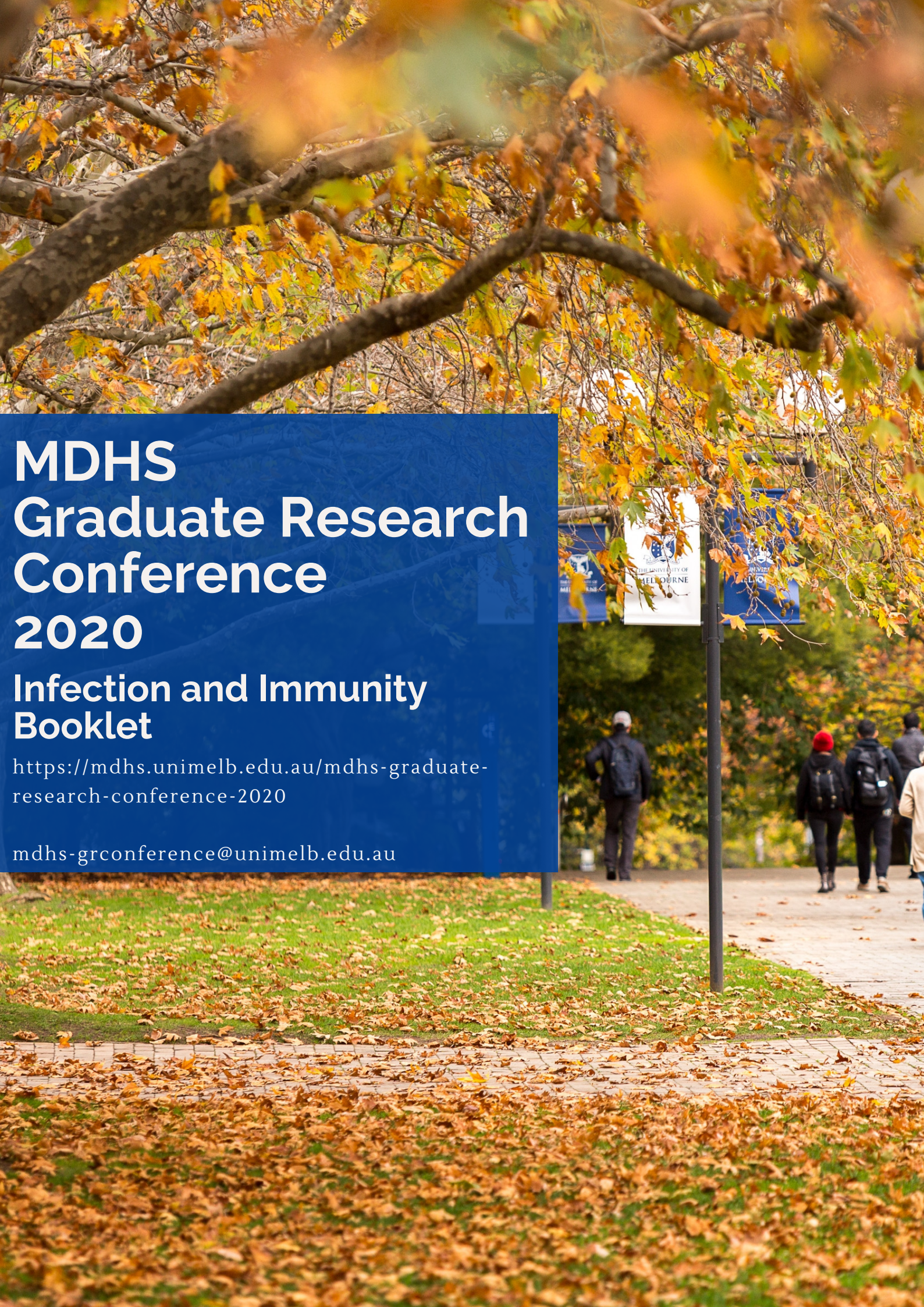


MDHS Graduate Research Conference 2020

Infection and Immunity Booklet

https://mdhs.unimelb.edu.au/mdhs-graduate-research-conference-2020

mdhs-grconference@unimelb.edu.au

MESSAGE FROM THE CHAIRS

Dear Delegates,

Welcome to the virtual inaugural Medicine, Dentistry and Health Science Graduate Research Conference 2020 (MDHS GR Conference), a student conference for all biomedical graduate research students that are part of the MDHS Faculty of the University of Melbourne. The organising committee is made up of members from 11 different student society across the MDHS faculty campus. The conference schedule consists out of 12 parallel session covering a variety of interesting topics and accommodating our student talks as well as national and international keynote speakers, Science Communication workshop and a Career Panel Discussion. This event was only possible due to the generous support of the University of Melbourne and the Graduate Student Association (GSA).

We hope that MDHS GR Conference will provide you with opportunities to listen to national and international leaders talking about their ground-breaking research in different biomedical fields and communicate your research to a broad scientific audience. Despite the fact that this conference will be virtually it will give you a unique chance to meet and network with peers from different research fields engage in discussions. We hope that the MDHS GR Conference will inspire you with new possibilities for your future career by listening to our invited speakers from academia and industry.

We wish you all the best for your presentation and hope you enjoy the event and get novel project ideas, career opportunities and new connections out of it.

Martha Blank & Alexander Anderson

(Chair & Deputy-Chair of the Medicine, Dentistry and Health Science Graduate Research Conference 2020)

GENERAL PROGRAM

08.00 - 08.15 Conference Opening & Welcoming Address
Professor Alex Boussioutas and Martha Blank

08.15 - 10.00 Session 1

10.00 - 10.30 Break

10.30 - 12.30 Session 2

12.30 - 13.00 Break
Virtual Socialise

13.00 - 14.30 Science Communication Workshop
Dr. Shane Huntington

14.30 - 16.00 Break
Virtual Socialise | Networking | Games

16.00 - 17.00 Careers Panel Discussion
A/Prof. Nicholas Opie | Dr. Danijela Mirosa | Dr. Ashish Sethi
Dr. Maryam Hussain | Dr. Simranpreet Kaur

17.00 - 19.00 Session 3

19.00 - 20.00 Award Ceremony & Conference Closing
Martha Blank and Alexander Anderson

SCIENCE COMMUNICATION WORKSHOP

Dr. Shane Huntington

Dr. Shane Huntington has been providing consulting services in communication and strategy for over 20 years. As a successful broadcaster, business owner, academic and strategist he draws together experience from multiple sectors, offering clients a more detailed and analytical approach than competitors. Shane has trained thousands of people to communicate more effectively, especially in fields of research. His unique and engaging style has led to him delivering programs to some of Australia's most prestigious institutions.

CAREERS PANEL DISCUSSION

A/Professor Nicholas Opie

Synchron Founding Director and CTO
Co-Lab Head of the Vascular Bionics Laboratory, The University of Melbourne

Dr. Danijela Mirosa

Franchise Director of Oncology for the Oceanic Cluster
Takeda Pharmaceuticals

Dr. Ashish Sethi

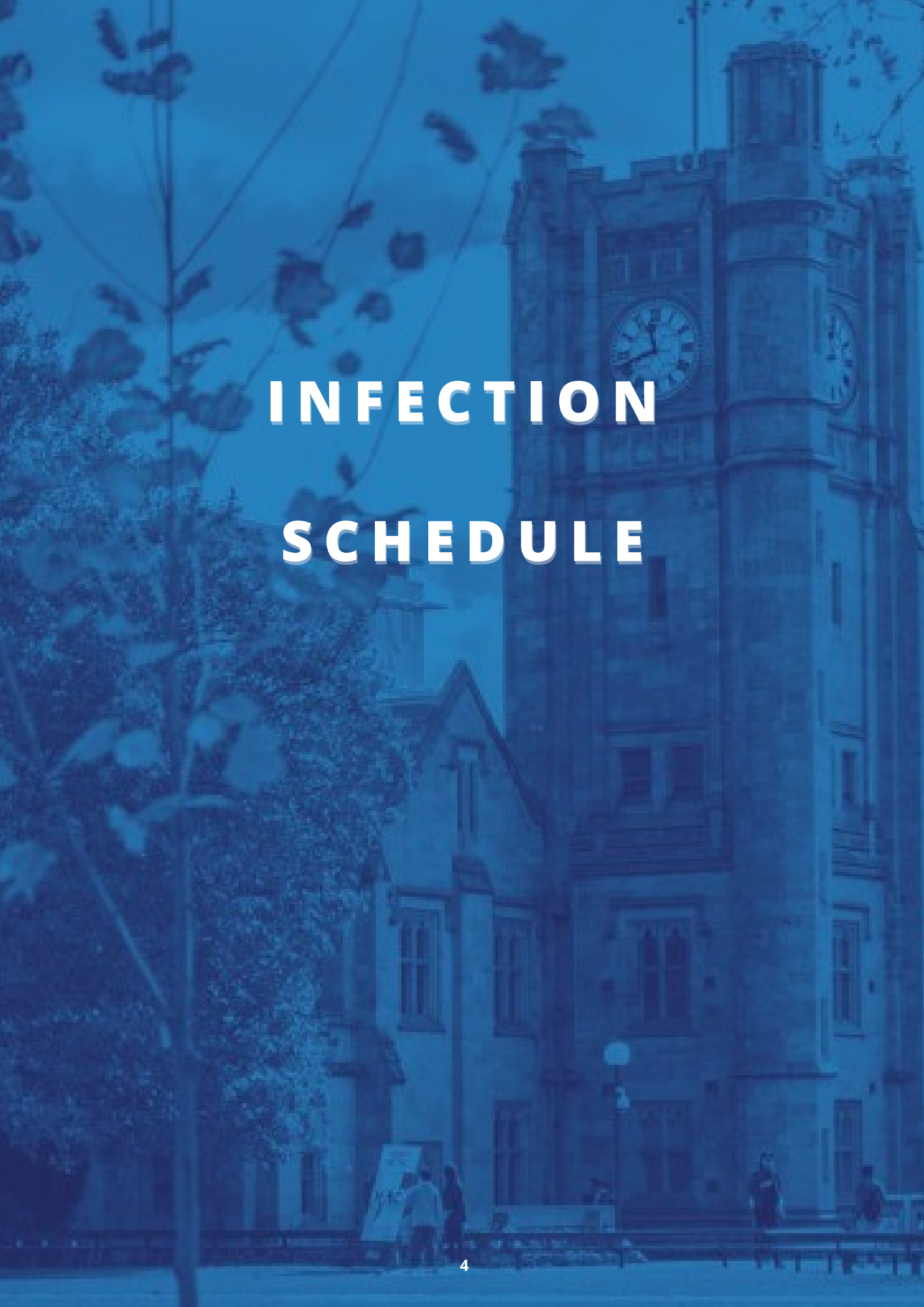
Postdoctoral Research Fellow
Department of Biochemistry & Molecular Biology, TheUniversity of Melbourne

Dr. Maryam Hussain

Medical Science Liaison
Boehringer Ingelheim

Dr. Simranpreet Kaur

Postdoctoral Researcher
MitoBrain Murdoch Children's Research Institute



INFECTION SCHEDULE

SCHEDULE

SESSION 1

08.15 – 08.45	**Modeling Immune Constraints of Antibiotic Resistance** **Keynote Speaker:** Dr. Jason Rosch	
08.45 – 09.00	**The molecular basis for zinc uptake via Streptococcus pneumoniae AdcAII** Marina Zupan	9
09.00 – 09.15	**Host macrophage omega-3 and -6 fatty acid metabolism is important for intracellular survival of leishmania parasites** Erin McGowan	10
09.15 – 09.30	**Artemisinins and delayed death drugs act antagonistically in Plasmodium falciparum** Emily Crisafulli	11
09.30 – 10.00	**Advances in immunisation leading to optimism for a COVID vaccine** **Keynote Speaker:** Prof. Tony Cunningham	

SESSION 2

10.30 – 11.00	**Strain variation in medically relevant prion disease** **Keynote Speaker:** A/Prof. Vicki Lawson	
11.00 – 11.15	**The role of Kelch 13 protein in the malaria parasite Plasmodium falciparum** Madel Tutor	12
11.15 – 11.30	**Ultrastructure and function of nuclear microtubules in Plasmodium falciparum gametocytes** Jiahong Li	13
11.30 – 11.45	**Formation of a novel two-component pore complex by a homologous pair of pore-forming toxins from Elizabethkingia anopheles** Bronte Johnstone	14
11.45 – 12.00	**A rapid, whole blood flow cytometry assay for the quantitation of antibody-dependent neutrophil phagocytosis of Plasmodium falciparum-infected erythrocytes** Dilini Rathnayake	15

SESSION 3

17.00 – 17.15	**Adaptive introgression and cryptic population structure of Cryptosporidium hominis in Africa revealed by comparative genomics** Swapnil Tichkule	16
17.15 – 17.30	**Quantifying the health-related quality of life burden of scabies** Susanna Lake	17
17.30 – 17.45	**Genome-informed vaccine design of Group A Streptococcus for remote Indigenous Australian communities** Taylah James	18
17.45 – 18.00	**CD8+ T-cell landscape in Indigenous and non-Indigenous people restricted by influenza mortality-associated HLA-A*24:02 allomorph** Luca Hensen	19
18.00 – 18.30	**Flux-sensing by kinase/transporter pairs - need-based activation of antibiotic resistance** **Keynote Speaker:** Dr. Susanne Gebhard	

Infection and Immunity

Infection
Keynote Speakers

Dr. Jason Rosch
Dept. of Infectious Diseases
St. Jude Children's Research Hospital

Session 1 08.15 - 08.45 am

Dr. Rosch received his undergraduate degree from the College of Wooster and his doctoral degree in molecular microbiology and microbial pathogenesis from Washington University in St Louis. He continued his career as a postdoctoral fellow in the lab of Elaine Tuomanen at St Jude Children's Research Hospital focusing on the pathogenesis of Streptococcus pneumoniae. He is currently an Associate Member in the Department of Infectious Disease at St Jude Children's Hospital. A major aspect of his research program focuses on bacterial and host factors that promote invasive bacterial infections. This includes the utilization of genetic approaches for the identification and characterization of novel virulence determinants. Mechanistic characterization of these virulence strategies provides insight into the intricacies underlying the various disease manifestations of the major human pathogen, Streptococcus pneumoniae. His most recent focus is modeling the impact of influenza co-infection on various aspects of pneumococcal host-pathogen interactions.
He has a longstanding interest in therapeutic interventions based on these discoveries, both through vaccine development and tailored interventions to exploit specific virulence strategies. He also has a longstanding interest on understanding antibiotic resistance in the context of impaired immunity. This work encompasses translational projects dissecting molecular mechanisms of resistance that have emerged as well as understanding the immune constraints in the acquisition and development of antibiotic resistance in bacterial pathogens. Through these areas of investigation his group has provided insight into the pathogenic strategies of the pneumococcus during disease.

Direct Interactions with Influenza Promote Bacterial Adherence during Respiratory Infections
Rowe, H.M., Meliopoulos, V.A., Iverson, A., Bomme, P., Schultz-Cherry, S., **Rosch, J.W.** *Nature Microbiology* 2019 August; 4(8): 1328-1336. PMID: 31110359

Professor Tony Cunningham
Centre for Virus Research
The Westmead Institute for Medical Research
Australian Centre for HIV and Hepatitis Virology Research
University of Sydney

Session 1 09.30 - 10.00 am

Professor Cunningham AO, FAHMS, MD, FRACP, FRCPA is an infectious diseases physician, clinical virologist and scientist, well known internationally for his research on the immunobiology of HIV and herpesviruses, his work on vaccine development and trialling, especially for shingles and herpes, and as an antivirals expert. He has served on numerous international expert panels on HIV/HSV, antivirals and vaccines. Professor Cunningham has previously worked at Stanford University, USA, built up the State Reference Laboratory in Virology and a state reference HIV laboratory at Westmead Hospital in the mid-1980s, before assuming directorship of WIMR in 1996.
In 2010 he was awarded Officer of the Order of Australia (AO) for 'service to medicine, particularly in the field of viral research and through the development and leadership of medical and biomedical research' and in 2015 was elected as an inaugural fellow of the Australian Academy of Health and Medical Sciences. Since stepping down from the WIMR director role in 2019 Professing Cunningham has continued his research on an NHMRC senior Investigator Grant and become immersed in COVID-19 research and other issues, including:

- A member of the NSW COVID-19 Vaccine Committee, the NSW Waratah COVID vaccines trial alliance and the national Vax4COVID trials alliance.
- A contributing author and lead author respectively: Papers on 'The risk of COVID19 recurrence in Winter' and 'Vaccines for COVID19' commissioned from the Australian Academies of Science and Health and Medical Science by the Chief Scientist of Australia for the Commonwealth Ministers of Health and Science.
- A member of the Safety Review Board for University of Queensland Phase 1 clinical trial of SARS-CoV-2 Sclamp vaccine and of the Seqirus global COVID Vaccines Advisory Committee setting up phase 2/3 trials.

Efficacy of the herpes zoster subunit vaccine in adults 70 years of age or older
AL Cunningham, H Lal, M Kovac et al *New England Journal of Medicine* 2016

Diversity of receptors binding HIV on dendritic cell subsets
Stuart G Turville, Paul U Cameron, Amanda Handley, George Lin, Stefan Pöhlmann, Robert W Doms, **Anthony L Cunningham** *Nature Immunology* 2002

Infection and Immunity

Infection
Keynote Speakers

A/Professor Vicki Lawson
The Peter Doherty Institute for Infection and Immunity
University of Melbourne

Session 2 10.30 - 11.00 am

Vicki Lawson is an Associate Professor in the discipline of pathology in The Department of Microbiology and Immunology at The University of Melbourne with research interests in how infectious agents are transmitted and cause disease. She received a PhD from the University of Melbourne for investigating the role of the HIV-1 envelope glycoprotein in the transmission and pathogenesis of AIDS and completed post-doctoral training at the Rocky Mountain Laboratories, NIAID, NIH, where she identified regions of the prion protein that are essential for the protein misfolding that defines prion disease before returning to Melbourne to establish a research laboratory with a focus on ransmissible neurodegeneration. Her research broadly seeks to understand how protein misfolding in the central and enteric nervous system gives rise to neurodegeneration, with a focus on diagnosis, treatment and prevention of prion and Parkinson's disease. In recent years our prion disease research has focused on understanding the cause and consequence of prion strain variation in medically relevant prion diseases as well as understanding how the normal function of the prion protein may contribute to diseases such as cancer.

The brain to gut pathway: a possible route of prion transmission
Lawson VA, Furness JB, Klemm HM, Pontell L, Chan E, Hill AF, et al. *Gut* 2010; 59: 1643-51.

Dr. Susanne Gebhard
Milner Centre for Evolution
University of Bath

Session 3 6.00 - 6.30 pm

Susanne trained in molecular microbiology in Germany and New Zealand. Her PhD work was focused on transport systems and their regulation, while her first postdoctoral project saw her shift her research towards regulation of antibiotic resistance. With her move back to Germany in 2009, Susanne combined these two interests and began to investigate how a transport system can play an active role in bacterial signaling. With this new project, she established an independent junior research group in Munich. In 2014, she moved to the UK to take up a lectureship in Medical Microbiology at the University of Bath, where she is continuing and expanding this work.

Conformation control of the histidine kinase BceS of Bacillus subtilis by its cognate ABC-transporter facilitates need-based activation of antibiotic resistance
Koh, A, Gibbon, MJ, Van der Kamp, MW, Pudney, CR, **Gebhard, S.** *Mol Microbiol.* 2020; 00: 1– 18.

ABSTRACTS

The molecular basis for zinc uptake via Streptococcus pneumoniae AdcAII

Marina Zupan[1] ,Zhenyao Luo[2,3,4],Aimee Tan[1], Victoria Pederick[5], Katherine Ganio[1], Evelyne Deplazes [6,7], Bostjan Kobe[2,3,4]& Christopher McDevitt[1]

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[2] School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland, Australia
[3] Australian Infectious Diseases Research Centre, The University of Queensland, Brisbane, Queensland, Australia
[4] Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, Australia
[5] Research Centre for Infectious Diseases, School of Biological Sciences, The University of Adelaide, Adelaide, South Australia, Australia
[6] School of Life Sciences, University of Technology Sydney, Sydney, NSW, Australia
[7] School of Pharmacy and Biomedical Sciences, Curtin Health Innovation Research Institute, Curtin University, Western Australia, Australia

Introduction: Streptococcus pneumoniae is a globally significant human pathogen responsible for more than a million deaths annually. To colonise and persist within the host, the bacterium must acquire the transition metal ion zinc [Zn(II)], which is poorly abundant in the host environment. In S. pneumoniae , Zn(II) import is facilitated by the ATP-binding cassette transporter, AdcCB, and two solute-binding proteins (SBPs), AdcA and AdcAII. Although both SBPs deliver Zn(II) to the AdcCB transporter, AdcAII has a greater role during initial infection and survival in response to Zn(II) starvation. Despite this, the molecular details of how AdcAII acquires Zn(II) ions remain poorly defined. This can be attributed to the inability of crystallographic approaches to determine a high-resolution structure of ligand-free AdcAII.

Methods: Here, we overcame the lack of structural information by systematically mutating each of the four Zn(II)-coordinating residues, and performing structural and biochemical analyses on the variant isoforms. X-ray crystallography and molecular dynamics simulations of AdcAII variant proteins identified structural elements involved in the binding mechanism. Further, quantitative in vitro metal-binding experiments were used to probe the role of each residue in ligand-induced conformational changes. Phenotypic assays of adcAII mutant S. pneumoniae strains provided insight into the effect of binding site mutations on Zn(II) import.

Results: Structural analyses revealed how specific regions within the protein undergo conformational changes via their direct coupling to two of the four metal-binding residues. In vitro metal-binding experiments revealed that each of the Zn(II)-coordinating residues make varied contributions to metal binding and affinity. These analyses also revealed that in contrast to AdcA, AdcAII is permissive for interacting with other first-row transition metal ions. Intriguingly, the impact of mutant adcAII alleles on the growth of S. pneumoniae did not generally correlate with SBP affinity, but was instead consistent with the degree of structural perturbation exhibited in mutant AdcAII proteins.

Conclusion: Taken together, our data show, for the first time, that SBP conformation rather than affinity is the primary determinant of efficacious Zn(II) uptake in S. pneumoniae. Collectively, our data reveal a novel metal-binding mechanism for AdcAII and highlight how ligand affinity and protein conformational changes are coupled within ligand-receptor proteins. These mechanistic insights provide a foundation for novel antimicrobial design to disrupt this process in bacterial metal-receptor proteins.

Host macrophage omega-3 and 6-fatty acid metabolism is important for intracellular survival of Leishmania parasites

Erin McGowan[1] , Eleanor Saunders[1] , Kaylene Simpson[2], Malcolm McConville[1] & Katrina Binger [3]

[1] The Bio21 Institute of Molecular Science and Biotechnology, Melbourne, Victoria, Australia
[2] Victorian Comprehensive Cancer Centre, Melbourne, Victoria, Australia
[3] La Trobe Institute for Molecular Sciences, Melbourne, Victoria, Australia

Introduction: Leishmania is the causative parasite of a spectrum of diseases that cause significant global morbidity and mortality. Current antileishmanial drugs are limited and increasingly undermined by the emergence of multi-drug resistance. As an intracellular parasite, Leishmania depends on the host macrophage for many of its nutrient and energy resources. Interventions that attenuate parasite growth by modulating macrophage metabolism are thus emerging as attractive alternative therapeutic strategies.

Methods: We performed a genome-wide small interfering RNA screen on a human monocyte cell line infected with two Leishmania species and identified knockdown of fatty acid desaturase-1 decreased the intracellular growth of both species, indicating a role for long-chain unsaturated fatty acids in promoting Leishmania survival. We next generated a L. mexicana strain expressing TurboRFP to quantify infection by flow cytometry. This assay was then used to screen the in vitro antileishmanial capacity of a range of inhibitors and agonists of omega-3 and -6 fatty acid metabolism.

Results: Chemical perturbation of enzymes and transcription factors in this metabolic network dramatically influenced infection in bone marrow derived macrophages and THP1 cells. Significant reductions in infection were observed with inhibition of phospholipase A2, fatty acid desaturase 2, 5- and 15-lipoxygenase, peroxisome proliferator-activated receptor α (PPARα) and activation of PPARγ. Furthermore, we have utilised TurboRFP-L. mexicana in cutaneous leishmaniasis murine models where conventional immunophenotyping by flow cytometry in conjunction with infection with a stably fluorescent parasite allows observation of the immune response, identification of infected cells and quantification of infection on a cellular level.

Conclusion: Here, we established a robust and unbiased method to screen antileishmanials and measure intracellular Leishmania infection in vitro and in vivo. Using these methods, we have revealed a role for macrophage omega-3 and -6 fatty acid metabolism in Leishmania survival and begun to elucidate important host cell factors by which this may occur.

Artemisinins and delayed death drugs act antagonistically in Plasmodium falciparum

Emily M. Crisafulli[1], Kit Kennedy[1], Simon A. Cobbold[1], Matthew P. Challis[2], Amanda De Paoli[2], Carlo Giannangelo[2], Darren J. Creek[2], Leann Tilley[1] & Stuart A. Ralph[1]

[1] Department of Biochemistry & Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Victoria 3010, Australia
[2] Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, Victoria 3051, Australia

'Delayed death' is a phenomenon observed in some apicomplexan species, such as Plasmodium falciparum, whereby parasites treated with inhibitors of apicoplast housekeeping successfully survive the first erythrocytic cycle following drug treatment but die in the subsequent cycle. The universal isoprenoid precursor, isopentenyl-pyrophosphate (IPP), is the only essential product of the apicoplast in the P. falciparum blood-stages. IPP plays a key role in various cellular processes, including protein prenylation. The isoprenoid, geranylgeranyl pyrophosphate (GGPP), is required for prenylation of Rabs that mediate cellular trafficking, notably the haemoglobin uptake pathway. The disruption in haemoglobin trafficking that results from apicoplast loss has been implicated as the proximate cause of delayed death. It is hypothesised that disruption of this pathway reduces the digestion of haemoglobin into free haem. This has possible implications for use of delayed death drugs in combination with artemisinins, which rely on free haem for activation. Here, we demonstrate that these two classes of drugs behave antagonistically, and we present preliminary data that directly implicates GGPP depletion in this antagonistic interaction. These data have potentially serious clinical implications due to the widespread use of delayed death drugs and artemisinins as antimalarials, in malaria prophylaxis, and in the treatment of other conditions.

The role of Kelch 13 protein in the malaria parasite Plasmodium falciparum

Madel V Tutor[1], Paul McMillan[2], Leann Tilley[1] & Stuart A. Ralph [2]

[1] Department of Biochemistry & Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Victoria 3010, Australia
[2] Centre for Advanced Histology & Microscopy, Peter MacCallum Cancer Centre, VIC, Australia

Malaria is a mosquito-borne infectious disease that took about half a million lives in 2018. It is caused by the Plasmodium parasite, the most life-threatening species of which is P. falciparum. Resistance to antimalarials is a recurring hurdle towards effective management and/or elimination of malaria. Decreased susceptibility to artemisinin, the most effective antimalarial currently available, has emerged and its global spread is a serious risk to malaria control. Mutations in the P. falciparum Kelch 13 (PfK13) protein lead to decreased susceptibility to artemisinin. Multiple hypotheses on what the role of this protein is in the parasite exist, but recent evidence shows involvement in the parasite's haemoglobin uptake process. Digestion of haemoglobin taken up by the parasite produces free haem, which is necessary to activate artemisinin, and initiates the events leading to parasite death. Using indirect immunofluorescence visualised by super-resolution microscopy, we show that PfK13 forms doughnut-shaped structures located at the parasite periphery. This indicates that PfK13 is located in the parasite's primary uptake structure called the cytostome. Furthermore, fluorescent live cell imaging of GFP-tagged PfK13 (GFP-PfK13) parasite line grown in fluorescent dextran-loaded red blood cells (RBC) show that GFP-PfK13 is closely associated with fluorescent dextran-loaded structures that form inside the parasite as it feeds from the RBC host. In mid-ring stage, fluorescent dextran-loaded structures are also found associated with hemozoin pigments that form prior to the formation of the digestive vacuole. GFP-PfK13 is also seen associated with these structures, indicating involvement of PfK13 in haemoglobin digestion and hemozoin formation. As previously shown, in late ring or trophozoite stages the hemozoin pigments coalesce to form a single digestive vacuole that is fed by haemoglobin-containing vesicles. We show that GFP-PfK13 is associated with such structures, indicating PfK13's involvement in digestive vacuole biogenesis. Taken together, our results show that PfK13 is closely associated with the haemoglobin uptake process and digestive vacuole biogenesis. Mutations in PfK13 lead to a decreased abundance of haemoglobin peptides in the parasite, which can lead to decreased activation of artemisinin and, ultimately, decreased parasite susceptibility.

INFECTION AND IMMUNITY
Infection

Ultrastructure and function of nuclear microtubules in Plasmodium falciparum gametocytes

Jiahong Li [1], Gerry Shami [1], Boyin Liu [1], Eric Hanssen [2], Matt Dixon [1] & Leann Tilley [1]

[1] Department of Biochemistry and Molecular Biology & Bio21 Molecular Science & Biotechnology Institute, The University of Melbourne
[2] Melbourne Advanced Microscopy Facility & Bio21 Molecular Science & Biotechnology Institute, The University of Melbourne

Plasmodium falciparum is the most prevalent and fatal species among human malaria parasites. The parasite has a complex lifecycle in its human host, including asexual and sexual stages. The sexual-stage parasites (gametocytes) undergo a dramatic morphological change during their maturation. The shape-changing ability of gametocytes is regulated by their cytoskeletons, with microtubules playing a crucial role. We have identified an unusual microtubule-based structure in the nucleus of early-stage gametocytes emanating from a microtubule-organizing center (MTOC) associated with the nuclear membrane. In this project, we aim to study the nuclear microtubules' dynamics and function in P. falciparum gametocytes. We hypothesis that nuclear microtubules play an important role in the shape and organization of the gametocyte's nucleus. We propose that the MTOC serves as a nucleation point for the cytoplasmic microtubules, thereby initiating the formation of a structure that controls gametocyte morphology during its 12-day development period.

Formation of a novel two-component pore complex by a homologous pair of pore-forming toxins from Elizabethkingia anophelis

Bronte A. Johnstone[1], Sara L. Lawrence[2], Craig J. Morton[1], Jordan C. Evans[3], Michelle P. Christie[1], Rodney K. Tweten[3] & Michael W. Parker[2,1]

[1] Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, VIC, 3010, Australia
[2] ACRF Rational Drug Discovery Centre, St. Vincent's Institute of Medical Research, Fitzroy, VIC 3065, Australia
[3] Department of Microbiology and Immunology, University of Oklahoma, Health Sciences Center, Oklahoma City, OK 73104, USA

Introduction: Cholesterol-dependent cytolysins (CDCs) are bacterial pore-forming toxins that are secreted as soluble monomers and oligomerise into large circular pre-pores on the surface of cholesterol-rich membranes. Various structural changes and transitions results in insertion of β-hairpins into the lipid bilayer, forming a large β-barrel pore that results in cell lysis [1]. We have identified a highly conserved structural motif of CDCs that plays a critical role in the prepore-to-pore transition. Furthermore, this motif is also highly conserved in a in a large, diverse family of uncharacterised proteins from over 220 species, which we have designated the name "CDC-like" (CDCL) proteins [2]. Many of these CDCLs exist as homologous pairs. One partner of the CDCL pair, termed CDCL long, consists of four domains: three similar to CDCs and a unique fourth domain. The other partner, CDCL short, possesses three domains, all similar to CDCs. We have identified and characterised a novel CDCL pair, referred to as ALY long (ALYL) and ALY short (ALYS), that originates from the species Elizabethkingia anophelis, a commensal bacterium of the Anopheles mosquito.

Methods: Structural characterisation of the novel pore complex is being conducted using an integrative structural biology approach, which includes the application of X-ray crystallography, negative-stain electron microscopy, cryo-EM, cross-linking mass spectrometry (XL-MS) and small angle X-ray scattering (SAXS). Further investigation into conditions affecting pore formation and function have been conducted using a range of liposome-based biophysical assays, including fluorescent liposome leakage assays and SDS-AGE experiments.

Results: X-ray crystallography revealed the structure of monomeric ALYL consists of characteristic CDC domain 1 – 3 structure; however, domain 4 differs from that of CDCs significantly. In the presence of lipids, ALYL and ALYS show weak pore-forming activity and analysis by negative-staining electron microscopy reveals a large circular oligomeric complex reminiscent of CDC pore complexes. ALYS also forms a non-lytic pore-like oligomer in the absence of ALYL with further investigation suggesting ALYS to be the membrane-inserting subunit of the heterocomplex. XL-MS data reveals a putative binding interface between ALYS and ALYL in addition to providing an initial insight into the structural changes between the monomeric and heterooligomeric states, which will be further supported by the high-resolution cryo-EM structure of the complex.

Conclusion: We have identified a pair of novel pore-forming toxins that share a conserved motif with the CDC family of β-barrel forming toxins. Furthermore, we have shown using multiple structural techniques that the toxins share some structural resemblance to CDCs, but also form a two-component pore complex that is unique to the CDC family. This study establishes the beginning of an investigation into the large family of novel CDC-like proteins present in a wide range of bacterial species and are suspected to play key roles in microbial survival and human disease.

References
[1] Christie, M.P., Johnstone, B.A., Tweten, R.K. et al. Cholesterol-dependent cytolysins: from water-soluble state to membrane pore. Biophys Rev (2018),10, 1337–1348. https://doi.org/10.1007/s12551-018-0448-x
[2]Evans, J.C., Johnstone, B.A., Lawrence, S.L., Morton C.J., Christie, M.P., Parker, M.W., and Tweten, R.K. A Key Motif in the Cholesterol-Dependent Cytolysins Reveals a Large Family of Related Proteins. mBio (2020), 11 (5) e02351-20; DOI: 10.1128/mBio.02351-20

INFECTION AND IMMUNITY
Infection

A rapid, whole blood flow cytometry assay for the quantitation of antibody-dependent neutrophil phagocytosis of Plasmodium falciparum-infected erythrocytes

Dilini Rathnayake[1]& Stephen J. Rogerson[1]

[1] Department of Medicine at the Royal Melbourne Hospital, The Peter Doherty Institute of Infection and Immunity, University of Melbourne, Victoria 3010, Australia

Introduction: Plasmodium falciparum malaria remains a major global health problem causing severe morbidity and mortality. Antibody immunity against blood stage of P. falciparum-infected erythrocytes (IEs) seems effective, but non-sterile. Antibody-mediated neutralisation of IEs and antibody-dependent phagocytosis (ADP) of IEs by innate immune cells are important for protection. The neutrophils are the most abundant and the first cells to be recruited during infection; but not many studies report ADP of IEs by neutrophils in human malaria. We devised a novel flow cytometric assay to measure ADP of P. falciparum-IEs by neutrophils in human whole blood that represents a more realistic physiological scenario than the isolated neutrophils.

Methods: This no wash-no lyse assay utilised small volumes of heparinised blood (<1 ml) from healthy volunteers. The antibody-opsonised IEs were subjected to phagocytosis by neutrophils in prediluted whole blood in plate-based assays. The leukocytes were identified based on their differences in light scatter by flow cytometry using blue and violet lasers (CytoFlexS, Beckman Coulter). IEs were uniquely identified by labelling with the nucleic acid dye dihydroethidium (DHE), and neutrophils were identified using fluorescently labelled antibodies against CD16 and CD66b. A phagocytic score was derived that measured the percentage of neutrophils that engulfed the parasites multiplied by the geometric mean fluorescent intensity of DHE-labelled IEs within the neutrophils.

Results: The developed assay avoids pre-processing of whole blood, and we observed that uninfected erythrocyte lysis followed by fixation of leukocytes did not alter antibody-mediated uptake of IEs by neutrophils. Assay throughput is high, and using pooled malaria exposed sera and unexposed controls there is exposure-specific uptake of opsonised IEs by neutrophils, confirmed using 40 individual samples from malaria-exposed pregnant women from Malawi (Mann-Whitney U test, p=0.0139). The results indicated minimal intra- and inter-donor variation (Spearman r=0.6-0.8) among different donors (n=3) representing high reproducibility of the assay. We are extending the assay to simultaneously measure ADP of IEs by monocytes in human blood and to correlate the results of both neutrophils and monocytes with purified cells using the same pregnant women cohort.

Conclusion: We developed a rapid flow cytometry assay for identifying neutrophils in whole blood that could clearly differentiate exposure-specific opsonic phagocytosis of neutrophils with non-exposed individuals in human malaria. This assay has the potential to study both antibodies and neutrophils from malaria-exposed individuals and to identify novel correlates of protection from malaria.

INFECTION AND IMMUNITY
Infection

Adaptive introgression and cryptic population structure of Cryptosporidium hominis in Africa revealed by comparative genomics

Swapnil Tichkule[1,2], Aaron R. Jex[1,2], Cock van Oosterhout[3] & Simone M. Cacciò[4]

[1]Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC, Australia
[2]University of Melbourne, Melbourne, VIC, Australia
[3]University of East Anglia, Norwich, UK
[4]Istituto Superiore di Sanità, Rome, Italy

Introduction: Cryptosporidiosis is a major cause of diarrhoeal illness among African children, and it is associated with increased childhood mortality, malnutrition, cognitive development and growth retardation. Cryptosporidium hominis is the dominant pathogen for this disease in Africa. Genotyping at the glycoprotein 60 (gp60) gene has revealed a complex distribution of different subtypes across Africa. However, a comprehensive exploration of the metapopulation structure and evolution based on whole genome data has yet to be performed.

Methods: In this study, we sequenced and analysed the genomes of 26 C. hominis isolates, representing different gp60 subtypes, collected at rural sites in Gabon, Ghana, Madagascar and Tanzania. These whole genomes were subjected to variant calling with GATK pipeline, followed by PCA, Phylogeny, STRUCTURE and network analysis to infer population structure. HybridCheck and RDP programs were employed to investigate introgression and recombination among the isolates. Polymorphic genes were then evaluated with population genetic metrices and tested their significance towards host-parasite co-evolution.

Results: Phylogenetic and cluster analyses based on Single Nucleotide Polymorphisms showed that isolates predominantly clustered by their country of origin, irrespective of their gp60 subtype. We found a significant isolation-by-distance signature that shows the importance of local transmission, but we also detected evidence of hybridization between isolates of different geographic regions. We identified 37 outlier genes with exceptionally high nucleotide diversity, and this group is significantly enriched for genes encoding extracellular proteins and signal peptides. Furthermore, these genes are found more often than expected in recombinant regions, and they show a distinct signature of positive- or balancing selection.

Conclusion: Our study showed that: 1) the metapopulation structure of C. hominis can only be accurately captured by whole genome analyses; 2) local anthroponotic transmission underpins the spread of this pathogen in Africa; 3) hybridization occurs between distinct geographical lineages; and 4) genetic introgression provides novel substrate for positive- or balancing selection in genes involved in host-parasite coevolution

Quantifying the health-related quality of life burden of scabies

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Background: Scabies is caused by a mite that burrows into the skin causing intense itch and skin lesions. The lesions can become infected and may lead to serious immune-mediated disease. Limited studies have shown that scabies impacts health-related quality of life (HRQoL), however no studies have been conducted in the Pacific region, an area with a high burden of scabies.
The aim of this study is to assess the impact of scabies on HRQoL in a high prevalence setting using the Children's Dermatology Life Quality Index (CDLQI) and Dermatology Life Quality Index (DLQI).

Methods: The study was conducted in Western Province, Solomon Islands where 15% of the population have active scabies cases. We went to 20 villages and examined the whole population for scabies. We conducted Dermatology Life Quality Index and Children's Dermatology Life Quality Index questionnaires with participants both with and without scabies.

Results: We surveyed 1051 adults and 604 children, 91 and 103 with scabies, respectively. Scabies had a small impact on HRQoL with an average DLQI score of 3.1 (95%CI 2.4-3.8) and CDLQI score of 2.8 (2.3-3.4) (out of 30) in participants with scabies compared to a DLQI score of 0.4 (0.3-0.5) and CDLQI score of 0.4 (0.2-0.5) in participants without scabies. The score increased linearly with the severity of scabies with an average DLQI score of 7.8 (4.2-11.4) and CDLQI score of 4.8 (3.0-6.7) in participants with severe scabies. The greatest impact on quality of life was due to the symptoms, impact on school and work, and sleep

Conclusions: Our study demonstrates that scabies has a small, but measurable, impact on HRQoL in Solomon Islands. We observed impacts on school, work and gardening which, in this setting, impact an individual's future and ability to support their family. In a setting where population prevalence of scabies is 20% this accounts for a large impact on the community. There is scope to develop a modified DLQI and CDLQI for scabies that is more culturally appropriate for Pacific Island countries and may better measure the true impact of this disease. The results of this study provide further evidence of the need for elimination of scabies as a public health problem in Solomon Islands and other settings where the disease is endemic.

INFECTION AND IMMUNITY
Infection

Genome-informed vaccine design of Group A Streptococcus for remote Indigenous Australian communities

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[2]Doherty Department at the Peter Doherty Institute for Infection and Immunity, The University of Melbourne

Introduction: Skin infections caused by group A streptococcus (GAS) are prevalent at hyperendemic levels in remote Indigenous communities within the top end of Australia. Recurrent, uncontrolled skin infections can result in rheumatic heart disease (RHD); the largest contributor of acquired heart disease in children. RHD is now responsible for the largest gap in life expectancy disparities seen between Indigenous and non-Indigenous Australians. A national effort into producing a protective vaccine against GAS is underway, which will provide an important step in closing the gap and preventing RHD. Several vaccines candidates are in various stages of development, including peptide antigen and whole-protein antigen vaccines. However, the epidemiology informing vaccine design is centered around high-income counties (HIC); and the theoretical coverage of these vaccine candidates in remote endemic regions, where disease levels are highest, is poorly understood.

Methods: We aimed to further inform vaccine design by utilizing whole genome sequences of 1,051 GAS isolates from remote Indigenous communities within the Northern Territory (NT). We screened for three major groups of vaccine antigens: a 30-valent M-protein vaccine; 74 peptide antigens from three different peptide vaccine candidates; and 23 whole-protein antigen candidates. Using novel molecular epidemiological and statistical analyses, we then examined the distributions of these major groups of vaccine antigens in NT GAS and compared these to 711 HIC GAS isolates, to determine which antigens would make the most appropriate vaccine candidates in both HIC and endemic contexts.

Results: We determined the efficacy of the 30-valent vaccine in the NT would be poor, covering only 50.9% of NT isolates compared to 80.2% of HIC isolates. Screening for peptide vaccine antigens showed that the distribution and prevalences varied significantly between NT and HIC regions. SpyCEP peptides S2 and S2.1 were found to be the best peptide candidates; having the highest, most conserved coverage. Alternatively, we found the distribution of prevalences of potential whole-protein vaccine antigens was similar; with 14 whole-proteins having prevalence levels >90% in both endemic NT and non-endemic HIC isolates.

Conclusion: Using these reverse vaccinology techniques, we concluded the best vaccine candidates for GAS infections include ADI, TF, SpyAD and SpyCEP. Which were prevalent in >99% of all NT and HIC isolates screened for. A multiantigen vaccine formula including a combination of these whole-proteins could theoretically increase levels of protective coverage of GAS isolates in hyperendemic regions like the NT; and reduce the burden of RHD in Indigenous Australians.

INFECTION AND IMMUNITY
Infection

CD8+ T-cell landscape in Indigenous and non-Indigenous people restricted by influenza mortality-associated HLA-A*24:02 allomorph

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[12] Immunology and Diabetes Unit, St Vincent's Institute of Medical Research, Fitzroy, Victoria 3065, Australia
[13] Sydney Medical School, University of Sydney, Sydney
[14] Chris O'Brien Lifehouse Cancer Centre, Royal Prince Alfred Hospital, Sydney
[15] Immunology Division, Garvan Institute of Medical Research, Darlinghurst 2010, New South Wales, Australia
[16] St. Vincent's Clinical School, University of New South Wales, Sydney 2052, New South Wales, Australia
[17] Institute of Infection and Immunity, Cardiff University School of Medicine, Heath Park, Cardiff CF14 4XN, United Kingdom
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[20] Indigenous Engagement, CQUniversity, Townsville Australia
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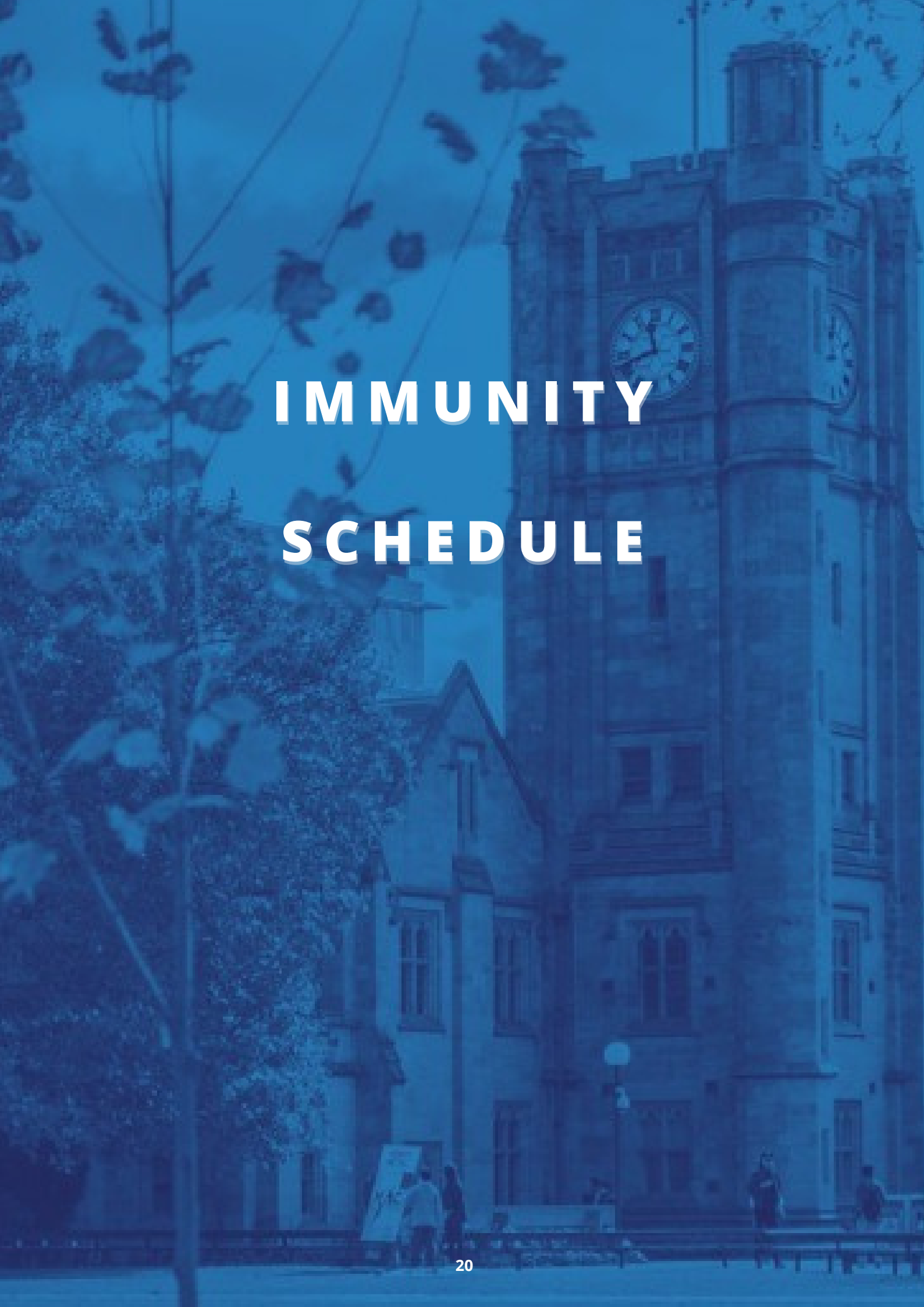
Indigenous people worldwide are at high-risk of developing severe influenza disease. HLA-A*24:02 allele, highly prevalent in Indigenous populations, is associated with influenza-induced mortality, although the basis for this association is unclear. We defined CD8+ T-cell immune landscapes against influenza A (IAV) and B (IBV) viruses in HLA-A*24:02-expressing Indigenous and non-Indigenous individuals, human tissues, influenza-infected patients and HLA-A*24:02-transgenic mice. We identified 7 immunodominant CD8+ T-cell epitopes, one towards IAV and six towards IBV, with A24/PB2550-558-specific CD8+ T-cells cells being cross-reactive between IAV and IBV. Memory CD8+ T-cells towards these specificities were present in blood (CD27+CD45RA- phenotype) and tissues (CD103+CD69+ phenotype) of healthy subjects, and effector CD27-CD45RA-PD-1+CD38+CD8+ T-cells in IAV/IBV patients. Our data present the first evidence of influenza-specific CD8+ T-cell responses in Indigenous Australians, and advocate for T-cell-mediated vaccines that target and boost the breadth of IAV/IBV-specific CD8+ T-cells to protect high-risk HLA-A*24:02-expressing Indigenous and non-Indigenous populations from severe influenza disease.



IMMUNITY SCHEDULE

SCHEDULE

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SESSION 2		
10.30 – 10.45	**De novo designed receptor transmembrane domains enhance car-t cell cytotoxicity and attenuate cytokine release** Nicholas Chandler	28
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12.00 - 12.30	**The emerging role of growth factors in cancer immune surveillance** **Keynote Speaker:** Dr. Alexander Barrow	
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Infection and Immunity

Immunity
Keynote Speakers

Professor JoAnne L. Flynn
Department of Microbiology and Molecular Genetics
University of Pittsburgh School of Medicine

Session 1 09.30 - 10.00 am

JoAnne Flynn has a Bachelor of Science in Biochemistry, from the University of California at Davis and a PhD from University of California at Berkeley in Microbiology and Immunology. Dr. Flynn's first post-doc was with Dr. Magdalene So at the Scripps Clinic Research Institute and then joined the lab of Dr. Barry Bloom at Albert Einstein College of Medicine, as a Howard Hughes Research Associate, where she began her studies in tuberculosis. Dr. Flynn joined the Department of Microbiology and Molecular Genetics at the University of Pittsburgh School of Medicine in 1994 and in 2019 was been awarded the title of Distinguished Professor. Dr. Flynn directs a NIH T32 Training Grant and has grants from NIH and the Bill and Melinda Gates Foundation. She was an Editor at Infection and Immunity from 2004-2014 and is a Section Editor for PLOS Pathogens and a member of the NIAID Board of Scientific Counselors. She was the 2018-19 President of the American Association of Immunologists, and is a Fellow of the American Academy of Microbiologists. She has published over 180 papers. Her research in tuberculosis is focused on immunology, host-pathogen interactions, vaccines, and drugs, and she has developed and used non-human primate models for tuberculosis research over the past 20 years. Dr. Flynn's research uses multiple cutting-edge tools and technologies to investigate the complexities of infection, with a particular focus on lung and lymph node granulomas.

Prevention of tuberculosis in nonhuman primates following intravenous BCG immunization.
Darrah PA, Zeppa JJ, Maiello P, Hackney JA, Wadsworth MH, Hughes TK, Pokkali S, Swanson PA, Grant NL, Rodgers MA, Kamath M, Causgrove CM, Laddy DJ, Bonavia A, Casimiro D, Lin PL, Klein E, White AG, Scanga CA, Shalek AK, Roederer M, F**lynn JL**, Seder RA. Nature, 2020. Jan;577(7788):95-102. doi: 10.1038/s41586-019-1817-8. Epub 2020 Jan 1. PMID: 31894150

Professor Carola G. Vinuesa
John Curtin School of Medical Research
The Australian National University
Australian Academy of Science
Shanghai Renji Hospital
NHMRC Centre for Research Exellence

Session 2 11.00 - 11.30 am

Professor Vinuesa has defined how antibody production by B cells reflects complex interactions with antigen-specific T follicular helper (TFH) cells within and outside germinal centers (GCs). Her team's discovery of BCL6 function in TFH -cells established that TFH, rather than Th2-cells, provide help for B cells. Vinuesa also discovered that excess TFH-cell numbers or activity causes systemic autoimmunity. This was followed by the identification of "follicular regulatory T (TFR) cells", a unique subset of regulatory T cells (Tregs) that modulates TFH-cell function and prevents the selection of B cells with rogue specificities. These discoveries are now being applied to autoimmunity, vaccination, elimination of HIV reservoirs, transplantation, immunodeficiency, cancer and allergies. Through her research program at the Centre for Personalised Immunology, Vinuesa has uncovered an important role of rare gene variants in human autoimmunity.

Infection and Immunity

Immunity
Keynote Speakers

Dr. Alexander Barrow
The Peter Doherty Institute for Infection and Immunity
The University of Melbourne

Session 2 12.00 - 12.30 pm

Dr Alexander Barrow, PhD (Bristol, UK) performed his post-doctoral training at the University of Cambridge and as a Marie Curie International Fellow in the lab of Marco Colonna at Washington University in St Louis School of Medicine. Alex is interested in how the immune system distinguishes malignant or virus-infected cells from normal healthy cells and much of this work has focused on identifying ligands for orphan receptors expressed by natural killer (NK) cells. Alex was also the first to show that NK cells express the activating NKp44 receptor that can sense growth factors over-expressed by malignant cells to activate the anti-tumour functions of NK cells. He has published in Cell, Nature Immunology, Journal of Clinical Investigation, Blood and Journal of Immunology and has contributed review articles for Current Opinion in Immunology, Immunological Reviews, Seminars in Immunology and Frontiers in Immunology. He has 26 papers and over 2400 citations and is a member of the international Siglec Nomenclature Group and peer reviewer for the Reactome database. Alex is currently an associate editor for Frontiers in Immunology and an editor for Pathogens. He is currently a mid-career researcher and newly appointed lab head and senior lecturer in immune-oncology in the Department of Microbiology and Immunology, University of Melbourne at the Peter Doherty Institute for Infection and Immunity. Alex is currently the lead chief investigator on a three-year MRFF Research Acceleration project grant to study the role of NK cell 'growth factor immune surveillance' in brain cancer.

Natural Killer Cells Control Tumor Growth by Sensing a Growth Factor.
Barrow AD, Edeling MA, Trifonov V, Luo J, Goyal P, Bohl B, Bando JK, Kim AH, Walker J, Andahazy M, Bugatti M, Melocchi L, Vermi W, Fremont DH, Cox S, Cella M, Schmedt C, Colonna M. C*ell.* 2018 Jan 25;172(3):534-548.e19. doi: 10.1016/j.cell.2017.11.037.

Professor Ines Swoboda
FH Campus Wien
University of Applied Sciences
Biotechnology Section
Campus Vienna Biocentre

Session 3 6.30 - 7.00 pm

Ines Swoboda graduated in Genetics in 1990 and obtained her PhD in 1995 at the University of Vienna. She was postdoctoral research fellow at the University of Melbourne (1995-1998) and at the Medical University of Vienna (1999-2003). In 2004 she received her Habilitation in Allergology. Her Habilitation thesis was awarded with the Kardinal-Innitzer-Prize for Human and Veterinary Medicine. In 2005 she established the research group of "Immune recognition" and in 2010 became Assistant Professor at the Medical University of Vienna. In 2011 she moved to the FH Campus Wien, where she is currently Professor of Immunology, teaching the subjects Virology, Immunology, Allergies & Autoimmune Diseases and Infection Biology to Molecular Biotechnology students. At the FH Campus Wien she established the research group of "Immunology" and since 2017 she is also head of the "Competence Center of Molecular Biotechnology". In 2019 she was listed by the "ZukunftInstitut" as one of six "Future People" of the year. After initial research in the field of Plant Molecular Biology studying the biological function of allergens, Ines Swoboda has been working in the field of Molecular Allergology for more than 20 years. Her research focuses on the analysis of the pathomechanisms of allergic diseases as well as the characterization of the disease eliciting molecules and the development of recombinant allergens for specific allergy diagnosis and for safe and efficient therapy of allergic patients. Ines Swoboda is author on more than 130 scientific publications and is often invited as speaker at national and international conferences.

Gal d 7 - the major allergen in primary chicken meat allergy
Klug, C., Hemmer, W., Román-Carrasco, P., Focke-Tejkl, M., Quirce S., Boyano-Martínez, T., Gaubitzer, E., Wank, H. and **Swoboda, I.** (2020). Journal of Allergy and Clinical Immunology, 146(1): 169-179.

Serum IgA inhibits HIV-specific broadly neutralising antibody Fc functions

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[2]Melbourne Sexual Health Centre, Department of Infectious Diseases, Central Clinical School, Monash University, Melbourne, Victoria
[3]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, University of Melbourne, Melbourne, Victoria

Introduction: Macaque passive transfer studies of HIV-broadly neutralizing antibodies (BnAbs) suggest a vital role of Fc functions in protection. The importance of antibody Fc functions were highlighted in the human HIV RV144 vaccine trial, however, serum IgA reduced vaccine efficacy and protective Fc functions. Serum IgA can influence Fc effector cell functions, such as phagocytosis, via FcαR. When in complex with antigen, serum IgA can induce Fc functions, however, free IgA can inhibit Fc functions. Elucidating how serum IgA modulates Fc responses is essential. Here we endeavour to determine if serum IgA influences the Fc capacity of IgG from people living with HIV or BnAbs.

Methods: Pooled purified IgG from HIV individuals (HIVIG) along with a panel of HIV BnAbs including PGT121 and VRC01, currently in human clinical trials, were assessed for their Fc functional capacity. The influence of IgA upon IgG was assessed by adding pooled HIV-specific IgA (n=10), pooled HIV-negative IgA (n=10), IgA1 and IgA2.

Results: HIV-specific IgA showed minor inhibition of phagocytosis (median=10.38%, IQR=8.09%, $p>0.05$). Intriguingly, significant inhibition was observed when HIV-negative IgA was added (median=21.24%, IQR=14.28%, $p<0.001$). Similarly, significant inhibition was observed with IgA1 (median=23.11%, IQR=18.18%, $p<0.001$) and IgA2 (median=19.88%, IQR=4.60%, $p<0.001$) when added to HIVIG and BnAbs. Addition of FcαR block to these assays was capable of reconstituting Fc functions, suggesting that IgA inhibition is mediated through IgA-FcαR binding.

Conclusion: HIV-negative serum IgA, and to a lesser extent HIV-positive IgA, reduced the functional capacity of HIVIG and BnAbs, suggesting IgA may inhibit through IgA-FcαR mediated inhibitory mechanisms. Understanding the mechanisms behind why IgA inhibits Fc responses could lead to improved future HIV vaccine design and educate passive transfer monoclonal antibody therapies. Elucidating the extent of IgA inhibition of Fc functions of different BnAbs will help inform tailor-made passive transfer treatments for HIV prevention, control and cure.

Intestinal immune response to acute and chronic viral infection

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Chronic viruses, such as HIV, represent major global health issues. The large intestine (LI) is a major site of infection for this virus, and a reservoir for immune cells that are important for the elimination of virally infected cells (e.g. T cells). However, little is known about the dynamics and roles of T cells in the LI compared to the small intestine (SI) during immune responses. Tissue-resident memory T cells (TRM) form and permanently reside in non-lymphoid tissues post-clearance of infection, providing accelerated protective immunity against secondary infections. In the SI epithelium, most TRM are CD8+CD103+CD69+ and their formation requires TGF-ß signalling. We used the acute and chronic strains of Lymphocytic Choriomeningitis Virus (LCMV) to characterise and compare T cell kinetics in the SI and LI during acute and chronic LCMV infection. After acute infection, few LCMV-specific TRM formed in the LI due to differential responses to TGF-ß and regulation of markers such as CD69, CD103 and P2XR7. Additionally, there was a large influx of regulatory T cells during chronic infection in the SI that was not seen in the LI. As Tregs are a key contributor to the establishment of chronic viral infection, we are now investigating the implication of Tregs during chronic infection in both gut compartments. These studies will contribute to a greater understanding of the development of T cell memory in the small and large intestine, and how this impacts the immune response to acute and chronic viral infection.

Guiding rational antibody engineering using graph-based signatures

Yoochan Myung[1,2], Carlos, H. Rodrigues[1,2], Douglas, E. V. Pires[1,3] & David, B. Ascher[1,2]

[1]Structural Computational Biology and Clinical Informatics, Baker Heart and Diabetes Institute, Melbourne, VIC 3004, Australia
[2]Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Victoria 3010, Australia
[3]School of Computing and Information Systems, University of Melbourne, Melbourne, VIC 3010, Australia

Introduction: Antibodies are major therapeutic and diagnostic agents for various diseases. While there have been improvements in conventional antibody engineering techniques, many bottlenecks still remain such as in high-throughput mutagenesis screening. The recent rapid growth of machine learning algorithms and massive public databases enabled better machine-learning based models to be built for the purpose of studying the effects of mutations on the antibody-antigen binding affinity and specificity. More specifically, the existing computational approaches mostly focused on single-point mutations but replacing individual residues may not be enough to yield viable affinity changes and may lead to unwanted physicochemical property changes. There have been many efforts in developing in silico tools to guide rational antibody engineering, but most approaches are inaccurate when applied to antibody design, and have largely been limited by analysing single point mutations at a time.

Methods: Using a novel way to computationally encode the antibody-antigen interaction interface, using graph-based signature, we developed a highly accurate tool for analysing the consequence of point mutations on antigen binding affinity.

Results: In terms of predicting binding affinity changes upon single point mutations, the model outperformed available tools showing a Pearson's correlation of 0.73 on 10-fold cross validation, 0.64 on experimental blind tests and 0.77 on homology model blind tests. To overcome the gap in understanding whether single point mutation datasets can be used to predict binding affinity changes of multiple mutations, we curated a dataset of 334 mutations in antibodies with experimentally determined changes in binding affinity (100 stabilising, 200 destabilising). Our approach outperformed other available tools on both 116 double/triple mutation dataset and 334 constructs showing Pearson and Spearman correlations of 0.90 and 0.81 and 0.73 and 0.56, respectively.

Conclusion: We have implemented our new approaches as web-servers that enable rapid and deep evaluations of specific mutations or systematic exploration of all possible combinations of a single or a set of double and triple mutations across antibody-antigen interface residues. mCSM-AB2 and mmCSM-AB will help to guide rational antibody engineering by analysing the effects of introducing mutations. These user-friendly web-servers are freely available at http://biosig.unimelb.edu.au/mcsm_ab2 and http://biosig.unimelb.edu.au/mmcsm_ab.

Using Polyhydroxyalkanoate Beads as Vaccine Delivery Carriers

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[2]Center for Cell Factories and Biopolymers, Griffith Institute for Drug Discovery, Griffith University, Brisbane, QLD, Australia

Introduction: Nanoparticles have been shown to be an effective vaccine carrier capable of inducing immunity against infectious pathogens and cancer. Polyhydroxyalkanoate (PHA) beads are inclusions formed by many archaea and bacteria that function as a carbon reserve used during starvation. PHA beads can be genetically engineered to express a model antigen, ovalbumin (OVA), on their surface. Here, we tested the immunogenicity of OVA-PHA beads in assays of T cell immunity and vaccination against cancer.

Methods: Antigen presentation assays involve labelling OVA-specific OT-I and OT-II T cells with cell trace violet (CTV) that can be used to track the division of cells. CTV OT-I and OT-II are injected into mice and following intravenous or subcutaneous vaccination with OVA- PHA beads, their division is analysed by flow cytometry. In cytotoxic T cell assays, mice are vaccinated with OVA-PHA beads and injected with OVA+ target cells to analyse endogenous cytotoxic T cells response. For tumor models, mice were therapeutically vaccinated prior to inoculation of tumour and tumour growth analysed.

Results: OVA-PHA beads were capable of inducing antigen-specific proliferation of OVA-specific OT-I and OT-II cells in vivo following intravenous and subcutaneous vaccination. OVA-PHA beads were also capable of inducing endogenous T cell responses, and could elicit anti-OVA cytotoxic T cells (CTL). Further investigation revealed that the CTL response elicited by PHA beads combined with an adjuvant could enhance CTL responses. To assess the ability of OVA-PHA to be used as vaccines against cancer, we inoculated tested their immunogenicity in two tumour models: OVA-expressing B16 melanoma and E□-myc-OVA lymphoma. In both models, OVA-PHA bead vaccination significantly reduced the tumor burden compared to mice that received no vaccination.

Conclusion: Overall, these findings suggest that PHA beads are an effective vaccine carrier that can elicity T cell immunity and anti-tumor responses.

De novo designed receptor transmembrane domains enhance car-t cell cytotoxicity and attenuate cytokine release

Nicholas J. Chandler[1,3], Assaf Elazar[1] , Ashleigh S. Davey[1,3], Jonathan Y. Weinstein[4], Julie V. Nguyen[1], Raphael Trenker[1,3], Misty R. Jenkins[2,3,5], Melissa J. Call[1,3], Matthew E. Call[1,3] & Sarel J. Fleishman[4]

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[2]Immunology Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia
[3]Department of Medical Biology, The University of Melbourne, Parkville, Victoria 3052, Australia
[4]Department of Biomolecular Sciences, Weizmann Institute of Science, Rehovot, Israel.
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Introduction: Chimeric antigen receptor (CAR) T cell therapy has revolutionized the treatment of B cell malignancies by redirecting patient T cells to destroy cancer cells using engineered receptors. However, CAR therapies carry significant risk of inducing cytokine release syndrome (CRS), a potentially deadly toxicity caused by excessive release of inflammatory cytokines. The ability to minimize CRS toxicity whilst guaranteeing adequate tumour cell-killing is therefore vital to the continued improvement of CAR therapies. We aimed to investigate the currently undefined relationship between CAR oligomeric state and potency, with the aim of leveraging this knowledge to predictably modulate CAR activity.

Methods: Alongside de-novo protein design collaborators we generated synthetic transmembrane domain (TMD) sequences that predictably formed homo-oligomeric structures. X-ray protein crystallography was used to determine the structures of these domains, to confirm they were forming their predicted oligomeric state. Structurally validated TMD sequences were then inserted into a well-established anti-HER2 CAR construct (comprised of an anti-HER2 scFv attached via stalk/transmembrane domains to a stimulatory tail sequence) and tested in a mouse T cell line and primary mouse T cells.

Results: Atomic structures of dimeric and trimeric TMD peptides were determined and agreed closely with their predicted structures. These oligomeric TMDs facilitated expression in the context of a HER2-specific CAR construct expressed in a mouse T cell line and triggered signalling in response to HER2+ target cells. When expressed in primary CD8+ mouse T cells and incubated with HER2+ target cells, dimeric and trimeric CARs exhibited enhanced target cell killing compared to a reference anti-HER2 CAR and remarkably also expressed dramatically reduced levels of inflammatory, CRS-associated cytokines.

Conclusions: We optimised a de novo protein design pipeline to generate highly accurate and tuneable homo-oligomeric TMD sequences capable of enhancing CAR T cell cytotoxicity and dramatically reducing secretion of CRS-associated inflammatory cytokines. These findings present an opportunity to immediately improve efficacy and safety of CAR T cell therapies and warrant further validation in in vivo mouse tumour models.

Single cell analysis of Alpha/Beta versus Gamma/Delta T cell development

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Introduction: T cells develop in the thymus, where they acquire their functional identities via a hierarchy of genetic programs that regulate dynamic transcriptional changes. Studies over the years have defined, at a population level, many of the specific genes that have to be activated or silenced as T cell progenitors differentiate toward either the αβ (alphabeta) or γd (gammadelta) lineages. However, it is still unknown whether the combination of silencing/activation of gene regulators and lineage specification is indeed a step-wise process occurring in every cell as they differentiate. In addition, the precise stage at which the αβ versus γd lineage decision is made remains contentious. To investigate this, single cell RNA sequencing (scRNAseq) technology has been employed to re-assemble de novo a model of the early stage in T cell development based on the transcriptional profiles of individual cells.

Methods: The experimental setup involved harvesting thymocytes from four C57BL/6 mice at 6 weeks of age. The pooled thymocytes population was then depleted of late stages αβ thymocytes (CD4+CD8+ double positive cells and later) on MACS magnetic bead columns. This resulted in an enriched pool of CD4-CD8- double negative (DN) plus γd thymocytes. These were then FACS sorted based on low cell surface CD4, CD8 but high CD90 expression to obtain a pure pool of DN plus γd thymocytes. 10,000 purified thymocytes were then loaded onto a Chromium 10x Genomics chip for the construction of scRNAseq libraries.

Results: From scRNAseq 5,527 high quality transcriptomes were obtained. Hierarchical clustering identified 19 sub-populations within the sample. This is much more complex than standard view of early T cell development, which implies only 4 DN stages plus γd thymocytes. Assembly of these novel thymocyte populations into developmental trajectories based on gene expression revealed key findings. It indicated that the decision to differentiate into αβ or γd T cells occurs at a much earlier stage than the current model and that there are distinct DN2 sub-populations and potentially DN1 that are specific to only one of the two developmental pathways. Analysis in the OP9-DL1 cultures confirmed that specific sub-populations of DN1s and DN2s differentiated into only αβ or γd thymocytes.

Conclusion: A comprehensive understanding of T cell development has important health and medical implications. It has the potential power to facilitate the development of novel immune based therapies, and for understanding and intervening in conditions caused by impaired T cell development, including immunodeficiency and autoimmunity.

MAIT cells expand in the absence of NKT and gamma-delta T cells

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Introduction: Unconventional T cells, namely MAIT, NKT, and gamma-delta T cells (gdT cells), recognise non-peptide antigens presented by non-classical MHC-like molecules, and can rapidly mount robust cytokine responses after activation, leading to their implication in modulating the host immune response to disease. In particular, MAIT cells recognise riboflavin biosynthesis metabolites, such as 5-OP-RU, presented by MR1, and utilise a semi-invariant TCR comprising a Trav1-Traj33+ TCRalpha chain that pairs with a limited range of TCRbeta chains. Despite their relative abundance, MAIT cell frequencies vary widely between individuals, ranging from 0.1-10% of total blood T cells in humans, the cause of which is understudied. Recent evidence has suggested that MAIT and other unconventional T cells may co-exist in the body within a shared developmental or homeostatic niche and are regulated by similar genetic and/or environmental factors.

Methods: Using fluorophore-conjugated tetramers of MR1 loaded with 5-OP-RU in addition to a panel of fluorophore-conjugated monoclonal antibodies, we characterised the frequency and phenotype of MAIT cells in CD1d-deficient and TCRdelta-deficient mice, which lack CD1d-restricted NKT cells and gdT cells, respectively, and in CD1d/TCRdelta-doubly deficient mice using flow cytometry. Additionally, we measured the relative abundance of Trav1-Traj33 mRNA transcripts within developing thymocytes in the aforementioned mice using qPCR.

Results: We confirm earlier findings that CD1d-deficient mice have increased MAIT cells and show that this is due to the loss of NKT cells rather than CD1d itself. Likewise, MAIT cells are also markedly increased in TCRdelta-deficient mice, and further expand in CD1d/TCRdelta-doubly deficient mice. Expanded MAIT cells phenotypically and functionally resemble their WT counterparts. Accordingly, we hypothesise that MAIT cells may compete with NKT and gdT cells for similar factors and subsequently expand in their absence. As increased MAIT cells were also observed in the thymus, we sought to investigate Trav1-Traj33 rearrangements and found that they were ~2X more abundant within developing thymocytes from TCRdelta- and CD1d/TCRdelta-doubly deficient mice. Consequently, we hypothesise that modification of the TCRdelta locus to create TCRdelta-deficient mice may have affected TCRalpha rearrangement during T cell development, in particular resulting in greater usage of distal Trav gene segments such as Trav1.

Conclusion: Thus, in addition to providing insight into factors that affect MAIT cell frequencies, our work also sheds light on previously unappreciated alterations in TCRalpha chain rearrangements in TCRdelta-deficient mice and cautions the use of CD1d- and TCRdelta-deficient mice for studying the role of NKT and gdT cells in disease, respectively.

CD1a-restricted T cells: unique “unconventional” T cells in allergy

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Introduction: Allergic contact dermatitis (ADC) is a common inflammatory skin disease mediated by T cells. Sunscreens protect against sun damage but can cause ADC. A group of “unconventional” T cells recognise lipid antigens presented by CD1 molecules. The most widely studied are CD1d-restricted, natural killer T (NKT) cells, which play an important role in bridging innate and adaptive immunity. Another subset, CD1a-restricted T cells, represent up to 10% of T cells yet remain an enigma in comparison. We therefore aimed to characterise this important immune population. Recent studies suggest that they play a role in skin immunity. Given the abundance of lipids in sunscreens and their similarity to known CD1a antigens (e.g. lipids in poison ivy), we hypothesised that they may also activate CD1a-restricted T cells.

Methods: CD1a tetramers were produced to isolate CD1a-restricted T cells in healthy blood donors using flow cytometry. Single cell RNA-sequencing was performed to compare CD1a-restricted T cells to other T cell subsets at a transcriptomic level. CD1a-restricted T cell lines were also generated for in vitro activation and tetramer binding assays.

Results: Populations of CD1a-restricted T cells that recognise endogenous, self-lipids were isolated from 52 donors. Transcriptomic analysis of these cells revealed that they do not express innate-like T cell markers similar to NKT cells, thereby distinguishing them as a unique population of unconventional T cells. We identified alkyl benzoate as a potential CD1a allergen commonly found in dermatological products. To determine whether it activates CD1a-restricted T cells, cell lines were co-cultured with CD1a-expressing antigen presenting cells with and without alkyl benzoate. Activation levels were correlated with increasing concentrations of alkyl benzoate. This was supported by enhanced tetramer labelling of the same cell lines with CD1a tetramers loaded with alkyl benzoate. As alkyl benzoate may be presented directly or metabolised into a different lipid, CD1a molecules were produced in a human expression cell line in the presence of alkyl benzoate. Mass spectrometry confirmed the presence of endogenous CD1a lipid antigens captured by CD1a produced normally. In contrast, endogenous lipids were notably absent from CD1a produced in the presence of alkyl benzoate, which appeared to be loaded preferentially with alkyl benzoate.

Conclusion: The results indicate that alkyl benzoate displaces endogenous lipid antigens and is presented to CD1a-restricted T cells, a unique population of lipid-reactive T cells. This work represents a step forward in understanding the role of CD1a-restricted T cells in ADC to skincare products.

Conventional type 1 dendritic cells present native antigen to B cells for generation of T-dependent humoral immunity

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Targeting antigen (Ag) to dendritic cells (DC) is a novel immunisation strategy of strong potential for vaccine development. DC are known to support antibody responses by priming T follicular helper cells (TFH), which support B cell proliferation and differentiation into antibody-secreting cells. However, there is also clear evidence that DC can present native Ag to B cells, which has been linked to their capacity to display Ag on their surface. Targeting Ag to Clec9A on conventional type 1 DC (cDC1) has been long known to induce potent humoral responses even in the absence of adjuvants. Clec9A is almost exclusively expressed by cDC1 and is conserved in mice, non-human primates and humans, which makes it a targeted to be exploited for vaccination. Interestingly, we observed that Ag targeted to Clec9A was retained in its native form on the surface of cDC1, which allowed for a direct interaction with B cells for native Ag presentation. This enabled efficient B cell activation and migration to the T/B border, which was essential for effective humoral immunity. Surprisingly, display of native Ag on Clec9A was redundant for B cells Ag capture, but was critical for enhanced Ag processing and presentation. These findings suggest that cDC1 can mediate humoral responses and that the role of individual DC subsets may be dictated by their ability to display the tested antigen. Targeting Ag to Clec9A on cDC1 represents an efficient mechanism for B cell activation and generation of humoral immunity, which can be exploited by novel vaccination approaches.

MHC II ubiquitination is required for dendritic cell function

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Introduction: Major histocompatibility complex class II (MHC II) is an antigen presenting molecule found on professional antigen presenting cells, such as dendritic cells (DCs). It plays a critical role in initiating adaptive immune responses by presenting antigen peptide to CD4+ T cells. The cell surface expression of MHC II is regulated by ubiquitination, with elevated MHC II expression observed in cells that lack MHC II ubiquitination. We have investigated the impact of MHC II ubiquitination on immunity using MHCIIKRKI/KI mice that express mutant MHC II molecules unable to be ubiquitinated.

Methods: To investigate immune cells in vivo, splenic immune cells were isolated from wild type and MHCIIKRKI/KI mice and analysed by flow cytometry. The number of DCs was quantitated and their cell surface phenotype was characterised. To investigate the impact of the MHC II ubiquitination on DC function, splenic DC were isolated and analysed ex vivo. Antigen uptake was assessed using Ovalbumin-Cy5 and endosomal pH was determined using the pH sensitive probe, pHrodo. Antigen degradation was also determined using DQ-Ovalbumin, which increases fluorescence as it is degraded. We determined how a lack of MHC II ubiquitination impacts immune outcomes by immunising mice with soluble, cell-associated and/or DC-targeted ovalbumin via mAb specific for DC surface receptor Clec9A (anti-Clec9a-OVA) and quantitating CD4+ and CD8+ T cell priming. Finally, we have investigated the impact on antibody production in response to anti-Clec9A mAb-targeted antigen.

Results: Surprisingly, the number of conventional DC (cDC)1, cDC2 and plasmacytoid (pDC) were significantly reduced in MHCIIKRKI/KI spleen. The remaining MHCIIKRKI/KI DC expressed an altered surface phenotype and function. While no changes to antigen uptake or endosomal pH were observed, the MHCIIKRKI/KI DC had significant defects in antigen proteolysis. Immunisation of MHCIIKRKI/KI mice revealed significant defects in MHC II and MHC I presentation and failed to elicit significant antibody production in response to anti-Clec9A mAb-targeted antigen

Conclusion: MHC II ubiquitination in DCs impacts the homeostasis, phenotype and antigen proteolysis by DC with significant consequences for antigen presentation, T cell and antibody-mediated immunity.

The JAK1 selective inhibitor ABT 317 blocks signaling through interferon and common gamma chain cytokine receptors to reverse autoimmune diabetes in NOD mice

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Cytokines that signal through the JAK-STAT pathway, such as interferon-gamma (IFN-g) and common gamma chain cytokines, contribute to the destruction of insulin-secreting beta cells by CD8+ T cells in type 1 diabetes (T1D). We previously showed that JAK1/JAK2 inhibitors reversed autoimmune insulitis in non-obese diabetic (NOD) mice and also blocked IFN-g mediated MHC class I upregulation on beta cells. Blocking interferons on their own does not prevent diabetes in knockout NOD mice, so we tested whether JAK inhibitor action on signaling downstream of common gamma chain cytokines, including IL-2, IL-7 IL-15 and IL-21, may also affect the progression of diabetes in NOD mice. Common gamma chain cytokines activate JAK1 and JAK3 to regulate T cell proliferation. We used a JAK1-selective inhibitor, ABT 317, to better understand the specific role of JAK1 signaling in autoimmune diabetes. ABT 317 reduced IL-21, IL-2 and IL-7 signaling in T cells and IFN-g signaling in beta cells, but ABT 317 did not affect GM-CSF signaling in granulocytes. When given in vivo to NOD mice, ABT 317 reduced CD8+ T cell proliferation as well as the number of KLRG+ effector and CD44hiCD62Llo effector memory CD8+ T cells in islets and spleen. ABT 317 also prevented MHC class I upregulation on cells. Newly diagnosed diabetes was reversed in 94% NOD mice treated twice daily with ABT 317. Our results indicate that ABT 317 blocks common gamma chain cytokines in lymphocytes and interferons in lymphocytes and cells and are thus more effective against diabetes pathogenesis than IFN-g receptor deficiency alone. Our studies suggest use of this class of drug for the treatment of type 1 diabetes.

In vivo expansion of Vδ2+Vγ9+ T-cells following treatment with phosphoantigens or bisphosphonates in nonhuman primates

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Introduction: Vdelta2+Vgamma9+ T-cells are a subset of unconventional T-cells which recognise endogenous and exogenous phosphoantigens, and have garnered significant interest as immunotherapeutics against cancers and infectious diseases. While preclinical studies have yielded promising results, clinical efficacy of gamma-delta T-cell immunotherapeutics is predominately lacking. Improvements in gamma-delta T-cell therapies will likely require refinements to the pharmacological expansion of gamma-delta T-cells to generate a robust cell product that can; 1) persist for extended periods of time in vivo, 2) traffic to the target site of interest, and 3) mediate direct or indirect cytolysis of target cells.

Methods: In this study, we investigated the impact of different antigenic stimuli and routes of administration on Vdelta2+Vgamma9+ T-cell expansion in nonhuman primates. Adult male pigtail macaques were treated with intravenous (IV) or intratracheal (IT) zoledronate (n=2 per group), the microbial phosphoantigen HMB-PP (IV and IT; n=2), or the endogenous phosphoantigen IPP (IT; n=1), along with recombinant human IL-2 to induce in vivo expansion of Vdelta2+Vgamma9+ T-cells. Vdelta2+Vgamma9+ T-cell frequencies and phenotypes were evaluated in the peripheral blood, peripheral lymph nodes, and mucosal tissues (bronchoalveolar lavage fluid [BAL] and rectal mucosa) via flow cytometry and compared to paired baseline samples.

Results: Vdelta2+Vgamma9+ T-cells readily expanded in the peripheral blood in macaques treated with systemic zoledronate or HMB-PP, peaking at day 4 post-antigen administration (12.2-28.1% of CD3+ T-cells versus 0.7-2.9% at baseline), and returning to baseline levels by day 8. Vdelta2+Vgamma9+ T-cell frequencies were increased in the BAL fluid of macaques treated with IV and IT antigens at day 8 post-administration (3.7-16.8% of CD3+ T-cells versus 2.6-7.9% at baseline). Vdelta2+Vgamme9+ T-cell expansion coincided with increased CD69 and granzyme B expression in both the peripheral blood and BAL. Expanded peripheral blood Vdelta2+Vgamma9+ T-cells were predominately CCR6- (22.4-45.1% CCR6+ at day 4 versus 82.5-92.6% at baseline), although preferential expansion of CCR6- Vdelta2+Vgamma9+ T-cells was not observed in the BAL.

Conclusion: Phosphoantigen reactive Vdelta2+Vgamma9+ T-cells readily expand in the blood and BAL of pigtail macaques following treatment with phosphoantigens. Furthermore, phosphoantigen and bisphosphonate mediated expansion with IL-2 preferentially expands CCR6-Vdelta2+Vgamma9+ T-cells, which may reflect a specific subset of the phosphoantigen reactive T-cell population. Future work will identify the influence of different stimuli on activation, expansion, and function of different Vdelta2+Vgamma9+ T-cell subsets in macaques. This work has implications for improved gamma-delta T-cell immunotherapeutic efficacy, with potential applications for both infectious and neoplastic diseases.

Defining immune cell interactions in the spleen using single-cell spatial transcriptomics

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Introduction: Single-cell transcriptomics is gaining prominence as a discovery tool in cellular biology, including the immune system. We previously applied single cell RNA-seq (scRNA-seq) to study a specific immune cell-type, CD4+ T cells, during infection with malaria parasites. To develop a complete understanding of immune responses during malaria, we sought to expand our analysis to include other immune cell-types such as CD8+ T cells, B cells, and NK cells. Importantly, however, scRNA-seq does not capture cells' spatial context. Hence, we sought an approach allowing simultaneous assessment of transcriptomes and spatial localization within tissue.

Methods: We ran droplet-based scRNA-seq on splenic immune cells from C57BL/6J mice before or 7 days after infection with Plasmodium chabaudi parasites. Resulting data was analysed through existing bioinformatic and computational pipelines to determine at genome-scale the effect of infection on multiple cell-types. Next, we applied a recent method, Slide-seq v2, a spatial transcriptomics platform, firstly on a proof of concept samples from the mouse gut, and then using spleens from malaria-infected and naïve mice. Multiple computational tools were applied to examine cellular structure using unsupervised analysis of transcriptomes.

Results: Droplet-based scRNA-seq analysis revealed expected transcriptomic change among T cells, with specific subsets of activated CD4+ T helper and cytotoxic CD8+ T cells emerging by day 7 of infection. In addition to emergence of plasmablasts and activated B cells, we noted previously unreported global transcriptomic changes in all B cells. This suggested that infection elicited substantial change in B-cell follicles. To examine these structures, and possible interaction with T cells, we used our Slide-seq v2 dataset with several computational tools. We found that Slide-seq produced interpretable transcriptomic data that was amenable to common scRNA-seq analyses such as dimensionality reduction, unbiased clustering, and imputation of poorly captured genes. We next confirmed the presence of immune structures in the spleen, including T and B cell zones and red pulp, by inferring cell types for single cell spatial transcriptomes. Our analysis also identified erythrocyte and stromal cell transcriptomes, which is typically challenging for droplet-based scRNA-seq technologies. Finally, we noted that splenic structure changed substantially during infection, and that this change was identifiable from the data.

Conclusion: Our preliminary results show that emerging spatial transcriptomics platforms and analysis techniques can map biologically relevant microanatomical and transcriptomic features, as well as changes in response to infection.

Antigen dose and inflammation influences the size, composition, and quality of the influenza vaccine-induced pulmonary CD8+ T cell response

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Influenza is a highly pathogenic respiratory pathogen that inflicts an estimated 500,000 deaths on an annual basis. While current antibody-based influenza vaccines exist, these are strain-specific and thus fail to provide long-term cross-protection against seasonal as well as newly emerging pandemic strains. Vaccines that elicit CD8+ T cell immunity can provide this cross-protection. Tissue-resident memory CD8+ T cells (TRM) are of particular interest as they permanently reside in non-lymphoid tissues and provide immediate local protection. Although current vaccines can generate T cell responses, these are variable and vaccine-induced TRM responses are understudied. Here, we explore how an influenza T cell vaccine that is limited to a single round of replication called S-FLU influences the size, composition, and function of the vaccine-induced CD8+ T cell and TRM response. We report that S-FLU immunisation in mice resulted in a reduction in the size, quality, and sensitivity of the CD8+ T cell response in comparison to a natural infection, which was a consequence of reduced inflammation and antigen dose. However, by giving a prime-boost immunisation regime of S-FLU involving additional antigen and inflammation, the size and quality of the local CD8+ T cell response was boosted, which in turn, resulted in potent protection following a heterologous influenza challenge. These findings highlight a novel influenza vaccine and how the role of local inflammation and antigen conditions the CD8+ T cell response in terms of size and functional capacity, and thus informs the design for optimal vaccine-induced CD8+ T cell responses.

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