages and their potential role in HIV-related diseases. *J Leukoc Biol.* 2006, 80(5):1052-66.

(1) *J Virol* 2001 75(14): 6572-6583 (2) *J Neurovirol* 2000 6(2): 156-63 (3) *Nature Neuroscience* 2003 6(10): 1064-1071

## **Project Title : Is the binding of two Fc $\alpha$ RI receptors to each IgA necessary for inhibitory signalling?**

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### **Dual activating and inhibitory signaling through Fc $\alpha$ RI.**

Hypothesis: Unique features of IgA and Fc $\alpha$ RI enable Fc $\alpha$ RI convey both activating and inhibitory signals.

The IgA receptor **Fc $\alpha$ RI is the major myeloid receptor for the IgA class of antibodies.**

**Fc $\alpha$ RI associates with the small activating subunit FcR- $\gamma$  chain which contains**

**immunotyrosine activation motifs (1-3).** Immune complexes of IgA engage this receptor and stimulate myeloid cell effector functions such as respiratory burst, degranulation and phagocytosis.

**Recently it has been found that low avidity/stoichiometric engagement of the IgA receptor Fc $\alpha$ RI, produces an inhibitory signal that desensitises myeloid cells to activation via other Fc receptors.** We have established a mast cell degranulation assay and have shown that the anti-Fc $\alpha$ RI antibody A77, without crosslinking, inhibits the IgE triggered degranulation of RBL mast cells transfected with Fc $\alpha$ RI. **This project will examine if this unusual dualistic signaling by Fc $\alpha$ RI is determined by the unique receptor stoichiometry for ligand.** Fc $\alpha$ RI forms a receptor:ligand complex with a 2:1 stoichiometry which is unlike the 1:1 stoichiometry of every other leukocyte Fc receptor and may well be key to its unusual signaling properties.

**To determine if the 2:1 receptor to IgA interaction is essential for IgA to trigger activating and inhibitory signaling by Fc $\alpha$ RI we will engineer a hybrid IgA so that it can only bind to one receptor.** One heavy chain of this IgA will contain the native receptor binding site while the second chain will be mutant and inactive in receptor binding. This mixed heavy chain IgA will be produced by transfection of puromycin and hygromycin selectable vectors in HEK293E. Hybrid IgA will be purified by sequential affinity chromatography on SSL7-Sepharose and cobalt-NTA Sepharose. It will then be tested and compared with WT IgA for its ability in immune complexes to degranulate Fc $\alpha$ RI transfected RBL mast cells (colourimetric  $\beta$ -hexosaminidase release). Hybrid IgA will then be tested and compared with WT IgA for its ability to desensitize RBL mast cells to IgE mediated degranulation. If the hybrid IgA fails to inhibit IgE mediated degranulation then the unique 2:1 binding is essential for inhibitory signaling by this receptor.

1: Wines BD, et al. J Biol Chem. 2006. 281(25):17108-13

2: Wines BD, et al. J Biol Chem. 2004 279(25):26339-45.

3: Wines BD, et al. J Immunol. 2001. 166(3):1781-9.

## **Project Title: How does the secreted *Staphylococcal* protein SSL7 inhibit complement?**

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### **How does the secreted *Staphylococcal* protein SSL7 inhibit complement?**

Hypothesis: *S. aureus* superantigen like protein SSL7 binds to the C234C neutrin domain of C5, thus blocking C5 binding to the C5 convertase.

***Staphylococcus aureus* is a major human pathogen responsible for a range of conditions including skin infections, scarlet fever, sepsis and endocarditis. The emergence of antibiotic resistant strains of *S. aureus* underscores a need to better understand immune evasion by this pathogen.**

**Superantigens interfere with T cell function, protein A targets IgG antibody effector functions and other proteins target complement activation. In a collaborative study with Prof. John Fraser of the University of Auckland we have investigated the interaction of SSL7, a secreted *S. aureus* superantigen like protein, with C5, a key component of the complement pathway. One explanation for the inhibition of complement by this protein is that it binds the complement component C5 at the site required for tethering to the convertase, hence preventing proteolysis and subsequent activation. This will be tested by mutagenesis of C5, the production of C5 and C3 chimeras and the production of the c-terminal neutrin domain of C5. Research methodology will comprise constructing mammalian and bacterial expression constructs, biosensor and FACS binding assays and complement assays.**

1: Wines BD, Willoughby N, Fraser JD, Hogarth PM.

A competitive mechanism for staphylococcal toxin SSL7 inhibiting the leukocyte IgA receptor, Fc alphaRI, is revealed by SSL7 binding at the C alpha2/C alpha3 interface of IgA. J Biol Chem. 2006. 281(3):1389-93.

2: Langley R, Wines B, Willoughby N, Basu I, Proft T, Fraser JD.

The staphylococcal superantigen-like protein 7 binds IgA and complement C5 and inhibits IgA-Fc alpha RI binding and serum killing of bacteria. J Immunol. 2005. 174(5):2926-33.

## **Project Title: The development of plant based vaccines for measles and avian influenza**

Dr Diane Webster

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(Note: some projects may require a small amount of work to be undertaken at Monash University, Clayton Campus)

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Traditional systems for the expression of recombinant proteins are hampered by high costs, problems with post-translational modifications, and human pathogen contamination risks. Plants represent an alternative production system that produces high quality recombinant protein, in an affordable, safe and scalable way. Plant-made human proteins have been shown to be fully functional and structurally comparable to native protein, with a similar pattern of glycosylation. Moreover, plant-based expression systems are free from contamination by pathogens.

We are currently investigating plants for the production and delivery of vaccines for measles and avian influenza (poultry vaccine), and expression of a protein with potential therapeutic applications in diabetes. A key feature of all projects is the production of affordable, oral protein based pharmaceuticals that can be stored at room temperature. Specific projects can be tailored to match the interest of each student, please come and discuss the options with us.

Some potential projects include:

- Structural characterisation of the influenza HA protein produced in plants
- Evaluation of the immunogenicity of H5 influenza HA protein produced in plants
- Assessment of the oral immunogenicity of measles virus HA protein produced in root culture.
- Evaluation of the impact of glycosylation of plant-made measles virus HA protein stability – *in planta*, *in vitro* and *in vivo*.

Suggested reading: Webster et al. (2002). Appetising solutions: an edible vaccine for measles. *Med J Aust* 176:434-7. Techniques potentially covered; molecular biology (bacterial cloning, PCR, gel electrophoresis); plant transformation (plant projects only); protein analysis (ELISA, SDS-PAGE, Western blots, glycosylation studies); animal trials (animal handling, injection techniques, gavage and feeding); immunological analysis (ELISA, antibody isotyping, antibody affinity, IFNg ELISPOTs). Note; animal handling is optional for plant projects.

## Project Title: Optimisation and characterisation of functional soluble RAGE produced in transgenic plants

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Soluble RAGE (sRAGE) is an important novel reagent and potential new treatment that has been shown to attenuate the development of type 1 diabetes and its complications in experimental systems.

Plants represent a viable production system for sRAGE, which is safe, scalable, and produces highquality recombinant protein. We have clearly demonstrated that functional sRAGE can be made in transgenic plants. Clinical translation of these exciting findings now requires additional innovation to generate even larger quantities of sRAGE in plants with enhanced efficacy in preventing the damaging effects of AGEs.

This research aims to use *state-of-the-art* techniques to fully characterize the processing of soluble RAGE in transgenic plants, and then selectively modify these processing pathways to generate a soluble RAGE with enhanced bioactivity, improved safety profiles, and increased yields. The posttranslational processing of sRAGE, including glycosylation of the V-type domain and polymerization, is known to play an important role in ligand binding and clearance. Unlike human RAGE, previous experiments have shown that bacterially-derived sRAGE is not glycosylated and exists largely as a monomer. However, plant RAGE is able to polymerize and glycosylation can be selectively manipulated in transgenic systems to produce ‘humanized’ proteins, and optimised to improve safety, function, and/or protein yield. It is also possible to use transgenic plant technologies to manipulate the proportion of recombinant protein that forms polymers, potentially enhancing RAGE-ligand interactions.

Possible honours projects include:

- Structural characterisation of aspects of plant-made sRAGE
- Investigation of the effect of chemical modification of plant-made sRAGE on protein stability
- Expression, purification and characterisation of sRAGE in transgenic root cultures.

Techniques potentially covered include; molecular biology (bacterial cloning, PCR, gel electrophoresis); plant transformation (plant projects only); protein analysis (ELISA, SDS-PAGE,

## **Project Title: Review of systems for accessing HCV treatment in Victoria: How can it be improved?**

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Treatment for hepatitis C (HCV) has improved over the past few years but access to treatment remains an issue. Despite almost 200,000 Australians having ongoing HCV infection and over 1,000 new infections occurring annually it is estimated that only 2000 people are treated for HCV every year.

The vast majority of people infected with HCV in Australia are injecting drug users (IDUs). It is important that programs are developed that assist this population in accessing HCV treatment and support. In the long run, to ensure that increased numbers of people are treated for HCV and that those being treated are from the group at greatest risk (IDUs), treatment programs are needed outside of major tertiary hospitals. In Victoria, primary health care centres appear to be the logical location to increase HCV treatment and support.

This study will explore the barriers to increasing access to HCV treatment in primary health care centres. Factors to be examined include facilities, manpower (prescribing doctors, HCV nurse managers, outreach/peer support, psychiatric support) and funding. Structured interviews will be conducted with staff of primary health care services and key community stakeholders (HCV council, ANEX etc.) regarding what is required to increase IDUs access to HCV treatment.