The Department of Microbiology and Immunology

Honours Projects 2016

Bachelor of Biomedicine (B.Biomed) and Bachelor of Science (B.Sc.) students who obtain faculty honours in their third year will be welcome to join the Department of Microbiology and Immunology as BBiomed/BSc (Honours) candidates during 2016 provided that we can find supervisors and laboratory accommodation to meet the demand.

The course, which runs from late February until mid November, is designed to develop the research student's capacity to solve problems, to analyse data, to read and think critically, and to communicate clearly. A research project is undertaken in close collaboration with a senior member of staff. In addition, the coursework component will involve 30 hours of contact for the study of advanced microbiology and immunology.

Project information is available on the Departmental website: www.microbiol.unimelb.edu.au
Please check the website regularly for any updated information.

Course Codes:
BH-BMED - Bachelor of Biomedicine (Honours): for students who have successfully completed or are about to complete the Bachelor of Biomedicine at the University of Melbourne.
BH-SCI - Bachelor of Science (Honours): for all other applicants who have successfully completed or are about to complete a Bachelor of Science or equivalent.

Choosing a Supervisor and Research Area:
A critical element of success in Honours is choosing a research area that interests you. Department websites have information on the range of research areas they offer, as well as areas of interest of academic staff members who can supervise your project.
It is very important for you to talk to supervisors and current/previous Honours students. It's one thing to be interested in the project but you need to get along with your supervisor too. If possible, try to get some work experience in the lab to get an idea about the environment.

Department Information Sessions:
Details regarding upcoming Department Honours Information Sessions will be made available online from July each year on the MDHS Honours page at:
http://sc.mdhs.unimelb.edu.au/honours-info-sessions

Honours Applications and Tracking System (HATS)
The list of proposed research projects will be available in early September through HATS for the submission of preferences. HATS can be accessed online at: http://hats.mdhs.unimelb.edu.au/
Students will be issued with a HATS password to access HATS once they have submitted their online application for entry to one of the two Honours courses.

Honours Coordinators:
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HOW MICROBIOTA REGULATES T CELL IMMUNITY - BEDOUI LABORATORY

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Overview:
Research in the Bedoui laboratory focuses on resolving how specific innate immune mechanisms contribute to the induction of effective T cell immunity. In this context, the lab examines the role of dendritic cells, pattern recognition receptors and cytokines, utilizing a variety of models that include viral and bacterial pathogens.

Project 1: How Microbiota Regulates T Cell Immunity
Supervisors: Dr. Sammy Bedoui, Annabell Bachem

Development of autoimmune diseases has been linked to disturbances in the microbes that normally colonize our body surfaces. However, precisely how these microorganisms and the resulting stimulation of the immune system ultimately impact on T cells still remains elusive. Building on extensive preliminary data already available in the lab, this project will investigate how the microbiota influences particular cytokine responses that are highly relevant to our understanding of T cell-mediated autoimmune disease, such as type I diabetes. This project is ideally suited for a student with an interest in working at the intersection between Immunology and Microbiology.

The aims of this project will be to:
(i) To decipher the role of cytokines in the maintenance of CD8+ T cells in mice lacking microbiota
(ii) To investigate microbial colonization patterns and how these influence T cells responses

Techniques commonly used in this laboratory:
Animal experiments including work with germ-free mice, T cell transfers, FACS, cell sorting, intracellular cytokine assay, molecular biology such as RNA/DNA isolation, real-time PCR, western blotting, ELISA, DNA/RNA Sequencing

References/Publications/Recommended Reading:
1. Bedoui, S. et al. Different bacterial pathogens, different strategies, yet the aim is the same: evasion of intestinal dendritic cell recognition. Journal of immunology 184, 2237-2242 (2010).

2. Kupz, A. et al & Bedoui, S. NLRC4 inflammasomes in dendritic cells regulate noncognate effector function by memory CD8(+) T cells. Nature immunology 13, 162-169 (2012).

3. Maynard, C. L., Elson, C. O., Hatton, R. D. & Weaver, C. T. Reciprocal interactions of the intestinal microbiota and immune system. Nature 489, 231-241, doi:10.1038/nature11551 (2012).

Biosafety Statement:
This project will involve animal experimentation. All procedures will be carried out in a PC2 level laboratory following appropriate standard operating procedures. Appropriate training in PC2 level procedures, techniques and animal handling will be provided.

HOW NATURAL KILLER CELLS RESPOND TO THE ALTERED EXPRESSION OF HLA CLASS I PROTEINS - BROOKS LABORATORY

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Overview: Much of the research in the **Brooks** laboratory is centered around understanding how natural killer cells respond to viral infections and tumours. While part of the innate immune system, there are increasing suggestions that following certain infections, in particular cytomegalovirus (CMV) infection, certain NK cell subsets expand and possess superior functional capacity, similar to a "memory" phenotype. These expanded populations are most frequently characterised by the expression of distinct sets of activating receptors, many of which interact with HLA class I proteins. Our laboratory is investigating how these receptors impact on NK cell recognition of both transplanted tissues and virus-infected cells in the context of human lung transplantation as well as in cancer.

Project 1: Investigating natural killer cell function in human lung transplant recipients.
SUPERVISOR(S) Prof Andrew Brooks, Dr Lucy Sullivan, Dr Philippa Saunders & Assoc. Prof. Glen Westall

Lung transplantation provides a unique model to study the role of NK cells in response to active CMV infection as the process often necessitates the introduction of a CMV positive organ into CMV negative patient. This CMV mismatching between donors and recipients is a major problem and typically results in primary CMV disease, largely due to the use of immunosuppression. Furthermore, transplanted donor lungs are not HLA-matched with the recipient. Consequently differences in HLA alleles between the recipient and the graft have the potential to dampen the inhibitory signals received by recipient NK cells upon entering the transplanted lung. This lack of inhibitory signaling may drive the selective expansion of NK cells expressing receptors with specificity for recipient but not graft-encoded HLA class I proteins.

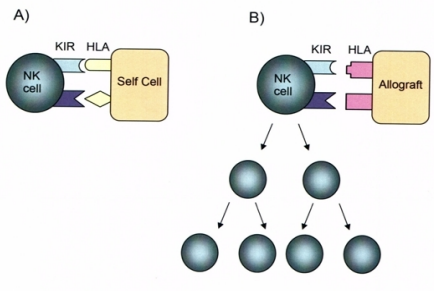

Figure 1. The lack of KIR ligands on transplanted tissue or tumours may drive the expansion of subpopulations of NK cells.

The aims of this project will be to:

(i) determine whether HLA mismatching and/or CMV infection drives the expansion of NK cell subsets in a clinically relevant setting

(ii) determine whether acute CMV infection results in the formation of "memory" NK cells with enhanced effector function.

References/Publications/Recommended Reading

Villard, J, The role of natural killer cells in human solid organ and tissue transplantation J. Innate Immunol 3: 395-402, 2011
Davis et al, Adaptive natural killer cell and killer cell immunoglobulin-like receptor expressing T cell responses are induced by cytomegalovirus and are associated with protection against cytomegalovirus reactivation after allogeneic donor hematopoietic cell transplantation. Biol Blood Marrow Transplant. 21:1653-1662, 2015

Project 2: The use of SMRT sequencing to define variation in the killer cell immunoglobulin-like receptor gene complex.
Supervisors Prof Andrew Brooks, A/Prof Tim Stinnear, Dr Philippa Saunders

The killer cell immunoglobulin-like receptors (KIR) regulate natural killer cell function in humans. These proteins are encoded by a large and highly variable gene complex. An increasing number of studies have associated either distinct combinations of KIR genes or particular alleles of KIR genes with clinical outcomes in cancer, infection and reproduction. This is perhaps most evident in patients who have received bone marrow transplants for certain types of leukemia and HIV-infected individuals. Nevertheless, given the extent of variation, our understanding of these linkages is still relatively rudimentary with more robust methods for determining gene content and allele level typing needed. This project will access a new technology, SMRT sequencing which potentially allows for the simultaneous sequencing of the entire KIR gene complex. Such a method can then be employed to assess the impact of polymorphisms in *KIR* loci on the outcomes in cancer patients or transplant recipients

The aims of this project will be to:

(i) *Develop a method for sequence based typing of KIR3DL1which can be applied to clinical cohorts in cancer and transplantation to examine disease associations.*

(ii) *Develop a method to sequence the entire KIR gene complex using SMRT sequencing*

References/Publications/Recommended Reading:

Parham and McQueen, Alloreactive killer cells: hindrance and help for haematopoietic transplants. Nature Rev Immunol 3:108-122, 2003
Parham et al, Phil. Trans. R. Soc. B Human-specific evolution of killer cell immunoglobulin-like receptor recognition of major histocompatibility complex class I molecules, 367, 800–811, 2012.

Techniques commonly used in this laboratory:

EXAMPLES: FACS, cell sorting, intracellular cytokine assays, tetramer staining, confocal microscopy, protein purification, surface plasmon resonance assays, ELISA, molecular biology such as gene cloning and expression analyses, RT-PCR, real-time PCR and DNA sequencing.

Biosafety Statement:

EXAMPLE: This project will involve handling of unfixed human tissue. All procedures will be carried out in a PC2 level laboratory following appropriate standard operating procedures. Appropriate training in PC2 level procedures will be provided.

ROTAVIRUS PATHOGENESIS AND IMMUNITY LABORATORY

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Overview: Rotaviruses are the major cause of acute severe infantile gastroenteritis worldwide. Research in the Coulson laboratory focuses on rotavirus pathogenesis and immunity. Major areas of interest are to understand rotavirus interactions with integrin and glycan cellular receptors, innate immune responses elicited and immune evasion mechanisms of rotaviruses, the functions of the rotavirus proteins during replication, and how rotavirus infection accelerates the onset of type 1 diabetes.

Techniques commonly used in this laboratory:
Cell and rotavirus culture; rotavirus purification; gel electrophoresis of viral RNA; rotavirus-cell binding, infection and infectivity assays; cell DNA transfection; cDNA cloning; real-time PCR; DNA sequencing; protein expression; laser scanning confocal microscopy; gene reporter assays; flow cytometry; intracellular cytokine staining; cell sorting; ELISA; immunoprecipitation; western blotting; animal experimentation, tissue harvest, cell transfer, mouse inoculation with rotavirus.

Biosafety Statement.
These projects will involve handling of infectious rotavirus. Students will not be handling pathogenic rotavirus, only culture-adapted strains. All procedures will be carried out in a PC2 level laboratory following appropriate standard operating procedures. Appropriate training in PC2 level procedures, virological techniques and the handling and infection of mice will be provided.

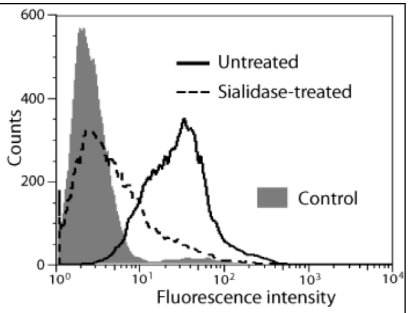

Fig. 1. Binding of recombinant rotavirus spike protein VP8* to cells is inhibited by cellular treatment with sialidase. Binding by VP8* protein with a GST tag is detected by flow cytometry of cells stained with anti-GST antibody. VP8* binding to untreated cells (solid line) and sialidase-treated cells (dashed line) is indicated. The control indicates the signal produced by cells reacted with GST.

Project 1: Rotavirus usage of integrins, sialic acids and gangliosides as cellular receptors. SUPERVISOR: A/Professor Barbara Coulson.

A major determinant of virus tropism for particular host tissues and cells is the identity of host cell receptors. Our group is a world leader in the discovery and analysis of rotavirus-receptor interactions. Rotavirus cell attachment and entry is a multi-step process that involves several cell surface integrins, sialic acids located on gangliosides and/or human blood group antigens. Sialidase treatment to remove cell surface sialic acids greatly inhibits infection by certain rotaviruses. Binding of recombinant virus spike protein VP8* to cells mediates this sialic acid recognition (Fig. 1).

The aim of this project is to further elucidate the roles of integrins, glycans and/or gangliosides in rotavirus cell binding and entry. Antibodies to integrins and glycans, recombinantly expressed rotavirus proteins, sialic acid mimetics, blood group antigens and other unique inhibitory compounds synthesized chemically by collaborators will be used as appropriate

to probe the receptors used by rotaviruses to bind and enter cells. The ability of purified rotaviruses to bind recombinant integrin protein will be analysed, in preparation for structural studies of rotavirus-integrin recognition.

Selected publications from our group that underpin Project 1.

Böhm R, Fleming FE, Maggioni A, Dang VT, Holloway G, Coulson BS, Itzstein Mv, Haselhorst T: **Revisiting the role of histo-blood group antigens in rotavirus host-cell invasion**. *Nat Commun* 2014, **6**:5907. doi: 5910.1038/ncomms6907.

Yu X, Mishra R, Holloway G, von Itzstein M, Coulson BS, Blanchard H: **Substantial receptor-induced structural rearrangement of rotavirus VP8*: potential implications for cross-species infection**. *Chembiochem* 2015. doi: 10.1002/cbic.201500360

Fleming FE, Bohm R, Dang VT, Holloway G, Haselhorst T, Madge PD, Deveryshetty J, Yu X, Blanchard H, von Itzstein M, Coulson BS. 2014. **Relative roles of GM1 ganglioside, N-acylneuraminic acids, and α2β1 integrin in mediating rotavirus infection**. J Virol **88:**4558-4571.

Project 2: Rotavirus protein sub-cellular localisation and function.
SUPERVISOR: Dr Gavan Holloway and A/Professor Barbara Coulson.

The roles of the 6 structural and 6 non-structural proteins of rotavirus during the replication cycle have not been completely defined. The localisation of rotavirus proteins within the host cell can give clues as to their possible function. For example, some rotavirus proteins can enter the host cell nucleus where they are actively exported back to the cytoplasm (Fig. 2). This suggests a novel nuclear element in rotavirus replication, which to date has been considered to be totally cytoplasmic. We have also observed that other rotavirus proteins show previously unrecognised localisation patterns, which may be important for rotavirus replication and pathogenesis, and might therefore be targeted for novel therapeutic strategies.

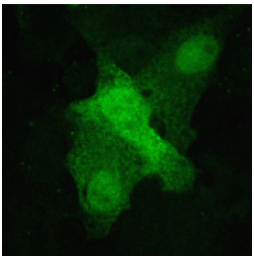

Fig. 2. Nuclear localisation of a rotavirus protein during infection.

The aims of this project are to:

1. Define the sub-cellular localization patterns of particular rotavirus proteins.
2. Identify the viral protein sequence motifs that are responsible for such localisation patterns.
3. Delineate the function of the protein at its subcellular location and establish whether preventing its proper distribution can affect its function and/or rotavirus replication.

Publications from our group and recommended reading for Project 2:

Holloway, G and Coulson, BS. 2013. Innate cellular responses to rotavirus infection. J Gen Virol **94**:1151-1160.

Holloway, G, Dang, VT, Jans, DA, Coulson, BS. 2014. **Rotavirus inhibits interferon-induced STAT nuclear translocation by a mechanism that acts after STAT binding to importin-α**. J Gen Virol **95**: 1723-1733.

Holloway, G, Truong, TT, and Coulson, BS. 2009. **Rotavirus antagonizes cellular antiviral responses by inhibiting the nuclear accumulation of STAT1, STAT2, and NF-κB**. J Virol **83**:4942-4951.

Alvisi, G, Rawlinson, SM, Ghildyal, R, Ripalti, A, and Jans, DA. 2008. **Regulated nucleocytoplasmic trafficking of viral gene products: a therapeutic target?** Biochim Biophys Acta **1784**:213-227.

Project 3: Understanding the mechanism behind virus acceleration of type 1 diabetes. SUPERVISOR: A/Professor Barbara Coulson.

At least 171 million people suffer from type 1 diabetes, with the incidence rising by 3% annually. As this increase in diabetes is not explained by genetic predisposition alone, focus has recently turned to environment factors such as viral infection. Viruses could affect diabetes develpment by multiple mechanisms (Fig. 3). A link between rotavirus infection and rises in pancreatic islet auto-antibodies in Australian children at risk of diabetes was discovered in our collaborative studies. This has been corroborated in Finnish and American children.

Our group demonstrated that rotavirus infection of adult NOD mice, which are the main animal model for human diabetes, results in acceleration of diabetes onset. Further extensive studies with this model have shown the importance of virus spread to regional lymph nodes, activation of antigen presenting cells at these sites, antibody responses and production of proinflammatory cytokines for rotavirus-mediated diabetes acceleration. More recently, we have shown that rotavirus infection induces B and T cell activation in the lymph nodes, spleen and pancreas. The aim of this project is to further analyse the important of lymphocyte activation for diabetes acceleration by rotavirus with a focus on the mechanism of bystander activation i.e the activation of cells independently of their specificity. In addition, the possibility of a similar mechanism for diabetes acceleration by influenza A and coxsackie B viruses will be investigated.

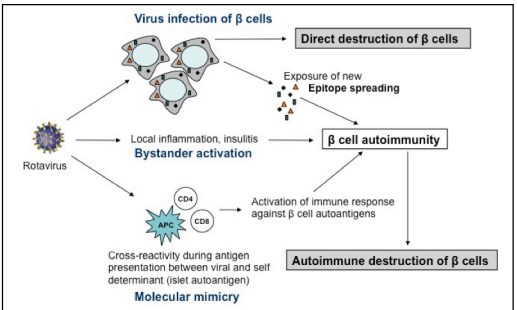

Fig. 3. Possible mechanisms involved in virus acceleration of type 1 diabetes. Direct pancreatic infection has been ruled out for rotavirus. Bystander activation and T cell mimicry are proposed for rotavirus acceleration of diabetes onset.

Selected publications from our group that underpin Project 3:

Pane JA, Coulson BS. **Lessons from the mouse: potential contribution of bystander lymphocyte activation by viruses to human type 1 diabetes**. *Diabetologia* 2015, **58**:1149-1159.

Pane JA, Webster NL, Coulson BS. 2014. **Rotavirus activates lymphocytes from non-obese diabetic mice by triggering toll-like receptor 7 signaling and interferon production in plasmacytoid dendritic cells.** PLoS Pathog **10**:e1003998.

Pane, JA, Webster NL, Graham, KL, Holloway, G, Zufferey C, Coulson BS. 2014. **Rotavirus acceleration of murine type 1 diabetes is associated with a T helper 1 dependent specific serum antibody response and virus effects in regional lymph nodes.** Diabetologia **56**: 573-82.