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Monash University, Clayton Campus

Generating innovative biological tools to assess lipid-sensing immune cells

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Winston Churchill Travelling Fellow 2017

"*Success* is not final, failure is not fatal: it is the courage to continue that counts."
- *Winston Churchill*

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Monash Biomedicine Discovery Institute (centre).

Acknowledgements

First and foremost, I would like to thank the Winston Churchill Memorial Trust for enabling me to visit Monash and Melbourne Universities in Melbourne, Australia. In particular, I would like to thank all the amazing people at the Winston Churchill Memorial Trust in London, specifically those who took their time to interview me at the Trust, namely, Mr Randolf Churchill, Prof. Alec Boksenberg, Prof. Chris Rapley and Ms Julia Weston or who kindly checked my report and assisted with the organization of my travels, namely, Mr Tristan Lawrence, Ms Sara Venerus and Ms Sara Canullo. In addition, I would like to thank members of the Welsh Association of Churchill Fellows and in particular, Dr. Kathy Seddon for her support and for putting me in touch with her son, Dr. Owen Seddon, a registrar in microbiology and infectious diseases here in Cardiff with whom I hope to study immune cells in patients with infectious diseases in the foreseeable future.

I feel very honoured to have been selected a Winston Churchill Travelling Fellow in the category Science and Technology in 2017. It has provided me with an unforgettable experience and an injection of enthusiasm about the scientific project that my visit related to, namely the study of lipid-sensing immune cells. In addition, I was unaware of how many excellent and well-known immunologists reside in Melbourne, many of whom I have now met in person and who share my research interests. New collaborations have resulted from my visit to Monash and Melbourne Universities, including collaborations related to my other research interest, the maintenance and maturation of memory T-cells that fight infections and cancer.

Obviously, I would not have had anywhere to go, if Prof. Jamie Rossjohn, FAA, FLSW, FMedSci had not welcomed me to his laboratory and research empire at Monash University. I feel very honored and am extremely grateful that he gave me this opportunity. I would also like to thank him for his very valuable help and advice and for his major contribution to making this journey immensely worthwhile and unforgettable (more on this in my report below).

Immense thanks also go to Associate Prof. Stephanie Gras, who very kindly shared her office at Monash University with me, made me feel very welcome indeed and who's research associate, Ms Shin Y Tin, showed me the laboratory technique I went to learn. Many thanks go to Shin for teaching me the technique and putting up with someone following her every step in the laboratory. Also, many thanks to both Stephanie and Shin for providing protocols and proof reading the sections on the method in this report. Furthermore, I am also grateful to Dr. Adam Shahine, who showed me how to displace lipids and load specific lipids onto CD1 antigen presenting molecules.

Special thanks also go to Jamie's PA, Mrs Jennifer Huyn, who assisted me with the organization of my visit and to members of Monash University Human Resources, who helped me with logistics regarding my stay and visa.

Furthermore, I would like to thank Prof. Nicola La Gruta, who organized the State of Victoria Immunology Retreat in the Yarra Valley in 2017, which took place during my visit. She kindly organized for me to present my work as one of three international speakers at this meeting. In addition, I would like to thank both Nicole and her husband, Prof. Stephen Turner for a lovely and long invitation to their house.

Another special thank you goes to Ms Sue Higgins, head teacher, and Mr Michael Johnstone, teacher, at St. Kilda Primary School in St. Kilda, Melbourne for allowing my son to attend St Kilda Primary School during my Fellowship. The parents of the other children in the class and my son's Australian classmates were also all amazing.

My Background

Currently I am a Clinical Senior Lecturer and an Honorary Clinical Fellow at Cardiff University School of Medicine and the Cardiff & Vale NHS Trust in Cardiff, UK. I studied medicine at and hold an MD doctorate with honors from the Charité, Humboldt University of Berlin, Germany. In addition, I hold a PhD from Cardiff University. For part of my foundation training I went to work at the Red Cross Children's Hospital and the Groote Schuur Hospital in Cape Town, South Africa. Following submission of my MD doctorate, I moved to Switzerland where I began my specialist training in infectious diseases and paediatrics at the Institute of Medical Virology, University of Zurich and the University Children's Hospital of Zurich, Switzerland. In the past, I have held research positions at the University of Zurich, Switzerland and at the University of California San Francisco, CA, USA. My main scientific interest since end of 2004 has been the study of peripheral and tissue-resident white blood cells called T-cells in infection, autoimmunity, cancer and ageing. For a list of my publications, please see:
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Abbreviations

CD1, Cluster of Differentiation 1
DNA, DeoxyNucleic Acid
FPLC, Fast Protein Liquid Chromatrography
HIV-1, Human Immunodeficiency Virus (HIV)-1
FACS, Fluorescent Activated Cell Sorter
KIR, Killer-Cell Immunoglobulin-like Receptors
NK, Natural Killer
MAIT, mucosa-associated invariant T (MAIT)

Fellowship

Background to my project

Hyperlipidemia is a metabolic abnormality that has become increasingly prevalent in the UK due to peoples' sedentary lifestyle and poor diet. The increased lipids or lipoproteins in the bloodstream lead to inflammation of the blood vessels and increase the risk of heart disease or stroke by causing plaque formation, which clogs blood vessels. I visited Prof. Jamie Rossjohn's laboratory at Monash University in Melbourne, Australia to learn how to generate innovative biological tools to enable me to identify lipid-sensing immune cells in people in the UK.

Purpose and Aims of my Fellowship

Due to the lack of available reagents for the identification of lipid-sensing immune cells, their role in blood vessel inflammation is not well understood. Jamie is a leading scientist in the field of antigen recognition by T-cells (where T stands for "thymus", which is an organ found in the chest just below the neck and which is important for the maturation of these cells) and molecular interaction of T-cells with antigen presenting cells. An antigen can be a peptide, which is a fraction of a protein, or a lipid, which is a fatty acid or a derivative thereof and insoluble in water, or a different compound. Antigens can originate from foreign organisms such as a virus or bacterium or can be self-antigens, which originate from tissues or cells. Lipid antigens can either be of self or foreign origin, as the cell membranes of most living organisms and many viruses are made up of a lipid bilayer. Antigen presenting cells are specialized cells found in the blood and in tissues. These cells can process antigens and

present them to T-cells. T-cells may then mediate an inflammatory response. Some T-cells instead of participating in inflammation can dampen inflammation. It is possible that lipid-sensing T-cells are either harmful and cause inflammation or that they are beneficial and dampen down inflammation. If their role is to dampen down inflammation, then it is possible that their function is impaired in atherosclerosis.

The role of lipid-sensing T-cells in human health and disease and the lipid ligands they recognize are not well understood.

Jamie's and his team's expertise also spans the identification of antigens recognized by T-cells, including lipid antigens.

The study of antigen-specific immune cells is an expert area in the UK, however, the focus has mostly been on cells that recognize peptides rather than lipids. Cluster of Differentiation (CD)1 molecules are glycoproteins (meaning they consist of a protein and sugars) that can carry lipid antigens and are found on different types of antigen presenting cells. T-cells can recognise lipids presented to them on CD1 (1) and there are four cell surface expressed forms of CD1, namely, CD1a, CD1b, CD1c and CD1d (reviewed in references 2 & 3). Some research in the UK has been done on immune cells that recognize lipids presented by CD1d (4) and recently, another group in the UK published data on the presentation of cholesteryl ester, a form of cholesterol (bad cholesterol is increased in the blood stream of patients with atherosclerosis), by CD1c (5). However, no data was published on the specific isolation of T-cells recognizing cholesteryl ester presented by CD1c.

A visit to Jamie's laboratory at Monash University, Australia, allowed me:

1. To learn how to generate lipid-loaded CD1 antigen presenting molecules. These reagents consist of a fluorescently-tagged version of a CD1 carrying a lipid. They are generated in human cell lines in the laboratory. These cell lines are engineered to make and secrete CD1. The CD1 is originally loaded with lipids from the cell it is made in and not with specific lipid. To load the CD1 with specific lipids, one has to displace the multiple different lipids it was loaded with in the cell line and then load with a specific lipid. This is a tricky process where a lot of the original CD1 generated and purified from the cell line is lost. In other words, the process is very labour intensive.

2. To find out how Jamie and his team identify lipid/lipoprotein ligands presented by CD1. This will involve using state of the art lipidomics-based assays incorporating mass spectrometry analysis and protein chemistry approaches. This is done by separating the lipids from the CD1 molecules and then analyzing the lipids using a technology called gas chromatography/mass spectrometry

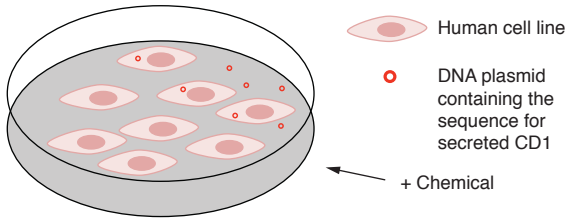
My main aim was to learn how to make secreted CD1 loaded with lipids.

The protocol for the generation of recombinant CD1 proteins loaded with lipids

Human cells are seeded into multilayer plastic cell culture flasks and a DNA (deoxynucleic acid) plasmid, which is a circular form of genetic material that in this case included a sequence for secreted CD1 as well as a histidine-tag, was added to the cell culture medium together with a chemical that facilitates that this DNA plasmid enters the cell (Figure 1a,b). This process is called "transfection".

The histidine-tag allows the purification of the secreted CD1 protein loaded with lipids using columns containing nickel ions, so called HisTRAP columns. Histidine-tagged proteins bind with high specificity and affinity to nickel in these columns and can subsequently be eluted from these columns as described below.

a



b

Figure 1. Transfection of a human cell line with a plasmid that contains the DNA sequence for secreted CD1. The secreted CD1 made in this cell line will carry lipids from the cell line. (**a**) Schematic showing the process of transfection in a cell culture dish. (**b**) Cell culture hood with flasks in a rack that contain the medium with the DNA plasmid and the chemical that facilitates entry of the DNA plasmid into the cell.

After a few days, the pink medium (liquid the cells are cultured in), which now contains the secreted CD1 made by the cells and loaded with lipids from the cells, is collected. To remove any cells that have detached from the tissue culture flask and are floating in the medium, the medium is spun down in a centrifuge (accelerated to high speed to pellet any cells) and the liquid above the cell pellet collected. This liquid is then filtered to remove further impurities (Figure 2a).

The protein in the liquid is then dialyzed against 10 mM TRIS, 500 mM NaCl at ph 8. The dialysis buffer is changed three times usually spanning 2-3 days. Following this, the dialyzed liquid, which is now much less pink then originally, is filtered (Figure 2b) and then loaded onto a nickel column, which prior to loading is equilibrated with a buffer containing 10 mM TRIS, 150 mM NaCl, 20 mM imidazole.

Imidazole is the competitive inhibitor for his-tagged proteins and at this low concentration prevents binding of other proteins such as Bovine Serum Albumin (which is present in this case, as human cell culture media contains it) to the column. The CD1 loaded with endogenous lipids is then eluted from the nickel column by Fast Protein Liquid Chromatrography (FPLC) in the presence of a high concentration of imidazole. The fractions containing the CD1 protein loaded with endogenous lipids are collected and pooled, then concentrated by centrifugation to retain proteins greater than 30kD in size, then subjected to size exclusion using size-exclusion chromatography, which again requires FPLC, but in conjunction with a Superdex 200 column to collect the pure protein, which again is concentrated by centrifugation.

The protein is then biotinylated, which allows the conjugation with a fluorescently-labeled streptavidin conjugate. This reagent can then be used to stain T-cells isolated from the blood of healthy volunteers and patients with abnormal lipid profiles.

a **b**

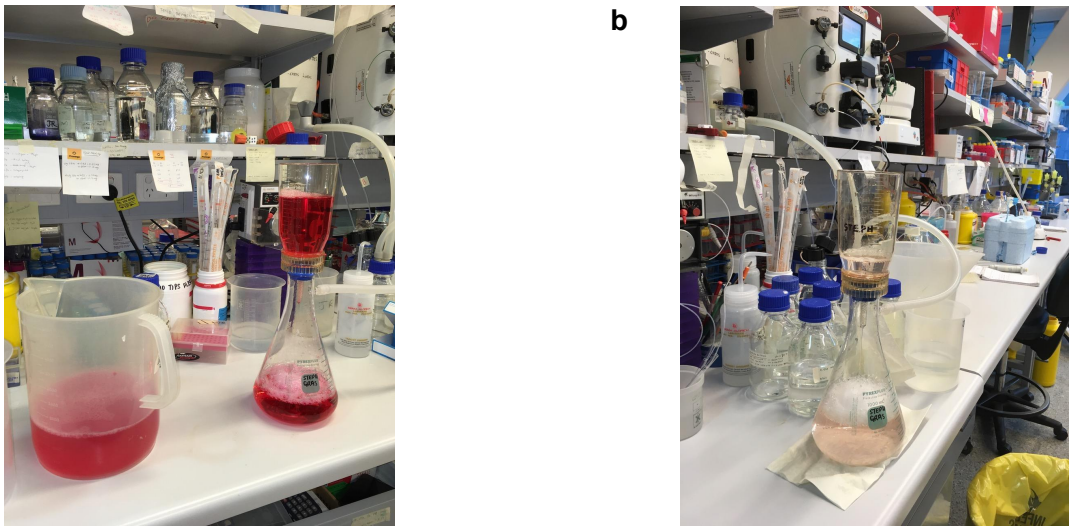


Figure 2. Harvested cell culture supernatant. (**a**) Filtering of the harvested medium that the cells were cultured in after they were given the DNA plasmid with the genetic sequence for the CD1 and the histidine tag in it. This medium now contains secreted CD1 loaded with lipids from the cell line. (**b**) Filtering of the liquid containing the secreted CD1 loaded with lipids after dialysation to enrich it for CD1 and get rid of other impurities. The liquid is losing colour (becoming more pure) when compared to Figure 2.

Lipid displacement from CD1 and loading with specific lipid

CD1 proteins can be loaded with specific lipids after pre-loading with charged lipids (e.g. the charged lipid C16 lyso-sulfatide), which is followed by incubation with an excess of the lipid of interest in the presence of e.g. saposin, which degrades specific lipids, and a detergent (6). Lipid loading is analyzed on an isoelectric focusing gel. Isoelectric focusing is an electrophoresis technique that separates protein based on their isoelectric point. The isoelectric point is the pH at which a protein has no net charge and does not move in an electric field. Such gels create a pH gradient. These gels can be used to detect a change in the CD1 protein after lipid displacement.

Benefits of my project to others and myself

There are a number of downstream benefits to others as well as myself. The study of lipid-sensing immune cells is important, as cardiovascular disease as a consequence of increased lipids in the blood stream, is the number one cause of death worldwide. I already have a local source of samples from patients with hyperlipidemia and my ethical approval to study these patients' blood samples has been reviewed and I am in the process of making some changes to it for approval. As I was asked to make some changes to the application for ethical approval, I have decided to add more patient groups to the application and this includes patients with Human Immunodeficiency Virus (HIV)-1 infection. There are two reasons to include this group of patients; first, HIV patients are prone to getting so called opportunistic infections. These opportunistic infections arise due to the impaired function of the immune system in these patients. The immune system can no longer control the outgrowth of microbes that will not make healthy people ill. Such microbes include certain types of mycobacteria and mycobacterial lipids are presented to T-cells by CD1 molecules (7-8). The second reason for including this patient group is a non-scientific one. Through being selected as a Winston Churchill Travelling Fellow, I met the physician Dr. Owen Seddon, who is the son of a former Winston Churchill Travelling Fellow, namely Dr. Kathy Seddon. Dr. Kathy Seddon chaired the Welsh branch of the Winston Churchill Fellows when I was awarded the Fellowship and she put me in touch with her son, who sees HIV-1-infected patients at the

University Hospital of Wales and who is happy to collaborate and collect blood samples for this project (obviously, after ethical approval is in place). In addition, I already collaborate with Prof. Andrew Godkin at the University Hospital of Wales with whom I study liver fine needle aspirates and matched peripheral blood samples from patients with liver diseases. The latter includes patients with fatty liver disease, another disease with lipid overload where lipid-sensing immune cells may play a role.

Recommendations

It is almost 2 decades ago that the first descriptions were published about T-cells recognizing antigens presented by CD1 cell surface glycoproteins (1) and it is only recently that scientists have managed to make reagents to specifically identify T-cells that recognize lipids presented by CD1a, CD1b and CD1c. Even though there is now some knowledge about the antigens presented by CD1, there is still much work to do on both the antigens that are presented by the different CD1s and the T-cells that respond to CD1 antigen presentation. It is important to highlight here that CD1 glycoproteins are found in every human individual. Thus therapies/diagnostics in the CD1 axis will be broadly applicable. Therefore, it makes sense to invest in this area of research.

Current and future work relating to my Fellowship

First, I am already involved in a study initiated by Jamie that involves other international experts in the CD1 field, namely, Prof. Branch Moody (Boston, MA, USA) and Assistant Prof. Ildiko van Rhijn (Boston, MA, USA) and other renowned scientists, many of whom I met in Melbourne during my Winston Churchill Travelling Fellowship. My contribution related to the use of the biological reagents, lipid-loaded CD1c, which I learned to make in Jamie's laboratory, to stain human blood samples here in the UK. A high impact publication from this collaboration has been accepted for publication in the high impact scientific journal "Nature Immunology".

Furthermore, after feedback from an ethics board at an NHS site in England, I am currently editing the ethical approval application for collection and assessment of samples from patient groups (as outlined above) in whom CD1-restricted T-cells may play a role.

I plan to carry out further exploratory studies in healthy volunteers and after successful ethical approval, in patient groups. This is to generate strong data for grant applications that will allow me to carry out larger studies in patients in the UK to gain a better understanding of lipid-sensing T-cells, which in turn will inform new therapeutic approaches. I plan to carry out cross-sectional and longitudinal patient studies as well as studies of these cells before and after therapeutic intervention, e.g. after patients have begun taking a drug, e.g. a statin, that lowers their cholesterol.

Unexpected new scientific contacts and collaborations not directly relating to the project I had proposed

I made some new contacts that I did not expect to make. After it was clear that I would visit Melbourne, my host, Jamie, put Prof. Nicole La Gruta in touch with me. Nicole was in the process of organizing the annual State of Victory Immunology Retreat (IgV), which she kindly invited me to as an international speaker. I sent her an email shortly after I arrived at Monash University. By coincidence, she was scheduled to give a seminar on the day I emailed her, which I was able to attend. We share many research interests and are already collaborating on a project studying flu-specific immune cells. She introduced me to "virtual memory T-cells", one of her current interests. At IgV, I met Dr. Kylie Quinn, a postdoctoral fellow in her laboratory, who is working on several of her projects, including the project on "virtual memory T-cells".

Other than this, Jamie's personal assistant, Ms Jennifer Huynh kindly organized a number of meetings with other scientists at Monash and Melbourne Universities for me. Early on, I met with Prof. Stephen Turner, who is currently Head of Department of Microbiology at Monash University and studies epigenetic regulation in the T-cell lineage to better understand what regulates the acquisition and maintenance of T cell effector function. The latter is required for the successful resolution of infections in humans. He has kindly agreed to be a collaborator on my next Fellowship application.

Other scientists I met at Monash University:

Dr. Julian Vivian is a group leader, who works with Jamie and I collaborate with him on at least two projects. The focus of his work is on Killer-Cell Immunoglobulin-like Receptors (KIR), which are expressed on T-cells and another white blood cell subset called Natural Killer (NK) cells. There are a number of different KIRs and the composition of KIRs is linked to control or lack of control of infectious and inflammatory diseases. Another area of interest is the study of antigenic ligands of different length and how these are presented by antigen presenting cells to T-cells. After I showed him some of my recently published data, he suggested that I should try to meet with Prof. Andrew Brooks, which I did (please see below).

Dr. Jerome Le Nours is another group leader working with Jamie. Together with Dr. Kwok Wu and Stephanie, he is involved in the collaboration on CD1-restricted T-cells with Jamie and Branch that I am involved in too.

Dr. Stephen Daley is interested in thymic T-cell selection. T-cells mature in the thymus, which is an organ that lies above and in front of the heart. His research aims to understand the separate but complementary functions of the 2 "waves" of T-cell negative selection in the thymus, and how autoimmune diseases may originate with failure of one or both "waves". It was very interesting to talk to him, as he has data that suggest a paradigm shift may be necessary.

Prof. Charles Mackay works in an area of research that has become quite "fashionable" in recent years and that is also very relevant to my project on lipid-sensing T-cells. He tries to understand the role of nutrition and the gut microbiome (i.e. the composition of bacteria in the intestine) in physiology. He suggests that there is potential to manipulate immune responses using "medicinal foods". He has demonstrated that dietary fibre and its breakdown products, the short chain fatty acids, influence the composition of the gut microbiota, immune tolerance, and inflammatory responses. He has developed antibodies to certain receptors that play dual roles in immune responses and metabolism of the cell. Charles proposes that the incidence of diabetes, cardiovascular disease, allergies and asthma, and autoimmune (where the immune system reacts to "self") diseases in Western countries relate to a changing diet.

Associate Professor Dr Meredith O'Keeffe works on an aspect of immunity that I do not think is directly linked to what I am working on, but that was very interesting to hear about. Her current research interests include investigating the function of dendritic cells (which are professional antigen presenting cells with characteristic protrusions called dendrites) in Lupus disease and malaria infection and examining the development and function of dendritic cells in Myelodysplasia. Myelodysplasia is a type of cancer in which the bone marrow does not make enough healthy blood cells. She is particularly interested in a factor that modulates the immune response called interferon-lambda. I had not yet heard much about interferon-lambda. She explores the contribution of interferon-lambda to autoimmune disease.

I met Prof. Tony Purcell together with other scientists. He applies cutting edge mass spectometry to identify ligands presented by antigen presenting molecules. He too is a co-

author on the accepted publication from the collaboration led by Jamie and Branch mentioned several times in this report.

I met Dr. Mierelle Lahoud at the IgV meeting. Her research focuses on the identification and study of molecules found on the surface of dendritic cells. She also explores how such cells could be used for therapeutic immune modulation.

Scientists I met at Melbourne University

I met with Prof. Katherine Kedzierska. She is based at the Peter Doherty Institute on the Melbourne University campus. She and her team research the immunity to viral infections and more specifically immunity to newly emerged flu viruses. She aims to identify key correlates of severe and fatal flu disease in high-risk groups including children, the elderly and Indigenous Australians.

Unfortunately, I was not able to meet Prof. Dale Godfrey, however, I met members of his team, namely Dr. Adam Uldrich and Dr. Daniel Pellicci. Both work on CD1 and T-cells that recognize lipid ligands presented to T-cells by CD1. Adam showed me unpublished data on T-cells that recognize lipid-ligands presented by CD1a. CD1a is mostly found on so-called Langerhans cells, i.e. professional antigen presenting cells found in the skin. Adam is also involved in the collaboration with Jamie and Branch on CD1c restricted T-cells that I am involved in. It was very interesting and useful to speak to Adam and Dan.

I also had the honour to meet Prof. Jim (James) McClusky and his co-workers, Dr. Lars Kjer-Nielsen, Dr. Sidonia Eckle, and Dr. Alexandra Corbett. Jim, Lars, Jamie and colleagues described that Vitamin B metabolites from microbes are presented by the non-classical antigen presenting molecule called MR-1 to mucosa-associated invariant T (MAIT) cells (9). MAIT cells are found in internal mucosal surfaces, which are exposed to microbes and this includes the lining of the gut. They are also usually abundant in the liver, which is the organ that helps clear toxins from the body. MAIT cells seem to play a protective role in anti-bacterial immunity. They are another unconventional T-cell subset and a lot of work is ongoing to better understand the role of MAIT cells in health and disease. I too have studied and am studying MAIT cells including in patients with liver disease. The latter in collaboration with Prof. Andrew Godkin here in Cardiff.

Dr. Susanne Heinzel is based at the Walter and Eliza Hall Institute of Medical Research on the Melbourne University Campus. She works with Prof. Phil Hodgkin. Their aim is to understand the molecular and cellular mechanics of immunity, which protect the body from infections and cancers. They combine experiment and theory and build computer models of the immune system. This is a similar approach to one I have taken in collaboration with Dr. Becca Asquith at Imperial College London and Prof. Derek Macallan at St. George's Hospital in London.

Dr. Laura Mackay leads a research group at the Peter Doherty Institute. Laura obtained her PhD from the University of Birmingham, UK, in the laboratory of Prof. Alan Rickinson. Her laboratory studies cellular immune responses, with a focus on the genes and signals that control tissue-resident memory T-cell differentiation, with a view to harness these cells to develop new treatments against infection, cancer, and autoimmune disease. She shared data on tissue-resident memory T-cells that reside in the skin and are involved in skin immunity. Laura's research aims to better understand tissue-resident T-cells.

As stated above, Dr. Julian Vivian recommended I meet with Prof Andrew Brooks, Deputy Head of the Peter Doherty Institute on the Melbourne University Campus. His laboratory examines how diverse arms of the immune system are integrated to provide effective anti-viral immunity, spanning NK and T-cell biology, soluble proteins of the innate arm of the

immune system and receptors involved in viral entry. I showed him the data I showed Julian at Monash, which are data I published in a reputable scientific journal in 2016 (10). Andrew immediately had an idea about what I could study using the techniques that I applied for that publication. I have already made contact with a Cardiff University-based haematologist to explore getting ethical approval to study NK cells in patients after bone marrow transplantation.

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Phillip & Churchill Islands, Apollo Bay & the Twelve Apostles, Yarra Valley/Healesville Sanctuary

Phillip & Churchill Islands

Already during my first days at Monash University, Stephanie suggested that we join her and her husband, Nico, on a Saturday on a whale-watching cruise at Phillip Island. Phillip Island lies off the Australian southern coast and it takes approximately two hours to drive there from Melbourne. As the whale-watching cruise was already booked, I booked a seal-watching trip instead. Unfortunately, during the drive to Phillip Island, the seal-watching cruise was cancelled due to high winds and swell. Instead, we were able to go on a scenic cruise in a relatively small and shallow eco speedboat (shown in the picture below). Not expecting much and hoping to avoid getting sea sick, we listened to the guide telling us about Churchill Island. Churchill Island lies adjacent to Phillip Island and is connected to it by a bridge and it is not named after Winston Churchill, but after John Churchill of Dawlish in Devon. It is an important historic place with regards to European settlement in Victoria.
The motor of the boat had just been revved up again when we noticed that the captain and guides were whispering. This was followed by the announcement that a whale and its calf had been spotted just outside of the shelter of the bay. We were asked whether we wanted to continue with the scenic cruise or try to find the whales. All passengers wanted to see the whale and its calf and I am very happy to be able to report that we found them. It was a Southern right whale with its calf. There are only approximately 10,000 of such whales in the Southern Hemisphere and we were very lucky to see them.
Before we returned to Melbourne that day, Stephanie and Nico took us to a place on Phillip Island where wallabies can be seen in the wild (picture below). We also saw beautiful mangroves (not shown).

The Eco speedboat we went on to see the whale with its calf (left) and a wild wallaby (right) on Phillip Island.

Apollo Bay & the Twelve Apostles

Jamie and his wife, Lisa, very kindly invited us to spend a weekend with them and their youngest daughter at Apollo Bay (pictures of the Bay are shown below). During the Australian summer, Apollo Bay is a bustling holiday destination, however, in winter, when we were there, it was not busy at all. We had a lovely time there with them and also, when we drove further up Great Ocean Road to see the Twelve Apostles (pictures of "some of them" below). We were extremely lucky with the weather that weekend.

Two views of Apollo Bay.

Twelve Apostles.

Yarra Valley/Healesville Sanctuary

On the last weekend, we went to Healesville Sanctuary. We had planned to do this during the IgV conference, as it was close by, but there was no time. We saw several Australasian animals at Healesville Sanctuary, including a Tasmanian devil (which amazingly was awake during the day even though these carnivorous marsupials are nocturnal), an echidna, koala bears and a platypus (shown in the pictures on the next page). It was a grey day, but luckily it mostly rained during the drive to Healesville and not while we were there. We also fed kangaroos there – they have very soft fur and one cannot touch their faces and ears as they are too sensitive. It was truly amazing.

Tasmanian devil (left) and echidna (right) at Healesville Sanctuary.

Koala (left) and platypus (right) at Healesville Sanctuary.

St Kilda Primary School

I am very grateful that my son was able to visit St. Kilda Primary School during my Fellowship. The school is amazing. The head teacher, Ms Sue Higgins is a very energetic and kind personality. Mr Johnston was the class teacher of a class with only 20 pupils. My son could not have had a better teacher (he was able to chose his teacher when we got there). Everything at this primary school seemed a bit more laid back than in his school in the UK. The pupils (even in year 1) were able to bring their football to school and play during the breaks. Children of all ages played football together and after school, many children stayed at the school for a bit longer with one of their parents and continued playing football. My son became part of the 1J family at St. Kilda Primary School and I think that he will always remember his time there.

St. Kilda Primary School.

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** Denotes equal contribution*