Victorian Infection & Immunity Network

Young Investigator Symposium

Wednesday 16 November 2022
Monash Institute of Pharmaceutical Sciences

www.viin.org.au
#viinyis, #YoungVIIN2022

Welcome to the 2022 VIIN Young Investigator Symposium

Dear Colleagues and Friends,

On behalf of the members of the Victorian Infection and Immunity Network's Executive Committee, we welcome you to the 2022 Young Investigator Symposium.

Special welcome to our keynote speakers: Prof Dena Lyras of Monash University and Associate Professor Catherine Satzke of the Murdoch Children's Research Institute.

This is the 15th year that the VIIN has convened a symposium for young investigators. We are delighted to be seeing you all in-person again and remind you to be vigilant about remaining COVID-safe by wearing your mask and sanitising regularly.

We are indebted to many who have been vital to bringing the 2022 meeting about, namely:

- The Symposium Organising Committee, who have worked tirelessly to review abstracts and organise the many logistical elements of the day:

Catarina Almeida, University of Melbourne
Aaron Brice, Australian Centre for Disease Preparedness, CSIRO
Carlo Giannangelo, Monash Institute of Pharmaceutical Sciences
Ella Johnston, La Trobe University
Gabriela Khoury, Monash University
Kevin Lee, University of Melbourne
San Lim, Hudson Institute of Medical Research
Rhea Longley, Walter and Eliza Hall Institute
Christophe Macri, University of Melbourne
Joyanta Modak, Deakin University
Melanie Neeland, Murdoch Children's Research Institute
Ursula Norman, Monash University
Jason Paxman, La Trobe University
Linda Reiling, Burnet Institute
Stephen Scally, Walter and Eliza Hall Institute
Rebecca Smith, Victorian Infection & Immunity Network
Jinxin Zhao, Monash University

- The 50+ Session chairs and judges for oral presentations, Science Bites and posters, which is a reflection of the ongoing popularity of this event. Thanks to each for your time and expertise.
- The sponsors and advertisers for this symposium. Your support is more and more important to the success of this event.
- The 15 Academic Institutions and government agencies that support VIIN annually through financial contributions. Without your support, our activities would cease.

Finally, thank you to everyone who is here as a delegate or to present a talk or poster. This meeting is for you. We always appreciate your participation and look forward to hearing your talks, Science Bites and posters.

Sincerely,

Prof Heidi Drummer and Prof Richard Ferrero, Co-Convenors of VIIN

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Victorian Infection & Immunity Network

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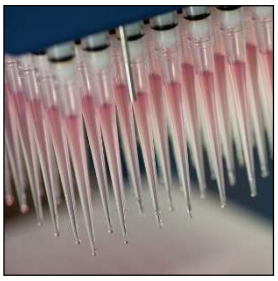
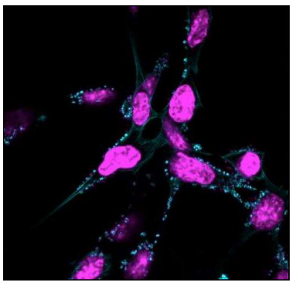
The Victorian Infection and Immunity Network (VIIN) brings together researchers from diverse disciplines across various nodes within Victoria who have an interest in infection and immunity. Through events such as the Lorne Infection and Immunity Conference and annual research symposia, VIIN connects established and emerging infection and immunity researchers with the goal of seeding new collaborations and strengthening research performance and capabilities.


The website at www.viin.org.au contains member profiles, contact details and fields of research, information on relevant news, events, platform technologies, career and funding opportunities.

VIIN Contributors include:

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For enquiries contact: info@viin.org.au

The Hartland Oration at Lorne

Professor Elizabeth Hartland was VIIN Co-Convenor between 2009 and 2017. Professor Hartland had key roles in co-convening the Lorne Infection and Immunity Conferences (2012-2017), the VIIN Industry Alliance (2012-2014) and the VIIN Young Investigator Symposia (2009 – 2016). Together with former Co-Convenor Professor Paul Hertzog, Liz oversaw the roll-out of VIIN's new website, its increasing presence on social media, implementation of the VIIN's annual careers evenings (2013-2017) and numerous other initiatives.

In honour of Liz's contribution to VIIN, the network is delighted to have established the Hartland Oration. This oration will be delivered at the Lorne Infection and Immunity Conference from 2018 onwards. The Hartland Orator will be selected at this VIIN Young Investigator Symposium and will be the post-doctoral researcher giving the best 10 minute oral presentation. This outstanding young researcher will receive free registration to the 2023 Lorne Infection and Immunity Conference and a cash contribution toward their accommodation, meals and transport.

Additional prizes at the VIIN Young Investigator Symposium

As in years past, prizes will also be awarded for:

- Poster prizes
- Science Bites prizes (3 minute oral presentations)
- Best VIIN Young Investigator Symposium PhD talk
- Best question prizes
- People's Choice Awards – stay tuned!

Would you like to join the VIIN Young Investigator Committee in 2023?

You have one week left to apply!

We are seeking expressions of interest from young investigators * who would like to join the VIIN Young Investigator Committee for 2023 for a 2 year term of commitment **.

Membership of the Young Investigator Committee is a prestigious opportunity for EMCRs to take leading roles in the infection and immunity community. By becoming part of the Young Investigator Committee, you are building your curriculum vitae in the areas of committee work, peer review, session chairing and skills relating to management, communication and influencing.

In this role, you will be required to:

- Attend Young Investigator Committee and Sub-Committee meetings (approximately 4 per year) (a mix of face-to-face and virtual mode).
- Participate in peer review for the annual Young Investigator Symposium and contribute to practical organisation of this event (including speaker management, sponsor recruitment, chairing sessions and other tasks).
- Contribute to a sub-committee responsible for the organisation of other workshops or networking activities throughout the year.
- After 12 months of service, a Young Investigator Committee member may be eligible for self-/nomination as the ECR Representative to the VIIN Executive Committee.

To apply, please click here: https://forms.gle/QSwmtLBffbwa9jjw8.

The deadline to apply is Wednesday 23 November, 11.59pm. Please note that these are voluntary roles.

* EMCRs within 10 years of graduation of their PhDs (career disruptions considered) are eligible to self-nominate.

** For more information, please email info@viin.org.au and see here: https://www.viin.org.au/news/expressions-of-interest-to-join-2023-viin-young-investigator-committee-1153 or scan the QR code:

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Program-at-a-Glance 2022

WEDNESDAY 16 NOVEMBER	
08:15 – 08:45	**Registration, Cossar Hall**
08:45 – 08:50	**Welcome and Acknowledgement of Country, Lecture Theatre**
08:50 – 10:40	**Session 1, Lecture Theatre** **Oral Presentations 1: Students and Research Assistants**
10:40 – 11:20	**Morning tea, Cossar Hall** **(includes transit time from and to Lecture Theatre)**
11:20 – 11:50	**Session 2, Lecture Theatre** **Keynote Speaker 1**
11:20	**Exploring the world with the pneumococcus** Associate Professor Catherine Satzke, Group Leader and Principal Research Fellow, Murdoch Children's Research Institute
11:50 – 12:40	**Session 3, Lecture Theatre** **Science Bites 1**
12:40 – 13:45	**Lunch and Poster Session 1, Cossar Hall** **(includes transit time from and to Lecture Theatre)**
13:45 – 14:55	**Session 4, Lecture Theatre** **Oral Presentations 2: Early-career researchers**
14:55 – 15:35	**Afternoon Tea, Cossar Hall** **(includes transit time from and to Lecture Theatre)**
15:35 – 16:05	**Session 5, Lecture Theatre** **Keynote Speaker 2**
15:35	**Gut microbes: from mobile DNA to pathogenesis** Professor Dena Lyras, Deputy Director, Monash Biomedicine Discovery Institute, Monash University
16:05 – 17:00	**Session 6, Lecture Theatre** **Science Bites 2**
17:00 – 18:00	**Evening networking and Poster Session 2, Cossar Hall**
18:00 – 18:15	**Prize-giving, thank yous and conclusion, Cossar Hall**

Notes

Capture and transmission of data: Please do not record or take photographs of any data slide in an oral presentation or Science Bite, or any data on a poster.

Social media: TWITTER: We encourage you to join the conversation on Twitter with #YoungVIIN2022 and #viinyis. Please follow us @The_VIIN where we will be live tweeting symposium highlights. Please remember: no data! Thank you.

Wifi login:
Monash Guest Wifi: https://www.monash.edu/esolutions/network/guest-wifi. Connect by registering your details.

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Please visit the Registration Desk for information about where to collect your special meal.

Storage of posters

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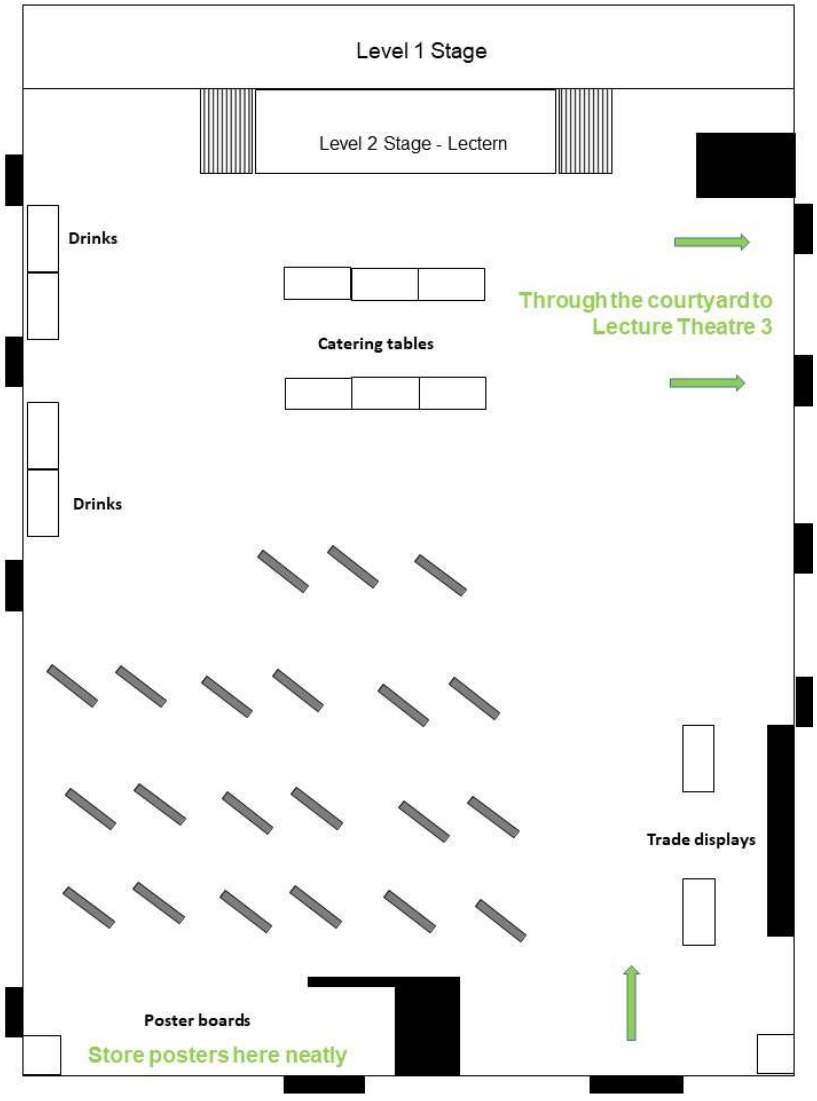

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Wednesday 16 November

Wednesday 16 November: 8.15am – 6.15pm

08:15 – 08:45	**Registration, Cossar Hall**
08:45 – 08:50	**Welcome and Acknowledgement of Country, Lecture Theatre** **Chairs: Rhea Longley, Walter and Eliza Hall Institute and Stephen Scally, Walter and Eliza Hall Institute**
08:50 – 10:40	**Session 1, Lecture Theatre** **Oral Presentations 1: Students and Research Assistants** **Chairs: Rhea Longley, Walter and Eliza Hall Institute and Stephen Scally, Walter and Eliza Hall Institute**
08:50	**Pirfenidone is more effective than glucocorticosteroids in limiting transforming growth factor-beta-enhanced viral infection and inflammation in the lungs** Julia Chitty, Department of Pharmacology, Monash University (PhD)
09:00	**Genetic and chemical validation of *Plasmodium falciparum* aminopeptidase PfA-M17 as an anti-malarial drug target in the haemoglobin digestion pathway** Rebecca Edgar, School of Medicine, Deakin University (PhD)
09:10	**Lower γδ T cell frequency in the colonic mucosa is an immunological signature of Hirschsprung disease** Ashleigh Gould, Drug Discovery Biology, Monash Institute of Pharmaceutical Science, Monash University (PhD)
09:20	**Accurate detection of house dust mite sensitisation in asthma and allergic rhinitis with a single Cytometric Basophil assay (CytoBas)** Lin Hsin, Department of Immunology and Pathology, Monash University (PhD)
09:30	**Using *Anopheles* salivary antibody biomarkers to assess spatial and temporal heterogeneity in vector exposure and malaria transmission in Southeast Myanmar** Ellen Kearney, Life Sciences, Burnet Institute (PhD)
09:40	**Identifying the mechanism of action of a novel invasion-blocking compound in Plasmodium falciparum** Dawson Ling, Malaria Virulence and Drug Discovery Group, Burnet Institute (PhD)
09:50	**Identification of influenza B virus HLA-B*07:02 and HLAB* 08:01 restricted CD8+ T cell epitopes** Tejas Menon, Department of Microbiology and Immunology, University of Melbourne, at the Peter Doherty Institute (PhD)
10:00	**Regulation of tissue-specific TRM cell responses** Grace Rodrigues, Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute (PhD)
10:10	***Salmonella* Typhimurium induces cIAP1 degradation to promote death in macrophages** Madeleine Wemyss, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research (PhD)
10:20	**The mechanism of *P. aeruginosa* outer membrane vesicle biogenesis determines their number, protein composition and biological functions** Lauren Zavan, Department of Microbiology, Anatomy, Physiology, and Pharmacology, La Trobe University (PhD)
10:40 – 11:20	**Morning tea, Cossar Hall** **(includes transit time from and to Lecture Theatre)**
11:20 – 11:50	**Session 2, Lecture Theatre** **Keynote Speaker 1**

	Chair: Linda Reiling, Burnet Institute and Catarina Almeida, University of Melbourne
11:20	**Exploring the world with the pneumococcus** Associate Professor Catherine Satzke, Group Leader and Principal Research Fellow, Murdoch Children's Research Institute
11:50 – 12:40	**Session 3, Lecture Theatre** **Science Bites 1** **Chairs: Joyanta Modak, Deakin University and San Lim, Hudson Institute of Medical Research**
11:50	**IgG allotypes influence IgG responses to SARS-CoV-2 vaccination** Carissa Aurelia, Department of Microbiology and Immunology, University of Melbourne, The Peter Doherty Institute for Infection and Immunity (Honours)
11:54	**Subversion of proteolytic capacity by lysosomal pathogens** Lauren Bird, Department of Microbiology and Immunology, University of Melbourne (PhD)
11:58	**Lipid droplets are transferred between cells using dynamin-dependent mechanisms** Camille Braganca, Department of Microbiology, Anatomy, Physiology and Pharmacology, La Trobe University (Masters)
12:02	**Interplay of type I and II interferons for the generation of CD8+ stem-like memory T cells** Benjamin Broomfield, Immunology Division, Walter and Eliza Hall Institute (PhD)
12:06	**Chronic immune activation and barrier dysfunction in the gut is associated with persistent neuroinflammation in ART-suppressed SIV-infected rhesus macaques** Sarah Byrnes, School of Health and Biomedical Science, RMIT University (PhD**)**
12:10	**Influenza virus infection induces high levels of CD52 expression on effector CD8+ T cells in the lungs** So Young Chang, Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity (PhD)
12:14	**Defining the factors that strategically position memory T cells within lymph nodes** Brigette Duckworth, Immunology Division, Walter and Eliza Hall Institute (PhD)
12:18	**RIPK1/3 regulates T cell responses in mucosal defence against bacterial gut infection** Vik Ven Eng, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research (PhD)
12:22	**Metal ionophores break macrolide resistance in *Streptococcus pneumoniae*** Chloe Estoque, Department of Microbiology and Immunology, University of Melbourne (PhD)
12:26	**Early life cystic fibrosis lung disease is associated with immune cell influx and reduced epithelial cell HLA-DR expression in the lower respiratory epithelium** Liam Gubbels, Infection and Immunity, Murdoch Children's Research Institute (Research assistant)
12:30	**Differential NK cell and γδ T cell activation in SARS-CoV-2-infected pregnant women** Jennifer Habel, Department of Microbiology and Immunology, University of Melbourne at the Peter Doherty Institute for Infection and Immunity (PhD)
12:40 – 13:45	**Lunch and Poster Session 1, Cossar Hall** **(includes transit time from and to Lecture Theatre)**

	See below for more information
13:45 – 14:55	**Session 4, Lecture Theatre** **Oral Presentations 2: Early-career researchers** **Chairs: Aaron Brice, CSIRO and Kevin Lee, University of Melbourne**
13:45	**Interferon epsilon limits ovarian cancer metastasis via regulation of peritoneal immune cells** Nicole Campbell, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research (ECR)
13:55	**The cytosolic DNA sensor AIM2 promotes gastric inflammation and tumourigenesis via inflammasome-dependent and -independent mechanisms** Ruby Dawson, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research (ECR)
14:05	**Impaired HA-specific T follicular helper cell and antibody responses to influenza vaccination are linked to inflammation in humans** Danika Hill, Department of Immunology and Pathology, Monash University (ECR)
14:15	**Unravelling the antiviral mechanisms by which *Wolbachia pipientis* restricts dengue virus replication** Robson Kriiger Loterio, Institute of Vector-Borne Disease , Monash University (ECR)
14:25	**REAPER: mosquito *in vivo* virus targeting to control viral transmission** Adam Lopez-Denman, Health & Biosecurity, CSIRO (ECR)
14:35	**The microbial metabolite indole-3-propionic acid protects against mitochondrial dysfunction in the airway epithelium and allergic asthma inflammation** Olaf Perdijk, Department of Immunology and Pathology, Monash University (ECR)
14:45	**Eat to compete: Understanding immunometabolic interactions that drive *Candida auris* infection** Harshini Weerasinghe, Infection Program and the Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University (ECR)
14:55 – 15:35	**Afternoon Tea, Cossar Hall** **(includes transit time from and to Lecture Theatre)**
15:35 – 16:05	**Session 5, Lecture Theatre** **Keynote Speaker 2** **Chair: Gabriela Khoury, Department of Microbiology, Monash Biomedicine Discovery Institute, Monash University and Ella Johnston, La Trobe University**
15:35	**Gut microbes: from mobile DNA to pathogenesis** Professor Dena Lyras, Deputy Director, Monash Biomedicine Discovery Institute, Monash University
16:05 – 17:00	**Session 6, Lecture Theatre** **Science Bites 2** **Chairs: Christophe Macri, University of Melbourne and Ursula Norman, Monash University**
16:05	**Redundant activity of caspase-1, 3, 7, and gasdermin-D drives cell death and IL-1b release in XIAP deficiency** Sebastian Hughes, Inflammation Division, Walter and Elisa Hall Institute (Research assistant)
16:09	**Lyn expression is increased in lupus patients and is associated with immune cell activation and disease severity** Elan L'Estrange-Stranieri, Department of Immunology and Pathology, Monash University (PhD)

16:13	**Insight into MR1T cell TCR specificity and recognition of MR1** SongYi Li, Department of Microbiology and Immunology, The University of Melbourne (Masters)
16:17	**Regulation of immune checkpoint molecules through ubiquitination** Pharvinderjit Kaur Manjit Singh, Department of Biochemistry and Pharmacology, The University of Melbourne (Masters)
16:21	**Investigating the effects of short chain fatty acids on fungal pathogen-host interactions** Christopher McCrory, Department of Biochemistry and Molecular Biology, Monash University (PhD)
16:25	**Dissecting mechanisms of immune-mediated control of melanoma** Lewis Newland, Department of Microbiology and Immunology, The University of Melbourne (PhD)
16:29	**Comparing the cell-specific innate immune response to RSV between preterm and term infants** Yan Yung Ng, Department of Paediatrics, Murdoch Children's Research Institute (Masters)
16:33	**Eliciting mucosal immunity to SARS-CoV-2 using recombinant influenza viruses** Devaki Pilapitiya, Department of Microbiology and Immunology, University of Melbourne (PhD)
16:37	**Regulation of T cell migration by novel CXCR3 chemokine niche formation** Raymond Qin, Immunology Division, Walter and Eliza Hall Institute (PhD)
16:41	**Novel bacteriophage depolymerase DpK2 degrades capsule of hypervirulent *Klebsiella pneumoniae* K2 strains** Abhinaba Ray, Department of Microbiology, Monash University (PhD)
16:45	**The lysine methyltransferase DOT1L is critical for regulatory T cell identity** Jessica Runting, Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute (PhD)
16:49	**Understanding immune responses towards COVID-19 BNT162b2 vaccines in Indigenous people** Wuji Zhang, Department of Microbiology and Immunology, University of Melbourne at the Peter Doherty Institute (PhD)
17:00 – 18:00	**Evening networking and Poster Session 2, Cossar Hall**
18:00 – 18:15	**Prize-giving, thank yous and conclusion, Cossar Hall**

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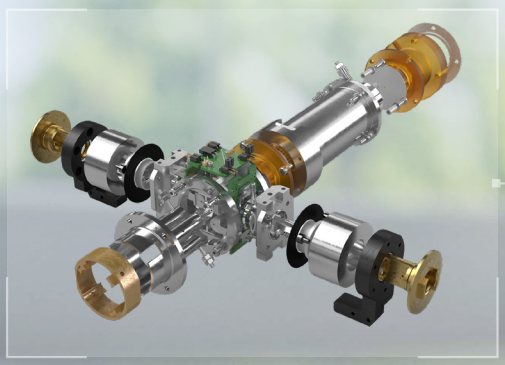

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POSTER 1

12:40 – 13:45	Lunch and Poster Session 1, Cossar Hall (includes transit time from and to Lecture Theatre)
1	**Breast immunity in development and cancer** Caleb Dawson, Immunology Division, Walter and Eliza Hall Institute (ECR)
2	**Revealing neurotoxicity pathways in response to intraventricular polymyxin B in rat** Jing Lu*, Department of Microbiology, Monash University (ECR) (*submitting author; TBC presenting author)
3	**Phage steering on antibiotics: Not always a seesaw effect** Sue Chin Nang, Department of Microbiology, Biomedicine Discovery Institute, Monash University (ECR)
4	**Human Respiratory airway model for bacterial infection and drug discovery** Pawan Parajuli, Health and Biosecurity, Australian Centre for Disease Preparedness, CSIRO (ECR)
5	**RIPK3 preferentially restrains MAIT cell abundance at steady state** Timothy Patton, Department of Microbiology and Immunology, University of Melbourne (ECR)
6	**Evolutionary rate of SARS-CoV-2 increases during zoonotic infection of farmed mink** Ash Porter, Department of Microbiology and Immunology, University of Melbourne (ECR)
7	**A High-affinity Gamma-delta T-cell Receptor for Cancer Therapy** Marc Rigau, Department of Microbiology and Immunology, University of Melbourne (ECR)
8	**An Integrative Analysis of RNA, Protein and Metabolite Levels Reveals Distinct Phases of Macrophage Polarization by IFNb** Sarah Straub, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research (ECR)
9	**Characterisation of host cell membrane biophysical changes and cytoskeletal rearrangements during *Toxoplasma gondii* invasion** Aurelie Tsee Dawson, Infection and Imaging, Walter and Eliza Hall Institute (ECR)
10	**Structural mechanism of SARS-CoV-2 entry and activation of spike glycoprotein by engaging unique host factors & potential interventions** Naveen Vankadari, Bio21 Institute, Department of Pharmacology and Biochemistry, University of Melbourne (ECR)
11	**A biparatopic nanobody with broad neutralisation against SARS-CoV-2 variants of concern** Amy Adair, Infectious Diseases and Immune Defence, Walter and Eliza Institute (Research assistant)
12	**Post-translational regulation of MHC Class II** Priscilla Agustina, Department of Biochemistry and Pharmacology, University of Melbourne (Masters)
13	**Extracellular Vesicle Purity Markers Found on Lipid Droplets** Irumi Amarasinghe, Department of Microbiology, Anatomy, Physiology and Pharmacology, La Trobe University (Honours)
14	**Neutrophil heterogeneity and autoantibody-mediated NETosis in COVID-19 severity risk**

	Aisah Resti Amelia, Infectious Disease and Immune Defence, Walter and Eliza Institute (Masters)
15	**Clonal selection defines an atypical human Vδ2+ γδ T cell compartment** Daniel Arsovski, Monash Biomedicine Discovery Institute, Monash University (PhD)
16	**Using CRISPR/Cas9 gene-editing technology to examine CD4+ T cell immune response against malaria infection** Takahiro Asatsuma, Department of Microbiology and Immunology, Peter Doherty Institute, University of Melbourne (PhD)
17	**Avian Adenovirus Evolution: What's New?** Ajani Athukorala, Department of Microbiology, Anatomy, Physiology, and Pharmacology, La Trobe University (PhD)
18	**Manufacture of a GMP-like bacterial challenge agent in a research facility for use in humans** Kristy Azzopardi, Infection and Immunity, Murdoch Children's Research Institute (Research assistant)
19	**Cephamycin derivatives as anti-sporulation agents against *Clostridioides difficile*** Clara Bate, Monash Biomedicine Discovery Institute and The Centre to Impact AMR (Microbiology), Monash University (PhD)
20	**Functional assessment of genetic variants affecting the NOD2 signalling pathway in patients with primary immunodeficiency** Ebony Blight, Department of Immunology, Monash University (PhD)
21	**Inhaled dosing regimens of aztreonam and tobramycin against multidrug-resistant hypermutable cystic fibrosis *Pseudomonas aeruginosa* isolates in a dynamic in vitro biofilm model** Siobhonne Breen, Drug Delivery, Disposition and Dynamics, Monash University (PhD)
22	**Importance of covalent antigen binding for MR1-mediated MAIT cell development and expansion** Charlene Chen, Department of Microbiology and Immunology, University of Melbourne (Honours)
23	**Dampening the inflammatory cytokine response in monocytes** Cassandra Cianciarulo, Department of Rural Clinical Sciences, La Trobe University (PhD)
24	**Cross-specific nanobodies against malaria parasite invasion ligands** Jaison D Sa, Infectious Diseases and Immune Defence, Walter and Eliza Hall Institute (PhD)
25	**Understanding interactions between interleukin-1 receptor associated kinase 3 (IRAK3) and mitochondria in inflammatory responses** Anushka Date, Rural Health School, La Trobe University (PhD)
26	**Variation in epigenetic profiles in newborns conceived with assisted reproduction - current evidence and future approaches** Jasmine Dennison, Molecular Immunity (Epigenetics), Murdoch Children's Research Institute and the University of Melbourne (Honours)
27	**Killer MAITs: Investigating the cytotoxic capacity of MAIT cells towards tumours** Eleanor Eddy, Department of Microbiology and Immunology, Peter Doherty Institute, University of Melbourne (PhD)
28	**Targeting human coronaviruses using nanobodies** Jackson Feng, Infectious Disease, Walter and Eliza Hall Institute (PhD)

29	**Novel approaches to the detection and surveillance of *Neisseria gonorrhoeae*** Janath Fernando, Department of Microbiology and Immunology, University of Melbourne (Honours)
30	**Perturbed S-palmitoylation cycles alters protein trafficking in dendritic cells** Ashley Firth, Department of Biochemistry and Pharmacology, University of Melbourne (PhD)
31	**The capacity of B-cell memory to recognise Omicron BA.2 and BA.4/5 following COVID-19 adenoviral vector vaccination** Holly Fryer, Department of Immunology and Pathology, Monash University (Honours)
32	**Differential immune detection of *Bacteroides fragilis* and their outer membrane vesicles** William Gilmore, Department of Microbiology, Anatomy, Physiology and Pharmacology, School of Agriculture, Biomedicine and Environment, La Trobe University (PhD)
33	**p-cresol sulfate acts on epithelial cells to reduce allergic airway inflammation** Rhiannon Grant, Department of Immunology and Pathology, Monash University (PhD)
34	**Similar antibody and higher T-cell responses after vaccination against SARS-CoV-2 in middle-aged people with well-controlled HIV-1 compared to demographically and lifestyle-comparable controls** Marloes Grobben, Laboratory of Experimental Virology, Department of Medical Microbiology, Amsterdam UMC (PhD)
35	**Lipid Droplets are Metabolic Hubs During Viral Infection** Meaghan Heyward, Department of Microbiology, Anatomy, Physiology and Pharmacology, La Trobe University (Honours)
36	**The role of the tetraspanin CD53 in type 2 asthma responses** Amy Hsu, Department of Immunology and Pathology, Monash University (PhD)
37	**Antibody responses to polymorphic regions of the lead malaria vaccine antigen** Alessia Hysa, Department of Infectious Diseases at the University of Melbourne and Burnet Institute (Masters)
38	**Investigating the role of serotonin in inflammatory responses induced by *Helicobacter pylori* infection** Sulaimon Idowu, School of Science, RMIT University (PhD)
39	**In Vivo Assembly of Epitope-coated biopolymer particles that induce anti-tumor responses** Devi Jenika, Department of Biochemistry and Pharmacology, University of Melbourne (PhD)
40	**The role of Type III interferon signalling in cDC1-targeting vaccination** Jung Hee Kang, Department of Biochemistry and Molecular Biology, Monash University (PhD)
41	**Immunity to malaria: association between antibodies and clinical protection in *Plasmodium vivax* low-endemic areas** Lejla Kartal, Population Health and Immunity, Walter and Eliza Hall Institute (Masters)
42	**Developing phage depolymerase enzymes to disarm *Klebsiella pneumoniae***

	Daniel Williams, Biomedicine Discovery Institute (Microbiology) Monash University (PhD)
13:45 – 14:55	**[Return to main program above]** **Session 4, Lecture Theatre**

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POSTER 2

17:00 – 18:00	Evening networking and Poster Session 2, Cossar Hall (includes transit time from and to Lecture Theatre)
43	**Risk factors for SARS-CoV-2 infection, by variant of concern: a systematic literature review** Matthew Harris, Melbourne School of Population and Global Health / Asia-Pacific Health, University of Melbourne and Murdoch Children's Research Institute (Masters)
44	**Deciphering the molecular mechanisms behind trans-synaptic transmission of rabies virus** Steph Keating, School of Medicine, Deakin University (PhD)
45	**Withdrawn**
46	**Using super resolution microscopy to visualise epigenetic control of T cell fate and function** Gavin Koh, Biomedicine Discovery Institute, Department of Biochemistry and Molecular Biology, Monash University (PhD)
47	**Lipid droplets act as platforms for innate immune signalling proteins** Jay Laws, Department Microbiology, Anatomy, Physiology and Pharmacology, La Trobe University (PhD)
48	**Investigating the role of γδ T cells and IL-4 in radiation- attenuated sporozoite vaccination** Shirley Le, Department of Microbiology and Immunology, Peter Doherty Institute, University of Melbourne (Masters)
49	**The PTRAMP-CSS heterodimer plays an essential role in *P. falciparum* invasion of human erythrocytes** Pailene Lim, Infectious Diseases and Immune Defences, Walter and Eliza Hall Institute (Research assistant)
50	**Nanobodies targeting malaria transmission-blocking candidate** Frankie Lyons, Infection and Immunity, Walter and Eliza Hall Institute (PhD)
51	**The off-target effects of BRAF inhibitors on murine dendritic cell activation** Zoe Magill, Department of Biochemistry & Molecular Biology, Monash University (PhD)
52	**Establishing Australia's Vaccine Safety Health Link** Hannah Morgan, Infection and Immunity, SAEFVIC Murdoch Children's Research Institute (PhD)
53	**Differential contribution of resident and infiltrating phagocytes in the defense against *L. longbeachae*** Lara Oberkircher, Hudson Institute of Medical Research, Monash University (PhD)
54	**Mapping the commitment of haematopoietic progenitors to the T cell lineage** Dhruti Parikh, Department of Medicine, University of Melbourne and St. Vincent's Institute of Medical Research (PhD)
55	**A potential role for ADAMTS versicanases in influenza virus infection** Jess Pedrina, School of Medicine, Deakin University (PhD)
56	**Eosinophil Hyperactivity in Early-Life Induces Lung Inflammation and Long-term Lung Defects** April Raftery, Department of Immunology and Pathology, Monash University (PhD)
57	**Role of urokinase plasminogen activator protein in *Helicobacter pylori* pathogenesis**

	Modhusudon Shaha, Biomedicine Discovery Institute and Department of Microbiology, Monash University (PhD)
58	**The role of butyrophilins in regulating αβ T cell responses** Kezia Christilla Singgih, Department of Microbiology and Immunology, University of Melbourne (PhD)
59	**Effect of childhood vaccination on antimicrobial resistance and pneumococcal populations among children in Mongolia** Paige Skoko, Translational Microbiology, Murdoch Children's Research Institute (Honours)
60	**Immunological memory response following reduced-dose pneumococcal vaccination schedule** Satvika Soppadandi, Infection and Immunity - New Vaccines Group, University of Melbourne and Murdoch Children's Research Institute (Honours)
61	**Impact of inflammasome-induced extracellular vesicles on different bystander cells** Lena Standke, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research (PhD)
62	**The more the merrier: bacteriophage cocktails provide superior suppression of Serratia marcescens compared to individual phages** Cassandra Stanton, Department of Microbiology, Anatomy, Physiology and Pharmacology, La Trobe University (PhD)
63	**Epidemiology of SARS-CoV-2 cases and COVID-19 hospitalisations and deaths in children and adolescents** Darren Suryawijaya Ong, Infection & Immunity, Murdoch Children's Research Institute (Masters)
64	**COVID-19 mitigation measures in early childhood education and care and schools: a systematic review** Darren Suryawijaya Ong, Infection & Immunity, Murdoch Children's Research Institute (Masters)
65	**Component-resolved diagnosis of peanut allergy through flowcytometric staining of basophils (CytoBas)** Liam Ta, Department of Immunology and Pathology, Monash University (Honours)
66	**Dynamic metabolic footprint analysis in a hollow-fibre infection model: ceftolozane-tazobactam versus a challenging *Pseudomonas aeruginosa* clinical isolate** Jessica Tait, Drug Delivery, Disposition, and Dynamics, Monash University (PhD)
67	**The lipidome of lipid droplets changes significantly during viral infection in the brain** Zahra Telikani, Department of Microbiology, Anatomy, Physiology and Pharmacology (MAPP), La Trobe University (PhD)
68	**Effect of different resistance mechanisms on bacterial killing and regrowth of *Pseudomonas aeruginosa* in response to meropenem** Alice Terrill, Drug Delivery Disposition and Dynamics, Monash University (Honours)
69	**Isolating transmission-blocking nobodies against the *Plasmodium falciparum* PfPSOP12 protein** Stephanie Trickey, Infection and immunity, Walter and Eliza Hall Institute (Research assistant)
70	**Determining new permeation pathway functionality of *Plasmodium berghei* in vivo using guanidinium hydrochloride** Mitchell Trickey, School of Medicine, Deakin University (PhD)
71	**Divergence in immune evasion capacity between different genotypes of Hendra and Nipah virus**

	Melanie Tripp, Department of Microbiology, Monash University and Australian Centre for Disease Preparedness, CSIRO (PhD)
72	**Rifaximin prophylaxis causes resistance to the last-resort antibiotic daptomycin** Adrianna Turner, Department of Microbiology and Immunology, University of Melbourne (PhD)
73	**Characterising the role of IKKe in STING signalling** Rajan Venkatraman, Department of Biochemistry and Molecular Biology, Monash University (PhD)
74	**Accelerated Bacille Calmette Guérin (BCG) reactions: more than meets the eye** Paola Villanueva, Department of Paediatrics, University of Melbourne; Infection and Immunity, Murdoch Children's Research InstituteUniversity of Melbourne (PhD)
75	**Don't take it for pomegranate: MR1 presents dietary antigens to modulate MAIT cell activation** Carl Wang, Department of Biochemistry and Molecular Biology, Monash University (PhD)
76	**Examining The Proteomic Shift in Molluscs Following Viral Challenge and Immune Priming** Angus Watson, Department of Microbiology, Anatomy, Physiology and Pharmacology, La Trobe University (Masters)
77	**The role of the inflammasome adaptor ASC in gastric tumourigenesis** Alice West, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research (PhD)
78	**CREB-regulated immunosuppression in the GBM tumour microenvironment** Samuel Widodo, Department of Surgery (RMH), University of Melbourne (PhD)
79	**Assessing the improvement in accuracy of *Plasmodium vivax* serological exposure markers through use of different genetic sequences** Wencong Wu, Division of Population Health and Immunity, Walter and Eliza Hall Institute of Medical Research (Honours)
80	**The ARTC2-P2X7 axis regulates the diverse function of MAIT, gdT, and NKT cells** Calvin Xu, Department of Microbiology and Immunology, Peter Doherty Institute, University of Melbourne (PhD)
81	**Characterisation of *Streptococcus pyogenes* reference strains** Serene Yeow (to be presented by Hannah Frost and Kristy Azzopardi), Murdoch Children's Research Institute (Research Assistant)
82	**Effects of Inflammation and Infection on MR1 Expression** Xiaoyue Zhang, Department of Microbiology and Immunology, University of Melbourne (PhD)
18:00 – 18:15	**Prize-giving, thank yous and conclusion, Cossar Hall**

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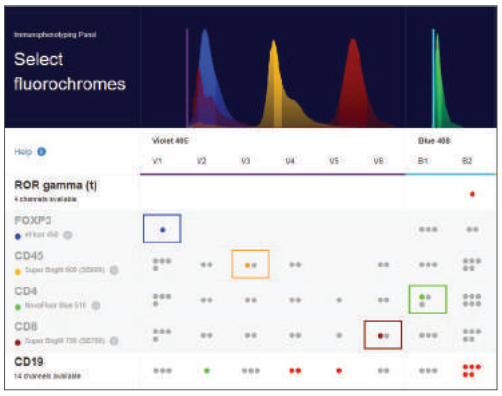

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ABSTRACTS
SESSION 1

Pirfenidone is more effective than glucocorticosteroids in limiting transforming growth factor-beta-enhanced viral infection and inflammation in the lungs

Julia G Chitty[1], Bruce Montgomery[2], Jack A Elias[3], Philip Bardin[4,5], Jane E Bourke[1], Belinda J Thomas[5]*

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* = presenting author

Introduction & Aims: Patients with respiratory diseases are more susceptible to viral infection, often developing more severe and prolonged symptoms. These exacerbations often lead to deleterious effects such as lung function decline and irreversible disease progression. Emerging evidence suggests that elevated transforming growth factor-beta (TGFβ), seen in patients with respiratory diseases, likely plays a crucial role in suppressing the immune response, thus resulting in enhanced viral infection and inflammation. While glucocorticosteroids (GCS) can effectively reduce inflammation, there is substantial evidence also demonstrating their immunosuppressive effects. Pirfenidone (PFD) is an anti-fibrotic small molecule used in patients with pulmonary fibrosis to slow disease progression. Our recent studies have shown that oral administration of PFD can reduce TGFβ-enhanced viral infection in a mouse model. However, the use of oral PFD comes with unpleasant side effects, therefore the aim of this study was to determine if inhaled PFD could also reduce inflammation and disease severity, and compare its effectiveness to that of GCS.

Methods: Transgenic C57Bl/6 mice with inducible lung-specific over-expression of TGFβ were treated intranasally with vehicle (control), PFD (13.3 mg/kg) or GCS (1 mg/kg) daily, starting 2 days prior to infection with IAV (10^2 PFU, HKx31). Mice were culled at day 3 post infection, and lung tissue and bronchoalveolar lavage fluid (BALF) were collected for assessment of infection, inflammation, and immune responses.

Results: Daily administration of PFD, but not GCS, was able to reduce TGFβ-enhanced viral load in lung homogenates ($p<0.05$), as measured by plaque assay. In BALF, the chemokine RANTES was reduced by both PFD and GCS, however IL-6, TNFα and KC were only reduced by PFD.

Conclusion: This study demonstrates that inhaled PFD was able to afford protection against TGFβ-enhanced viral infection and inflammation more effectively than the current standard treatment, GCS. These promising findings offer the possibility of repurposing PFD to treat patients with respiratory disease during viral exacerbations, offering protection from worse disease outcomes.

Genetic and chemical validation of *Plasmodium falciparum* aminopeptidase *Pf*A-M17 as an anti-malarial drug target in the haemoglobin digestion pathway

Rebecca C.S. Edgar[1,2]*, Ghizal Siddiqui[3], Kathryn Hjerrild[1], Tess R. Malcolm[4], Natalie B. Vinh[5], Chaille T. Webb[4,5], Clare Holmes[7], Christopher A. MacRaild[3], Hope C. Chernih[1,2], Willy W. Suen[7], Natalie A. Counihan[1,2], Darren J. Creek[3], Peter J Scammells[5], Sheena McGowan[4,6] and Tania F. de Koning-Ward[1,2]

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[3]*Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, 3052, Australia*
[4]*Biomedicine Discovery Institute and Department of Microbiology, Monash University, Clayton, 3052, Australia,*
[5]*Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University Parkville, 3052, Australia*
[6]*Centre to Impact AMR, Monash University, Melbourne, 3800, Australia*
[7]*CSIRO Australian Centre for Disease Preparedness, Geelong, VIC, 3219, Australia*

Plasmodium falciparum, a causative agent of malaria, remains a global health problem due to increasing drug resistance to therapeutics. Drugs with novel modes of action are desperately needed to combat this resistance. It is the intra-erythrocytic cycle of *P. falciparum* that is responsible for the clinical manifestations of disease. Here, *P. falciparum* infects human red blood cells and digests the cells main protein constituent, haemoglobin, in a specialised digestive vacuole. Digestion occurs in a step-wise process, with many of the enzymes in the cascade having overlapping, redundant functions. Leucine aminopeptidase *Pf*A-M17 is one of several aminopeptidases implicated in the final step of this digestive pathway, but currently there is little evidence of its essentiality and its biological function is unconfirmed.

Here we utilised reverse genetics to generate a parasite line in which *Pf*A-M17 can be conditionally depleted and showed that it is essential for *P. falciparum* survival. We additionally created a compound specifically designed to inhibit the activity of *Pf*A-M17, which we confirmed as on target using thermal proteomics profiling and found it to kill parasites in a sub-micromolar range. Using a metabolomic approach we found that *Pf*A-M17 provides parasites with free amino acids for growth, many of which are highly likely to originate from haemoglobin. Moreover, parasites grown in the absence of non-essential amino acids become more sensitive to our *Pf*A-M17 inhibitor, confirming *Pf*A-M17's function is to provide amino acids essential for parasite survival. A further novel finding was that loss of *Pf*A-M17 results in parasites exhibiting multiple digestive vacuoles at the trophozoite stage, which we were able to confirm via electron microscopy. In contrast to other haemoglobin-degrading proteases that have overlapping redundant functions, we validate *Pf*A-M17 as a potential novel drug target worthy of future antimalarial development.

Lower γδ T cell frequency in the colonic mucosa is an immunological signature of Hirschsprung disease

Ashleigh Gould*[1], Cameron Nowell[1], Sebastian King[2,3,4], Matthew Rowe[1], Simona Carbone[1], Daniel Poole[1], Emily Eriksson[5,6]

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3. *Surgical Research, Murdoch Children's Research Institute, Melbourne, Victoria, Australia,*
4. *Department of Paediatrics, The University of Melbourne, Victoria, Australia*
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* = presenting author

Hirschsprung disease (HSCR) is a congenital neuropathy affecting 1 in 5000 live births annually. During embryonic development, the enteric nervous system fails to populate and grow in the distal portion of the colon leading to an 'aganglionic' distal bowel and functional obstruction. The only intervention available is corrective surgery to remove the affected bowel, however, a large proportion of children continue to experience post-surgical complications that significantly impact their quality of life. Approximately 50% of patients will develop Hirschsprung-associated enterocolitis (HAEC), a potentially life-threatening infection of the mucosal lining of the colon. Given the high incidence of HAEC, we hypothesised that HSCR patients have a different immunological structure that underpins susceptibility to this condition.

To understand this, we studied the colonic mucosa of HSCR patients to characterise the composition and distribution of immune cells in the intraepithelial layer (IEL) and lamina propria (LPL). Analysis of isolated lymphocytes from these two regions using flow cytometry showed a significantly lower frequency of γδ T cells in the IEL and the LPL compartments of the mucosa of patients with HSCR compared to age-matched controls. This finding was further supported by immunofluorescent labelling and imaging of tissue sections, which confirmed the lower frequency of γδ T cells in both regions of the HSCR bowel. There was also a significant increase in the distance between γδ T cells and the epithelial barrier.

These novel findings not only support the hypothesis that there are differences in the mucosal immune system in patients with HSCR, but also establish that γδ T cells that are important for gut barrier integrity have a different spatial location and are present at lower numbers. Collectively, this provides important insight that may to some extent explain why HSCR patients are at risk of HAEC infection.

Accurate detection of house dust mite sensitisation in asthma and allergic rhinitis with a single Cytometric Basophil assay (CytoBas)

Lin Hsin[1], Nirupama Varese[1,2,3], Pei M. Aui[1], Bruce D. Wines[1,3], P. Mark Hogarth[1,3], Mark Hew[2,4], Robyn E. O'Hehir[1,2], Menno C. van Zelm[1,2]

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Background: House dust mite (HDM) is the most prevalent cause of perennial atopic asthma and allergic rhinitis globally. Disease management critically relies on accurate detection of allergen sensitisation and often requires multiple clinic appointments by patients. To improve diagnosis quality and patient care, we designed fluorescent protein tetramers for direct staining of IgE on basophils in a single flow cytometric assay to detect HDM sensitisation.

Methods: Recombinant proteins of Der p 1 and Der p 2, the major HDM allergen components, were produced as streptavidin-fluorochrome conjugated tetramers. Blood samples from 54 HDM-allergic patients and 24 non-atopic controls were incubated with allergen tetramers for evaluation of basophil binding and activation with flow cytometry. Patients had allergic rhinitis and/or asthma and serum HDM-specific IgE≥3.5kU$_A$/L (ImmunoCAP).

Results: Recombinant allergen tetramers effectively bound (median fluorescent intensity) and activated (CD63 expression) basophils from allergic patients but not non-atopic controls. The tetramers bound all basophils in HDM-allergic individuals, even the fraction that did not show activation (CD63$^-$) to the same degree as the CD63$^+$ fraction. Fluorescent staining using CytoBas was more sensitive with Der p 1 (91%) than with the basophil activation test (BAT; 87%); and more specific with Der p 2 (100%) than BAT (87%) in the detection of allergen sensitisation. Importantly, for HDM allergy sensitisation detection, CytoBas positivity for Der p 1 and Der p 2 was 100% sensitive and 100% specific, whereas it was only 96% sensitive and 87% specific with BAT.

Conclusion: Component-resolved diagnosis of asthma and allergic rhinitis with the CytoBas approach can provide advantages over serology IgE tests by rapidly detecting functional allergen-specific IgE bound to basophil effector cells. A single, multiplex CytoBas assay encompassing the prevalent aeroallergens (grass pollen, HDM, animal dander) can provide a rapid, sensitive and specific diagnostic test, enabling early start of optimal treatment.

Using Anopheles salivary antibody biomarkers to assess spatial and temporal heterogeneity in vector exposure and malaria transmission in Southeast Myanmar

Ellen Kearney*[1,2], Paul Agius[1,2,3], Punam Amratia[4], Kefyalew Addis Alene[4,5], Katherine O'Flaherty[1], Win Han Oo[1], Julia Cutts[1,6], Win Htike[1], Daniela Da Silva Goncalves[1], Aung Thi[7], Kyaw Zayar Aung[1], Htin Kyaw Thu[1], Myat Mon Thein[1], Nyi Nyi Zaw[1], Wai Yan Min Htay[1], Aung Paing Soe[1], Victor Chaumeau[8,9], Julie A Simpson[2], Peter Gething[4,5], Freya JI Fowkes[1,2,3]

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* = presenting author

Background: A strong surveillance system for malaria and its vectors has been identified by the World Health Organization as a cornerstone of the elimination agenda. Human antibodies to *Anopheles* salivary proteins and malaria parasites have potential to serve as sensitive and feasible biomarkers of vector exposure and to identify focal areas of ongoing transmission, however, evidence for this application is limited.

Methods: Antibodies to *Anopheles* salivary proteins and transmission-stage malaria parasites were measured by ELISA in 13,594 samples collected over 15 months from 104 villages in Southeast Myanmar as part of routine malaria testing. A Bayesian geostatistical modelling framework was used to identify environmental and climatic risk-factors and generate spatially continuous predictions of malarial and *Anopheles* salivary antibody seroprevalence, as a proxy for malaria transmission.

Results: The spatial distribution of *Anopheles* salivary antibodies was associated with the abundance of the dominant vectors of the region (*Anopheles minimus, An. maculatus, An. dirus*) and climatic variables: precipitation, population density and indices of vegetation and topographic water-accumulation. Antibodies to *Anopheles* salivary proteins and transmission-stage parasites were dynamic and followed seasonal trends in vector abundance and malaria transmission and varied over small spatial scales. Overlayed predicted *Anopheles* salivary and transmission-stage parasite antibody seroprevalence identified potential foci of ongoing transmission.

Significance: These findings support the use of *Anopheles* salivary antibodies as a serosurveillance tool to measure vector exposure and identify foci of malaria transmission and areas at risk of malaria rebound in low-transmission settings such as the Greater Mekong Subregion.

Identifying the mechanism of action of a novel invasion-blocking compound in *Plasmodium falciparum*

Dawson Ling[1,2*], Madeline Dans[1,3], Greta Weiss[1], Betty Kouskousis[1], Zahra Razook[1,4], Somya Mehra[1,4], Alyssa Barry[1,4], Brendan Crabb AC[1,2], Hayley Bullen[1,2], Paul Gilson[1,2]

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Plasmodium falciparum malaria accounted for nearly all the 627,000 reported malaria deaths in 2020 (World Health Organization, 2021). Emerging drug resistance against all currently available antimalarials threatens global progress towards malaria eradication, underpinning the need for new antimalarials with novel mechanisms of action. Parasite invasion of red blood cells (RBCs) is responsible for parasite proliferation and symptomatic malaria, presenting as an attractive drug target. To uncover compounds that block invasion, we screened an open-access library of 400 compounds called the Pathogen Box, released by Medicines for Malaria Venture. This led to the discovery of an invasion-blocking compound, MMV687794. At the same concentration, the compound blocked RBC invasion from mature schizonts but had no effect in the developmental ring and trophozoite stages (Dans *et al.*, 2020) and is therefore invasion-specific. Live-cell microscopy analysis of MMV687794-treated parasites revealed a RBC invasion defect whereby extracellular daughter merozoites remained stuck to the RBC surface and failed to develop into the ring stage, a phenotype enhanced by increased drug treatment duration. Genomic analysis of MMV687794-resistant parasites revealed mutations in an alpha/beta-hydrolase gene containing a lysophospholipase motif which we have termed ABH-83. To validate ABH-83 as the target of MMV687794, these mutations were engineered into wild-type parasites using CRISPR/Cas9. These transgenic mutant parasites recapitulated the drug resistance phenotype, confirming that ABH-83 is the target of MMV687794. We have found that ABH-83 localises to the invasion-related organelles known as rhoptries. We hypothesise that ABH-83 is involved in hydrolysing invasion-related rhoptry lipids during the schizont stage, essential for rhoptry fusion to facilitate the discharge of invasion-related proteins during RBC invasion. We are currently investigating this by examining how MMV687794-mediated inhibition of ABH-83 affects the discharge of other invasion-related proteins during RBC invasion.

Identification of influenza B virus HLA-B*07:02 and HLA-B*08:01 restricted CD8+ T cell epitopes

Tejas Menon[1#*], Patricia Illing[2#], Chloe Shepherd[2], Jan Peterson[2], Grace Khuu[2], Louise C Rowntree[1], Lilith F Allen[1], Jaime Rossjohn[2, 3, 4], Anthony Purcell[2†], Carolien E van de Sandt[1,5†] and Katherine Kedzierska[1†]

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Although seasonal influenza B viruses (IBV) cause significant morbidity, especially in young children, immune responses towards IBV are largely understudied. $CD8^+$ T cells play an essential role in clearing influenza virus infections and may recognize epitopes encompassing conserved peptides derived from internal IBV proteins. However, to date, a very limited array of $CD8^+$ T cell epitopes have been identified for IBVs. In our study, we sought to identify novel $CD8^+$ T cell epitopes restricted to prominent HLA-B*07:02 and HLA-B*08:01 alleles expressed in 10.5% and 8.4% of the global human population respectively. Immunopeptidomics identified eighteen candidate peptides for HLA-B*07:02 and thirty-six candidate HLA-B*08:01 epitopes derived from IBV. These were subsequently screened for their ability to expand $CD8^+$ T cells from HLA-B*07:02$^+$ and/or HLA-B*08:01$^+$ donors, followed by intracellular cytokine staining (ICS) and flow cytometry analysis. HLA restriction of identified peptides was confirmed by restimulation with peptide-pulsed C1R-B*07:02 and C1R-B*08:01 cells, resulting in the discovery of four novel HLA-B*07:02-restricted and three HLA-B*08:01-restricted IBV epitopes. All epitopes were immunodominant and highly conserved. With the use of peptide-HLA tetramers combined with Tetramer Associated Magnetic Enrichment (TAME) and single cell multiplex PCR-based analysis, we investigated the memory phenotype and the paired TCRαβ repertoire of IBV epitope-specific $CD8^+$ T cells directly ex vivo. We observed high frequencies of memory $CD8^+$ T cells, suggesting recruitment during previous IBV infections. In addition, we also established that individuals who co-expressed HLA-B*07:02 and HLA-B*08:01 had lower cytokine responses following stimulation with peptide-pulsed C1R-B*08:01 cells and lower HLA-B*08:01-epitope-specific $CD8^+$ T cell memory frequencies directly *ex vivo*. This indicates that the prominence of IBV-specific HLA-B*08:01-restricted $CD8^+$ T cells can be impacted by the presence of HLA-B*07:02. Overall, our work has identified new targets for T cell based IBV vaccine strategies and has described intriguing dynamics caused by the co-expression of HLA-B*07:02 and HLA-B*08:01.

Regulation of tissue-specific T_{RM} cell responses

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A cornerstone of the adaptive immune system is the ability to generate long-lived specialised cells that retain memory of previous infections, mediating rapid antigen-specific responses at a heightened intensity upon repeat encounter. While primarily understood to be comprised only of circulating memory subsets, tissue resident memory T (T_{RM}) cells have emerged as a distinct population within the memory T cell pool. T_{RM} cells are preferentially localised at the site of primary infection and in many cases are defined by the expression of the cell surface markers CD69 and CD103. Gene expression analysis has shown that T_{RM} cells in multiple tissues share a core transcriptome, characterised by the downregulation of a repertoire of pro-migratory genes, aiding retention. Although cytokines such as TGF-β and IL-15 have been proposed to promote T_{RM} cell development, it is clear that there are tissue-specific determinants of T_{RM} cell development and maintenance that remain elusive. By developing a novel methodology to generate T_{RM}-like cells *in vitro*, we identify a critical role for retinoic acid (RA) and the transcriptional repressor Hypermethylated in Cancer 1 (HIC1) in the development of T_{RM}-like cells. Transcriptomic analysis revealed that activation of $CD8^+$ T cells in the presence of IL-2, IL-15, TGF-β and RA resulted in faithful expression and repression of the core T_{RM} cell transcriptome, and that HIC1 was critical in regulating repression of the core gene program. Critically, we find that RA, TGF-β and RA/TGF-β conditions induce unique gene signatures that have similarities to *bona fide* T_{RM} cells from the gut, liver or lung, providing a unique insight into the factors that potentially regulate tissue-specific T_{RM} cell development. As T_{RM} cells are key mediators of immunity in recurrent infections and cancer, but are capable of facilitating autoimmune disease when dysregulated, understanding how best to modulate these cells will be crucial in informing the design of novel therapeutics.

Salmonella Typhimurium induces cIAP1 degradation to promote death in macrophages

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Bacterial gut pathogens such as *Salmonella enterica* are a major contributor to the global foodborne disease burden. In humans, Non-Typhoidal *Salmonella* serovars including *S*. Typhimurium infect the small intestine and underlying immune cells to cause gastroenteritis, and can induce systemic disease if left unchecked in immunocompromised individuals. Infected phagocytes such as macrophages facilitate the immune response against *Salmonella* infection by activating programmed cell death mechanisms. Death of the host macrophage halts intracellular bacterial replication, and enables the extracellular release of inflammatory cytokines and danger signals. However, *Salmonellae* use two specialised Type III Secretion Systems (T3SSs) to introduce effector proteins directly into the host cell cytosol, thus manipulating the cellular environment to suppress innate immune signalling and promote bacterial survival. As such, characterising novel functions of these effector proteins is crucial to understanding the success of these pathogens.

Our research discovered that *S*. Typhimurium induces the degradation of cellular inhibitor of apoptosis protein 1 (cIAP1), an important host cell adaptor of inflammatory signalling and inhibitor of apoptotic cell death. Using an *in vitro* model of human infection in THP-1 macrophage-like cells, we observed strong association between cIAP1 loss and increased cellular cytotoxicity, with corresponding caspase-8/-3 activation. Depletion of cIAP1 required functional *Salmonella* Pathogenicity Island 1 (SPI-1) T3SS effector translocation, and was not prevented by pan-caspase, proteasomal or lysosomal inhibitors. Anti-cIAP1 immunoblot detected a low molecular weight peptide following *S*. Typhimurium infection, suggesting that SPI-1 effector/s may cleave cIAP1 during infection. Current work combines molecular, *in vitro* and *in vivo* techniques to explore the cIAP cleavage mechanism and determine the responsible effector protein, with transfection screens suggesting several key candidates. This finding suggests a new role for *Salmonella* effector proteins in activating, rather than preventing, host cell death in macrophages, which we hypothesise may promote dissemination of the bacteria.

The mechanism of *P. aeruginosa* outer membrane vesicle biogenesis determines their number, protein composition and biological functions.

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Outer membrane vesicles are nanoparticles produced by Gram-negative bacteria to facilitate a number of biological processes. This includes contributing to the predation of other bacterial species due to the antimicrobial proteins contained within OMVs. The production of OMVs by bacteria occurs either via blebbing from the outer membrane during natural growth, or formation after prophage mediated explosive cell lysis. It is currently unknown whether the mechanism of OMV biogenesis can regulate the production, composition, and functions of OMVs. Therefore, in this study we examined the quantity, protein composition and antimicrobial functions of *Pseudomonas aeruginosa* OMVs produced via different mechanisms of biogenesis.

OMVs were isolated from *P. aeruginosa* strains that produced OMVs by (i) both blebbing and explosive cell lysis, (ii) by budding alone, (iii) or predominately by explosive cell lysis. The quantity and size of OMVs produced by each mechanism of biogenesis were characterised, revealing that the mechanism of OMV biogenesis dictated the number of OMVs released from *P. aeruginosa*. Furthermore, we analysed and compared the proteomes of OMVs produced via different mechanisms and their parent bacteria to determine if there was a selective enrichment of proteins packaged within OMVs. Our results determined that OMVs produced by various mechanisms of biogenesis were significantly different in their protein composition compared to each other and their parent bacteria, revealing that the mechanism of biogenesis dictates the OMV proteome. Finally, we compared the ability of OMVs to inhibit *P. aeruginosa* or *Staphylococcus aureus* growth and found that only OMVs produced by explosive cell lysis could kill *P. aeruginosa* whilst OMVs could kill *S. aureus* irrespective of their mechanism of biogenesis.

Together, our results reveal that the mechanism of OMV biogenesis is a novel regulator of OMV production and protein composition, suggesting there is a specific packaging of protein cargo into OMVs dependent on their biogenesis mechanism. Furthermore, our data shows that the changes in OMV proteomes may alter their ability to inhibit bacterial growth as only explosive cell lysis OMVs can inhibit *P. aeruginosa* growth. Therefore, our data provides new insights into the regulation of OMV cargo composition and biological functions by bacteria.

ABSTRACTS
SESSION 3

IgG allotypes influence IgG responses to SARS-CoV-2 vaccination

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Immunoglobulin G (IgG) is the most abundant antibody isotype in human serum and is divided into four structurally and functionally distinct subclasses (IgG1-4). IgG subclass heavy chains display variations, termed allotypes, which can differ between individuals and ethnic groups. Following infection and/or vaccination against a range of pathogens, IgG allotypes have been shown to influence serum IgG subclass levels and half-life. Furthermore, prior studies have demonstrated the impact of allotypes on functional IgG responses, particularly those mediated via Fcγ receptor (FcγR) engagement. However, the importance of IgG allotypes in the IgG response to SARS-CoV-2 has not yet been characterised. Here, we aimed to examine the relationship between IgG allotypes and vaccine-induced antibody responses to SARS-CoV-2.

Genomic DNA was extracted from granulocytes of individuals vaccinated against SARS-CoV-2 in Australia. Allotype identity was determined by PCR and sequencing of IgG1-4 constant heavy chain genes. Plasma samples were collected pre-vaccination (n=71), post second dose (n=67, median 28 days post second dose), and post booster (n=36, median 30 days post booster). SARS-CoV-2 specific IgG subclass titres and FcγR binding were evaluated using a multiplex assay.

The most common IgG1 and IgG3 allotypes in the vaccinees were G1m-1,3 (39%) and G3m5* (44%) respectively, mirroring previously reported distributions in Caucasian populations. SARS-CoV-2 spike-specific IgG1 titres were two-folds higher in G1m1/G1m1 vaccinees in contrast to G1m-1,3/G1m-1,3 vaccinees following both second and booster doses ($p<0.05$). Furthermore, spike-specific IgG3 titres were two-fold higher post second dose ($p=0.08$) and post booster ($p<0.05$) in G3m5*/G3m5* individuals compared to G3m21*/G3m21* individuals. In addition, engagement to FcγRIIIa was 1.5-fold higher in vaccinees who were homozygous for both G1m-1,3 and G3m5* compared to vaccinees homozygous for both G1m1 and G3m21* ($p<0.05$).

Overall, our study illustrates a potential link between IgG allotypes and IgG subclass distribution as well as FcγR binding following SARS-CoV-2 vaccination. Ongoing studies aim to explore how allotype driven differences in subclasses and FcγR engagement may modulate functional antibody responses and protection from infection. An understanding of allotypes may provide insight on mechanisms maximising vaccine-induced protection, and potentially improving vaccine boosting approaches in different populations.

Subversion of proteolytic capacity by lysosomal pathogens

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The mammalian lysosome is central to cellular homeostasis, acting as a control centre for nutrient recycling and destruction of foreign matter. Distinguishing features of the lysosome include an acidic pH and the presence of degradative hydrolases. The only known human pathogens to replicate inside a lysosome-derived niche are the Gram-negative bacterium *Coxiella burnetii* and protozoan parasites of the *Leishmania* species.

Here, we used activity-based probes to examine the activity of lysosomal proteases known as cathepsins during infection of macrophages with *C. burnetii* or *Leishmania mexicana*. This protease profiling revealed distinct changes to the host proteolytic capacity driven by each pathogen. Infection with *C. burnetii* led to a decrease in the activity of several cathepsins, while infection with *L. mexicana* largely increased cathepsin activity. The disparate host responses induced by these pathogens suggest divergent methods of avoiding lysosomal degradation.

Our team used SILAC-mass spectrometry to examine host proteome changes during *C. burnetii* infection. Using this method, we observed and experimentally validated a decrease in the abundance of several cathepsins during *C. burnetii* infection, though RT-qPCR demonstrated that cathepsin transcripts were not decreased. Furthermore, infection with a *C. burnetii* secretion system mutant revealed that cathepsin loss is dependent on a functional type IV secretion system, suggesting that a translocated bacterial effector protein may be destroying cathepsins to prevent bacterial degradation inside the lysosome.

Lastly, we developed a coinfection model to examine lysosomal proteolysis in cells infected with both *C. burnetii* and *L. mexicana*. Coinfection with *L. mexicana* led to a small rescue of cathepsins in *C. burnetii*-infected cells. The impact of cathepsin retention during co-infection is yet to be elucidated. This data suggests that *C. burnetii* and *L. mexicana* are actively modifying the lysosomal compartment in divergent ways. This likely reflects a mechanism of avoiding degradation in the lysosomal niche, shedding light on how these pathogens survive in such a harsh environment.

Lipid droplets are transferred between cells using dynamin-dependent mechanisms

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Lipid droplets (LDs) are ubiquitous organelles found throughout the cytoplasm of most cells. Recent work from our laboratory has shown that viral infection drives LD accumulation and increases LD speed in the host, underpinning a successful antiviral response through enhanced production of antiviral cytokines (Type I and III Interferon). We have also established that antiviral proteins (key ISGs) and membrane trafficking proteins (members of the RAB family) are significantly upregulated on virally driven LDs, which also contributes to our hypothesis that LDs can transport between cells to set up a “bystander effect” and this study sought to investigate this.

Determining intercellular LD movement is challenging due to diffusible dyes, so to address this, we created HeLa and primary immortalised astrocyte cell lines fluorescently expressing the LD resident protein; adipose differentiation-related protein (ADRP) tagged in either mCherry or GFP. Co-culture of these cells demonstrated a crossover of red and green LDs, and furthermore the uptake of isolated fluorescently tagged LDs could also be observed into naïve cells. To determine the endocytosis mechanisms utilised for LD uptake, primary immortalised astrocytes were treated with various concentrations of dynasore (dynamin inhibitor) and different concentrations of isolated mCherry-ADRP LDs. Preliminary data from fluorescent microscopy demonstrated that LD uptake decreases with increasing concentrations of dynasore, which was further supported with Western Blot analysis, probing for mCherry-ADRP. This suggests that the mechanism for LD entry into the cell is dynamin-dependent endocytosis such as clathrin-mediated or caveolin-dependent. To better understand the impact that isolated LDs have on neighbouring cells, isolated LDs from mock, or immune activated cells were placed on naïve cells before infection with the RNA virus, zika virus (ZIKV). Cells that received LDs isolated from mock infected cells had a 20% reduction in viral mRNA, with a 55% reduction in viral mRNA seen in cells that received LDs from immune activated cells.

Given the pro-host role of LDs during viral infection observed through our previous studies, understanding mechanisms for LD uptake could assist in the development of therapeutic agents to target viral pathways more efficiently to combat current and as yet unknown viral threats.

Interplay of type I and II interferons for the generation of CD8$^+$ stem-like memory T cells

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Following viral infection, the timing of interferon (IFN) signalling is precisely regulated within lymphoid organs to engage anti-viral responses. While type I IFN (IFN-I) signalling is required for viral clearance, deficiency or inhibition of the IFN-I receptor, IFNAR, drives the generation of a highly proliferative memory CD8$^+$ T cell subset termed stem-like memory T cells (T_{SL}). T_{SL} cells promote the generation of T effector cells following reinfection and PD-1 blockade, critical for pathogen clearance and cancer regression. Here, we show that blocking IFNAR signalling early during LCMV infection promotes potent formation of T_{SL}. Unintuitively, IFNAR blocking increased the expression of IFN-inducible chemokines CXCL9 and CXCL10, which are required for CXCR3-dependent intranodal movement of CD8$^+$ T cells. In turn, the chemokine gradient for T cell movement was disrupted, impeding cell migration. Thus, following IFNAR blocking T cells were retained within the lymph node paracortex where they differentiate into T_{SL}. We observed that IFNAR inhibition increased the IFNγ-dependent recruitment of monocyte-derived dendritic cells into lymph nodes which were crucial for the development of T_{SL}. Further, absence of both IFNAR and IFNγ ablate the expression of CXCR3 chemokines and maintained the differentiation of T_{SL} parallel to T cell exhaustion and increased viral load. Applying this knowledge, we used lipid nanoparticle encapsulated mRNA vaccination to show that early inhibition of IFNAR signalling directs exclusive generation of T_{SL} with loss of both effector and exhausted T cells. Combined, our findings demonstrate that the precise timing and interplay of IFN-I and IFN-II can be leveraged to inform vaccine design and immunotherapies to drive superior immune protection.

Chronic immune activation and barrier dysfunction in the gut is associated with persistent neuroinflammation in ART-suppressed SIV-infected rhesus macaques

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Introduction: HIV-associated neurocognitive disorders (HAND) affect ~40% of virally suppressed people with HIV (PWH), suggesting that HAND pathogenesis may be driven by mechanisms other than direct viral replication in the brain including chronic systemic inflammation. However, to date, the precise viral dependent and independent changes to the brain of virally suppressed PWH remains unclear. Here we comprehensively characterized the CNS reservoir and immune environment of SIV-infected (SIV+) rhesus macaques during acute (n=4), chronic (n=12) or ART-suppressed SIV infection (n=11).

Methods: Multiplex immunofluorescence for markers of SIV infection (vRNA/DNA) and immune activation was performed on frontal lobe and matched gut tissue. Brain and gut inflammation were also measured in an SIV-uninfected model of chronic colitis, validated to mimic SIV-induced gut damage, to determine the effect of gut damage on neuroinflammation independent of SIV infection.

Results: SIV+ animals contained detectable viral DNA+ cells that were not reduced in the brain or gut by ART ($P<0.05$), supporting the presence of a stable viral reservoir. SIV+ animals had heightened activation of astrocytes and myeloid cells as well as reduced blood-brain barrier (BBB) integrity compared to uninfected animals, and these dysfunctions were not abrogated by ART ($P<0.05$ for all). Interestingly, measures of brain immune activation and BBB integrity correlated with gut, but not brain, viremia and immune activation in virally suppressed animals. Furthermore, SIV-uninfected animals with experimentally induced gut damage showed a similar immune activation profile in the brain to animals with SIV, supporting the role of chronic gut damage as an independent source of neuroinflammation.

Discussion: Collectively, we show that ART-suppressed SIV+ rhesus macaques exhibit impaired BBB integrity and heightened myeloid cells (including microglia) and astrocyte activation which is associated in part with viral reservoirs and immune activation in the gut.

Conclusion: These findings provide the strongest evidence to date that the brain of SIV-infected animals remains in an activated state despite long-term ART treatment and that gut damage can directly induce neuroimmune activation. Thus, these findings have direct translational impact for PWH and provides rationale for the targeting of systemic inflammation as an adjunctive therapeutic approach to potentially improve brain health in PWH.

Influenza virus infection induces high levels of CD52 expression on effector CD8$^+$ T cells in the lungs

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PUBLISH CONSENT WITHHELD

CD8$^+$ T-cells provide broadly cross-reactive immunity against distinct influenza viruses, however key markers associated with optimal effector function of influenza-specific CD8$^+$ T-cells remain unclear. From single-cell RNA-sequencing, we identified *Cd52* was highly expressed on influenza-tetramer$^+$CD8$^+$ T-cells in the blood of a IBV-infected patient at 14 days post-infection. We investigated the novel role of CD52 on influenza-specific CD8$^+$ T-cells in mice to understand mechanisms underpinning generation of optimal effector CD8$^+$ T-cells. CD52 was also expressed at high levels on tetramer$^+$CD8$^+$ T-cells recruited to the site of infection during the effector phase in influenza A- (lung) and Semliki Forest virus-infected (brain) B6 mice. Functional, transcriptomic, and proteomic analyses of CD52^{hi}OT-I cells at the effector phase revealed signatures of exhausted-memory-like effector T-cell features, whereas CD52^{lo}OT-I cells displayed features of resting-memory CD8$^+$ T-cells and exhibited a more superior recall capacity at the memory stage (d30+). Co-transfer assays with control OT-I and CD52-depleting OT-I cells showed enhanced proliferation of CD52-depleting OT-I cells in comparison to control OT-I cells on day 8 post-infection. Moreover, co-expression of the activation marker, CD38, was higher on CD52-lacking OT-I cells than control at the site of infection. Our findings demonstrate how CD52 expression is tightly regulated within CD8$^+$ T-cell immune responses during influenza virus infection, thereby revealing novel mechanisms that underpin optimal CD8$^+$ T-cell immunity against influenza and other viruses.

Defining the factors that strategically position memory T cells within lymph nodes

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Immune memory is critical for providing superior protection against infection. Currently, vaccine strategies promote $CD8^+$ T cell responses with varying success, and vaccine outcomes for pathogens which require a strong T cell response are poor. Thus, there is an urgent need to understand the mechanisms which underpin the maintenance, function and longevity of T cell memory. Increasing evidence suggests that $CD8^+$ central memory T cells (T_{CM}) are positioned strategically in lymph nodes, however the mechanisms that regulate this location remain unknown. We used 3D light-sheet fluorescence microscopy of intact, draining lymph nodes to identify the location of T_{CM} cells following the resolution of viral infection. In steady-state lymph nodes, we showed that T_{CM} cells occupy a more peripheral region compared to naïve T cells. T_{CM} cells were quantified at a higher density in the cortical ridge and interfollicular regions than naïve T cells, which primarily reside in the T cell paracortex. This distinct T_{CM} location was observed following various infection challenges, including LCMV, HSV-1 and influenza, despite their unique modes of lymphatic drainage and dissemination. This finding emphasises the significance of a precise T_{CM} location and suggests a conserved mechanism of positioning that is independent of infection. Furthermore, in the lymph nodes of aged mice, this T_{CM} niche was disrupted and T_{CM} cells relocated to the T cell paracortex. To explore cell-cell contacts regulating T_{CM} location, we combined high-resolution confocal microscopy and single-cell RNA sequencing to identify the key interactions of T_{CM} with specific dendritic and stromal cells within this niche. Together, these findings suggest that T_{CM} occupy a conserved and precise lymph node memory niche which promotes cell-specific interactions and sustains long-term T_{CM} maintenance. In identifying the key mechanisms regulating the intranodal T_{CM} niche, our work will improve vaccine strategies grounded in robust and long-lived T cell memory.

RIPK1/3 regulates T cell responses in mucosal defence against bacterial gut infection

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Receptor interacting serine/threonine protein kinase (RIPK) 1 and 3 are key signaling factors in inflammation and programmed cell death. In particular, the interaction of RIPK1 and RIPK3 through their conserved RHIM domains results in necroptosis, where membrane pore formation releases danger-associated molecular patterns that drive an inflammatory response. Consequently, the diarrhoeagenic bacteria enteropathogenic *Escherichia coli* (EPEC) has evolved to express an effector that cleaves the RHIM domains of RIPK1/3 *in vitro*, however the physiological significance of this event to host immunity remains to be examined.

Using *Citrobacter rodentium* – the model organism for EPEC, evaluation of disease in a panel of RIPK knockout mice revealed that the absence of both RIPK1 and RIPK3 greatly heightened the diarrhoeal severity and bacterial burden experienced at peak infection. Notably, only RIPK3 played a significant role in moderating local gut pathology and in a manner independent from necroptosis. More interestingly, flow cytometry analysis in RIPK1/3-deficient mice also found a marked reduction in the T-helper 17 and T-regulatory cell populations in the colonic lamina propria following infection. This dysregulated T cell response is proposed to drive bacteria-induced colitis via RIPK1/3. In agreement with our results, challenge with an *espL* deleted *C. rodentium* mutant was sufficient to ameliorate disease in wildtype mice. Here, qPCR evaluation of inflammatory cytokine levels found an increased expression of *Il22*, but not *Il17a* in the colons of mutant *C. rodentium* infected mice, suggesting RIPK1/3 skews towards an anti-inflammatory response.

Here we show for the first time, a novel link between RIPK1/3 (innate immunity) and T cell responses (adaptive immunity) that is involved in host mucosal protection against enteric bacteria. Further characterisation of the underlying signaling pathways will be important to inform future management and treatment of serious gastrointestinal diseases.

Metal Ionophores Break Macrolide Resistance in *Streptococcus pneumoniae*

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Streptococcus pneumoniae (the pneumococcus) is a major human bacterial pathogen and a significant cause of morbidity and mortality worldwide especially in young children (≤ 5 years old), the elderly (≥65 years old), and those with underlying medical conditions. A rise in multidrug resistant isolates, coupled with a diminished antibiotic pipeline, and only limited protection provided by the current vaccines, highlights the need for novel and/or alternative approaches to treat *Streptococcus pneumoniae* infections. Globally, ~30% of pneumococcal isolates are resistant to at least one antibiotic, with macrolide resistance being highly prevalent.

Here, we investigated the use of a metal-shuttling ionophore to break antibiotic resistance in *S. pneumoniae*. Metal ions are innately antimicrobial and are an important component of the host immune response against invading pathogens. Ionophores, which facilitate unregulated transport of metal ions across the bacterial membrane, mediate significant toxicity in *S. pneumoniae*.

Through *in vitro* broth microdilution and time-dependent killing assays, we show breakage of antibiotic resistance in multiple clinical isolates when antibiotics are co-administered with an ionophore. This includes breakage of macrolide resistance, restoring the efficacy of the critical, frontline antibiotic, azithromycin. Current investigations are aiming to determine the cellular and molecular pathways facilitating this breakage of resistance. This will utilize a multi '-omic' approach including metalloproteomics, transcriptomics, and metabolomics, as well as *in vivo* analyses in established mice models of pneumococcal infection.

Collectively, this work describes how disruption of fundamental homeostasis pathways in bacteria may provide a mechanism to restore the efficacy of our current antibiotic arsenal to combat pneumococcal infection.

Early life cystic fibrosis lung disease is associated with immune cell influx and reduced epithelial cell HLA-DR expression in the lower respiratory epithelium

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Lung disease is the major cause of morbidity and mortality in cystic fibrosis (CF). It is characterised by an unresolving cycle of infection and inflammation that leads to progressive tissue damage. Myeloid cells in the lungs of adults with CF exhibit decreased HLA-DR expression, resulting in inadequate antigen presentation and recurrent infections [1]. Due to difficulties in obtaining respiratory samples in early life, this has not been explored in children. Further, it is unclear if the respiratory epithelium also displays alterations in HLA-DR. In this work, we used flow cytometry to assess the immune and epithelial cell landscape, as well as HLA-DR expression, in bronchial brushings from children with CF and non-CF controls. We discovered that bronchial brushings from all children had high proportions of immune cells [median immune% (non-CF = 90.46, CF = 69.05)], which is not seen in the respiratory epithelium of adults [2]. The percentage of immune cells was also significantly higher in CF children with structural lung disease (severe-CF) compared to CF children without structural lung disease (mild-CF) (p=0.045), indicating immune cell influx at epithelial sites in severe CF. Finally, we discovered that, HLA-DR is significantly downregulated in bronchial epithelial cells from children with CF compared to non-CF controls (p=0.0016). This work shows that the paediatric respiratory epithelium is enriched for immune cells, indicating that they may have an important role in maintaining the lung epithelium during early life. Poor antigen presentation by epithelial cells in combination with immune cell influx may contribute to early life CF lung disease.

1. Hofer, T.P., et al., *Decreased expression of HLA-DQ and HLA-DR on cells of the monocytic lineage in cystic fibrosis.* J Mol Med, 2014. **92**(12):p.1293-304.
2. Loske, J., et al., *Pre-activated antiviral innate immunity in the upper airways controls early SARS-CoV-2 infection in children.* Nat Biotechnol, 2022. **40**(3):p.319-324.

Differential NK cell and γδ T cell activation in SARS-CoV-2-infected pregnant women

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Pregnancy poses a greater risk for developing severe COVID-19, however the underlying mechanisms for increased disease severity with SARS-CoV-2 infection during pregnancy are poorly understood. To define the immune response to SARS-CoV-2 during pregnancy, we measured and analysed 217 immune parameters in unvaccinated pregnant and non-pregnant women during acute and convalescent COVID-19. Assessment of plasma cytokines revealed elevated levels of IL-8, IL-10 and IL-18 in healthy pregnant women, and these cytokine levels remained elevated in pregnant women with acute or convalescent COVID-19. Additionally, SARS-CoV-2-specific antibody titres were similar in pregnant and non-pregnant women, however cellular immune analyses revealed marked differences in the NK cell and γδ T cell responses in pregnant women with COVID-19. NK cells and γδ T cells displayed pre-activated phenotypes in healthy pregnant women when compared to age-matched non-pregnant women, however activation profiles of the pre-activated NK and γδ T cells remained unchanged in pregnant women during acute and convalescent COVID-19. Conversely, activation dynamics of NK and γδ T cells were prototypical in non-pregnant women with COVID-19. The activation of total αβ $CD4^+$ and $CD8^+$ T cells, T follicular helper cells and antibody-secreting B cells was similar in pregnant and non-pregnant women with COVID-19. Collectively, this study demonstrates perturbations within the innate immune compartment of unvaccinated pregnant women with COVID-19, while adaptive immunity was comparable to non-pregnant women. In-depth analysis of antigen-specific T and B cells using peptide-MHC tetramers and spike probes provides novel insights into virus-specific adaptive T cell and B cell immunity in pregnant and non-pregnant women. This work improves our understanding of pregnancy-specific immune responses and has the potential to inform patient management and education for those who are infected with SARS-CoV-2 during pregnancy.

ABSTRACTS
SESSION 4

Interferon epsilon limits ovarian cancer metastasis via regulation of peritoneal immune cells

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PUBLISH CONSENT WITHHELD.

The cytosolic DNA sensor AIM2 promotes gastric inflammation and tumourigenesis via inflammasome-dependent and -independent mechanisms

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Helicobacter pylori (*H. pylori*) infection can trigger chronic gastric inflammation perpetuated by overactivation of the innate immune system, leading to a cascade of precancerous lesions culminating in gastric cancer (GC). However, key molecular regulators of innate immunity that promote gastric carcinogenesis remain ill-defined. Here, we uncover divergent roles for the inflammasome-associated cytosolic DNA sensor Absent In Melanoma 2 (AIM2) in early-stage *Helicobacter*-induced gastric pathology and late-stage gastric tumourigenesis.

In independent clinical cohorts, *AIM2* gene expression was significantly upregulated in GC patient tumours and gastric tissue from *H. pylori*-positive (versus -negative) gastritis patients, and significantly correlated with worse GC patient survival. In addition, gastric *Aim2* mRNA levels were elevated in two independent disease models: 1) the spontaneous *gp130*$^{F/F}$ GC mouse model, and 2) wild-type (WT) mice subjected to chronic infection with *H felis*. *Aim2* ablation in *gp130*$^{F/F}$ mice significantly decreased tumour burden, however, inflammation and inflammasome activation were unaffected, suggesting the existence of a novel inflammasome-independent pro-tumourigenic mechanism for AIM2 in GC. Specifically, we identified a decrease in the migration potential of primary *gp130*$^{F/F}$ mouse gastric epithelial cells and GC patient organoids upon genetic ablation of AIM2. Critically, interaction with a microtubule regulatory protein, End Binding Protein 1 (EB1) was found to be important for mediating the migratory function of AIM2. These data support changes in epithelial cell migration as the underlying mechanism of the pro-tumourigenic role of AIM2 in GC.

Notably, *Helicobacter*-induced gastric inflammation and hyperplasia were less severe in *H. felis*-infected *Aim2*$^{-/-}$ versus WT mice, evidenced by reductions in immune cell infiltrates, mucosal thickness and pro-inflammatory cytokine and chemokine release. Additionally, *H. felis*-driven proliferation and apoptosis in both gastric epithelial and immune cells was largely attenuated in *Aim2*$^{-/-}$ mouse stomachs. These observations correlated with decreased inflammasome activity, supporting an inflammasome-dependent driving role for AIM2 in *Helicobacter*-associated inflammation.

Taken together, this work uncovers two distinct pathogenic roles for the AIM2 in *Helicobacter*-induced gastric disease and GC, and thus furthers our understanding of the host immune response to a common pathogen and the complex and varying roles of AIM2 at different stages of cancerous and pre-cancerous gastric disease.

Impaired HA-specific T follicular helper cell and antibody responses to influenza vaccination are linked to inflammation in humans

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Antibody production following vaccination can provide protective immunity to subsequent infection by pathogens such as influenza viruses. However, circumstances where antibody formation is impaired after vaccination, such as in older people, require us to better understand the cellular and molecular mechanisms that underpin successful vaccination in order to improve vaccine design for at-risk groups. Here, by studying the breadth of anti-haemagglutinin (HA) IgG, serum cytokines, and B and T cell responses by flow cytometry before and after influenza vaccination, we show that formation of circulating T follicular helper (cTfh) cells was associated with high-titre antibody responses. Using Major Histocompatability Complex (MHC) class II tetramers, we demonstrate that HA-specific cTfh cells can derive from pre-existing memory $CD4^+$ T cells and have a diverse T cell receptor (TCR) repertoire. In older people, the differentiation of HA-specific cells into cTfh cells was impaired. This age-dependent defect in cTfh cell formation was not due to a contraction of the TCR repertoire, but rather was linked with an increased inflammatory gene signature in cTfh cells. Together, this suggests that strategies that temporarily dampen inflammation at the time of vaccination may be a viable strategy to boost optimal antibody generation upon immunisation of older people.

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Unravelling the antiviral mechanisms by which *Wolbachia pipientis* restricts dengue virus replication

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Aedes aegypti mosquitoes are the major vector for human pathogenic viruses such as dengue virus (DENV). Over the past decade, the insect endosymbiotic bacterium *Wolbachia pipientis* has been introduced in *Ae. aegypti* populations as an antiviral biocontrol tool, significantly reducing the mosquito-human transmission of *Aedes*-borne viruses in regions where these mosquitoes are established [1, 2]. Despite the success of this intervention, we still do not fully understand how *Wolbachia* restricts viruses. Here, we sought to compare two antiviral and one non-antiviral *Wolbachia* strains stably infecting *Ae. aegypti*-derived cell lines (*Aag2*.*w*Mel, *Aag2*.*w*AlbB and *Aag2*.*w*Pip, respectively). *Aag2*.*w*Mel showed potent antiviral activity dramatically reducing DENV RNA copies and plaque titers compared to its matched *Wolbachia*-free line (*Aag2*.Tet; cells cured of *Wolbachia* infection by tetracycline). *w*AlbB also significantly inhibited DENV replication in *Aag2* cells but the antiviral activity was weaker than *w*Mel. By contrast, *w*Pip had no impact on viral replication or infectious virus. To investigate the mechanisms underlying the antiviral phenotype, *Wolbachia* subcellular localization in *Aag2* was determined by transmission electron microscopy. We observed that *w*Mel and *w*AlbB frequently associated with the host endoplasmic reticulum (ER), while *w*Pip did not. We also investigated *Wolbachia*'s impact on the ER-derived organelle, lipid droplets (LDs). Primarily, all *Wolbachia* strains increased the number of LDs compared to *Aag2*.Tet. *Aag2*.*w*Mel accumulated fewer but bigger LDs, while *Aag2*.*w*AlbB and *Aag2*.*w*Pip accumulated smaller LDs, with *Aag2*.*w*AlbB inducing more LD's per cell, suggesting that antiviral strains may increase the total volume of LDs. Interestingly, DENV infection caused LDs to further accumulate in *Aag2*.*w*Mel and *Aag2.w*AlbB, but not *Aag2*.*w*Pip. These results indicate that the antiviral impacts of *Wolbachia* may be associated with ER disruption and perturbed lipid storage. Further work is required to validate the role of these host impacts in viral inhibition. Understanding how *Wolbachia* generates an antiviral state in *Ae. aegypti* will fill a vital knowledge-gap to support the long-term success of *Wolbachia* in the field.

1. Indriani, C., et al., Reduced dengue incidence following deployments of Wolbachia-infected Aedes aegypti in Yogyakarta, Indonesia: a quasi-experimental trial using controlled interrupted time series analysis. Gates Open Research, 2020. **4**: p. 50.
2. Utarini, A., et al., Efficacy of Wolbachia-Infected Mosquito Deployments for the Control of Dengue. New England Journal of Medicine, 2021. **384**(23): p. 2177-2186.

REAPER: mosquito *in vivo* virus targeting to control viral transmission

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Publish consent withheld.

The microbial metabolite indole-3-propionic acid protects against mitochondrial dysfunction in the airway epithelium and allergic asthma inflammation

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Microbial colonisation in early life is instrumental in educating the immune system to mount tolerance to allergens. Dysbiosis in the gut microbiota during this crucial window increases risk of developing metabolic disorders, atopy and wheeze later in life. Animal models have revealed that depletion of the gut microbiota elevates allergic inflammation in the lung, but the underlying mechanisms remain poorly understood. We treated mice with antibiotics directly after weaning and co-housed them with non-treated SPF littermates for six weeks. Mice treated with antibiotics in early life were more susceptible to house-dust mite (HDM) induced allergic asthma directly after antibiotics treatment, which was maintained after cohousing, as shown by increased eosinophilia, dendritic cell activation in the lung and Th2 cytokine production upon restimulation of the mediastinal lymph nodes compared to their SPF littermates. Cohousing allowed for recolonization of the bacterial community and restored gut permeability, systemic inflammation and immune parameters in circulation and lung. Interestingly, we found a perturbed immune-structural interplay in antibiotic-treated mice in the lung after cohousing, as shown by an elevated chemokine production upon HDM stimulation. Single-cell RNA sequencing revealed significant changes restricted to the epithelial cell compartment, with the majority of differentially expressed genes involved in mitochondrial functioning. Epithelial mitochondrial dysfunction in the epithelium of antibiotic-treated mice was further shown by an increased mitochondrial membrane potential and superoxide production. To investigate what could be driving this dysfunction we employed untargeted metabolomics on serum. The microbial-derived metabolite indole-3-propionic acid (IPA) was 8-fold decreased upon antibiotics treatment. In vitro experiments confirmed that IPA inhibits mitochondrial respiration by the airway epithelium, which could dampen the production of reactive oxygen species and onset of allergic asthma. Our findings highlight a novel mechanism through which early-life antibiotics treatment can result in long-lived metabolic dysfunction of the airway epithelium and increased susceptibility to allergic asthma.

Eat to compete: Understanding immunometabolic interactions that drive *Candida auris* infection

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Opportunistic fungal pathogens that cause human disease pose a serious threat to public health. The rapid emergence of drug resistant pathogenic fungi such as *Candida auris,* along with the overuse of commercially available antifungal drugs and inadequacies in drug development, have meant that our therapeutic safety net for fungal infections has become limited [1, 2]. Recently, the study of immunometabolism (how immune cells remodel their metabolism when challenged by fungal pathogens) during fungal infection, has offered significant insights into host-pathogen metabolic crosstalk as well as potential for innovation in antifungal treatments. We investigate the metabolic interactions of *C. auris* with macrophages during infection. We show that *C. auris* replicates robustly in macrophages and is able to escape macrophages without killing them. However, after escape, *C. auris* will outcompete macrophages for nutrients, ultimately resulting in macrophage death without inducing a strong immunological response. We also focus on how macrophages maintain glucose homeostasis to fight off fungal infections and demonstrate that glucose supplementation improves immune-cell outcomes. Utilising various mutants affecting glycolytic metabolism of *C. auris*, we demonstrate that the ability to utilise glucose plays a key role in the establishment and progression of infection. Our data suggests that *C. auris* escapes immune containment by mechanisms that differ from those used by other *Candida* pathogens and can leverage host metabolic shifts for survival and proliferation.

1. Hanson, B.M., Dinh, A.Q., Tran, T.T., Arenas, S., Pronty, D., Gershengorn, H.B., Ferreira, T., Arias, C.A., and Shukla, B.S. (2021). Candida auris Invasive Infections during a COVID-19 Case Surge. Antimicrob Agents Chemother. 65(10), e0114621.
2. Jacobs, S.E., Jacobs, J.L., Dennis, E.K., Taimur, S., Rana, M., Patel, D., Gitman, M., Patel, G., Schaefer, S., Iyer, K., et al. (2022). Candida auris Pan-Drug-Resistant to Four Classes of Antifungal Agents. Antimicrob Agents Chemother.

ABSTRACTS
SESSION 6

Redundant activity of caspase-1, 3, 7, and gasdermin-D drives cell death and IL-1β release in XIAP deficiency

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Genetic lesions in X-linked Inhibitor of Apoptosis (XIAP) pre-dispose to cell death-associated inflammatory diseases in humans, marked by serum elevation in the pro-inflammatory cytokine IL-1β. Heightened IL-1β release in XIAP deficiency hinges upon the increased activation of apoptotic caspase-8, which processes pro-IL-1β into its bioactive fragment and allows for its release. Caspase-8 also activates the NLRP3-inflammasome complex, leading to caspase-1 activation which further drives IL-1β processing and release. However, the mechanisms by which caspase-8 triggers both IL-1β release and NLRP3-caspase-1 activation remain unclear. Here, we report that patients lacking XIAP that present with inflammatory bowel disease display heightened apoptotic caspase-8 and inflammatory IL-1β processing, and this correlates with pyroptotic gasdermin-D (GSDMD) activity in diseased tissue. Using models of XIAP deficiency, we show that excess caspase-8-driven cell death and bioactive IL-1β release is only abrogated by combined deletion of the caspase-8 substrates GSDMD, caspase-3 and caspase-7, alongside caspase-1. We further investigated the roles of caspase-3, -7 and GSDMD in caspase-8-dependent activation of NLRP3 and found that neither the single loss of GSDMD nor the caspase-3/-7 substrate pannexin-1 prevented NLRP3 inflammasome assembly downstream of caspase-8. Remarkably however, pannexin-1 was required for NLRP3 activity downstream of mitochondrial caspase-9-dependent apoptosis. These findings uncouple the mechanisms of cell death and NLRP3 activation resulting from extrinsic and intrinsic apoptosis, reveal how XIAP loss can co-opt dual cell death programs, and uncover strategies for targeting the cell death and inflammatory pathways that result from XIAP deficiency.

Lyn expression is increased in lupus patients and is associated with immune cell activation and disease severity

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The tyrosine kinase Lyn maintains a fine balance in the activation of B and myeloid cells; Lyn simultaneously promotes activation and prevents hyper-activation by propagating signalling from both activating and inhibitory immunoreceptors. Animal models have illustrated this duplicitous function, as both Lyn-deficient mice and mice expressing a constitutively active form of Lyn develop spontaneous autoimmune pathology that resembles lupus due to hyperactivated B and myeloid cells. Two early human studies suggested B cells from lupus patients exhibited reduced Lyn expression, and as a consequence, the 'Lyn-deficient' scenario was adopted as the dominant view of Lyn's role in human lupus disease. However, these studies only utilised a small number of patients and were relatively rudimentary. Therefore, we undertook analyses of five large public datasets to more extensively investigate the role of Lyn in lupus disease. Unexpectedly, we found Lyn expression to be increased in lupus patients compared to healthy controls in whole blood expression datasets. Gene Set Enrichment Analysis identified that overwhelmingly, Lyn expression was associated with immune cell activation pathways and correlated strongly with the type-I interferon signature. Lyn expression was also found to serve as a biomarker of disease severity, as patients with high Lyn expression showed more severe disease presentation than patients with low Lyn expression. Given that bulk sequencing datasets can be confounded by variations in immune cell frequencies, we also analysed a single cell sequencing dataset of kidney-infiltrating immune cells from lupus patients. This analysis reiterated our initial findings, showing that Lyn expression increased with the activation and interferon status of B and myeloid cell populations. This work suggests that contrary to current thinking, Lyn exerts a pathogenic effect in lupus patients through overexpression. We hypothesise that Lyn expression is induced by interferon exposure, with Lyn overexpression promoting further immune cell dysregulation.

Insight into MR1T cell TCR specificity and recognition of MR1

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The major histocompatibility complex class I-related protein 1 (MR1) is a non-polymorphic and specialised antigen presenting molecule that presents microbial-derived vitamin B_2 metabolites to an unconventional T cell subset known as mucosal-associated invariant T (MAIT) cells. Increasing studies suggest MR1 has the capacity to bind a more extensive repertoire of ligands than currently appreciated, including self-derived metabolites. Moreover, a novel subset of T cells, termed 'MR1T' cells that respond to MR1 expressed on mammalian cells has been recently identified. Despite intensive effort, determining the nature of MR1T antigens (Ags) has been hampered by lack of reagents and limited characterisation of the binding properties of MR1T cell antigens to MR1. In this study, we provide insights into the molecular properties of potential MR1T Ags by investigating the TCR specificity of novel MR1T cell clones to endogenous and defined MR1 ligands presented by MR1 expressing antigen presenting cells (APCs). We further dissect MR1T cell response using APCs expressing MR1 that has been mutated at residues along the $\alpha1$ and $\alpha2$ helices and across the A'- and F'-pockets of the MR1 ligand-binding groove. Taken together, this study has expanded our limited knowledge of MR1T cells TCR specificities and the requirement on MR1 for MR1T cell reactivity. The reagents developed in this study will significantly facilitate future work describing the precise chemistry of MR1T Ags.

Regulation of immune checkpoint molecules through ubiquitination

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Activation of T cells by antigen stimulation is regulated by stimulatory and inhibitory molecules known as immune checkpoints. Checkpoint molecules such as PD-L1 and CD86 are expressed on the surface of antigen-presenting cells. While the success of cancer therapies targeting immune checkpoint molecules has led to extensive research on immune checkpoint regulation in cancer cells, little has been done to understand regulation in immune cells[1].

In recent years, advancements in ubiquitination analysis have led to its recognition as a highly complex post-translational modification. The resulting "ubiquitin code" generates a strong diversity in protein function[2]. Here, we aim to explore the ubiquitin linkages and branching architecture of the ubiquitin code associated with checkpoint molecules such as CD86 and PD-L1 using absolute quantification mass spectrometry and ubiquitin clipping. We identified ubiquitin-like protein 3 (UBL3) as a novel regulator of CD86 ubiquitination in immune cells[3] and further aim to assess how UBL3 modifies the ubiquitin code of CD86. Overall, our findings will generate insight on the regulation of immune checkpoint molecules and further our understanding on how T cell activity is controlled in the context of autoimmunity, infection, and cancer.

References

1. Zerdes, I., Matikas, A., Bergh, J., Rassidakis, G. Z. & Foukakis, T. Genetic, transcriptional and post-translational regulation of the programmed death protein ligand 1 in cancer: biology and clinical correlations. *Oncogene 2018 37:34* **37**, 4639–4661 (2018).
2. Ohtake, F. & Tsuchiya, H. The emerging complexity of ubiquitin architecture. *The Journal of Biochemistry* **161**, 125–133 (2017).
3. Liu, H. *et al.* Ubiquitin-like protein 3 (UBL3) is required for MARCH ubiquitination of major histocompatibility complex class II and CD86. *Nature Communications 2022 13:1* **13**, 1–15 (2022).

Investigating the effects of short chain fatty acids on fungal pathogen-host interactions

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The human gastrointestinal (GI) tract hosts a diverse microbiome comprising of bacteria, viruses and fungi within complex bionetworks and dynamic microenvironments (1). Here microbes, fundamental to host digestion and metabolism of dietary macronutrients, coexist and compete for nutrients to survive (1). Of the many metabolite bi-products generated by microbes involved in these processes, short chain fatty acids (SCFAs) are known to impact both host and microbial physiology (2). The yeast *Candida albicans* is a commensal microbe of the GI tract which can also cause superficial to severe systemic disease (3). It's ability to morphologically transition from a yeast to a pathogenic hyphal form is correlated with its clinical manifestation (3).

We investigate the signalling pathways influenced by the metabolism of SCFAs, and how they affect gene expression via histone acylation and hyphal morphogenesis of *C. albicans*. We show that the SCFA crotonate, which causes the posttranslational modification of lysine crotonylation of histones and other proteins, represses hyphal morphogenesis of *C. albicans* in immune cell macrophages and in media mimicking the phagosomal environments. It also reduces hyphae-dependent macrophage killing by *C. albicans.* Furthermore, RNAseq analysis showed that crotonate upregulates fatty acid metabolic process and inhibits the expression of hyphal genes needed to drive pathogenicity in *C. albicans*. Our data suggests that crotonate acts in concert with hyphal transcriptional repressors to regulate hyphal morphogenesis. Collectively, these studies should shed light on the impact of SCFAs on *C. albicans* invasive hyphal morphogenesis and how it influences pathogenicity and virulence.

References

1. Koh A, De Vadder F, Kovatcheva-Datchary P, Backhed F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. Cell. 2016;165(6):1332-45.
2. Chen XF, Chen X, Tang X. Short-chain fatty acid, acylation and cardiovascular diseases. Clin Sci (Lond). 2020;134(6):657-76.
3. Mayer FL, Wilson D, Hube B. Candida albicans pathogenicity mechanisms. Virulence. 2013;4(2):119-28.

Dissecting mechanisms of immune-mediated control of melanoma

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It is now widely appreciated that the immune system, particularly T cells, can influence disease outcomes in cancer. To study this, our group recently developed a translatable epicutaneous melanoma model that shows variable primary tumour kinetics and outcomes, ranging from rapidly growing tumours to persisting microscopic lesions. This has since been extended to include curative-intent surgery of primary tumours, allowing the study of spontaneously developing melanoma metastases in the tumour-draining lymph nodes. Using this model, we aim to define the immune mechanisms that control persisting primary and metastatic tumours, which could be harnessed to combat occult melanoma and disease recurrence.

We have been able to induce stable control of both primary and metastatic tumours over several weeks through adoptive T cell therapy (ACT) with tumour-specific T cells. Furthermore, we have refined the model by selecting the time point of surgery based on tumour size, which we show increases the rate of metastatic disease for studying spontaneous immune responses. The immune contexture and T cell dynamics of these situations of spontaneous or induced tumour control are now being interrogated using high-dimensional spectral cytometry, single-cell RNA sequencing, and static and live imaging. In the skin cut-outs from surgery, a higher number of tumour-specific T cells strongly predicted these mice would not develop metastases. In the tumour-draining lymph node, high dimensional flow cytometry revealed that tumour-specific T cells in outgrowing metastases display phenotypic indicators of exhaustion or dysfunction. This population was highly localised to the outgrowing metastases and absent from mice with stably controlled lesions. These data allude to the importance of both the quantity and quality of the T cell response in disease outcome, which provides precedence for identifying biomarkers that predict metastases progression or control.

Comparing the cell-specific innate immune response to RSV between preterm and term infants

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Background: Respiratory syncytial virus (RSV) is the most common cause of childhood lower respiratory tract infection globally. A common risk factor for severe RSV disease includes premature birth, with a higher morbidity and mortality rate in preterm infants. As the adaptive immune response of preterm infants is still developing, the immaturity in innate immune response is postulated as an important factor for the increased risk of severe RSV disease. However, there is limited data on the susceptibility of specific innate immune cells to RSV infection.

Methods: We stimulated cord blood mononuclear cells (CBMCs) from 25 moderate preterm and 25 term infants with RSVA for 24 hours. Innate immune cell-specific intracellular cytokine production, including IL-6, IL-1b, IL-8, IL-10, TNF-a, IFN-a, IFN-g was examined by high-dimensional flow cytometry.

Results: Our interim analysis revealed that RSV predominantly infects myeloid dendritic cells (mDCs) (50% of total mDCs) and monocytes (61% of total monocytes). RSV stimulation significantly increased the production of IL-6, IL-1b and TNF-a in both mDCs (8-fold, 5-fold and 5-fold increase respectively) and monocytes (5-fold, 2-fold and 7-fold increase respectively) compared to unstimulated CBMCs. A similar level of IL-8 production was observed between RSV stimulated and unstimulated CBMCs. The analysis of the differences in cell-specific cytokine response to RSV between preterm and term infants is ongoing.

Conclusion: RSV can infect a major proportion of innate immune cells and influence their cytokine production. These differences may help to explain why some infants develop severe RSV disease and could be used to inform new strategies to protect high-risk groups.

Eliciting mucosal immunity to SARS-CoV-2 using recombinant influenza viruses

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ABSTRACT

Introduction:
Recombinant influenza viruses are promising mucosal vaccine vectors for combatting infectious respiratory diseases such as COVID-19. In contrast to intramuscular delivery, intranasal vaccine administration can trigger robust protective immune responses directly in the lung mucosa. These include secretory antibody and tissue-resident memory T and B cell responses. As the mucosa serves as the primary site of viral replication, elicitation of mucosal immunity has been proposed as a potential pathway to augment vaccine mediated protection by conferring sterilising immunity against infection and disrupting transmission.

Methods:
The NS genomic segment of influenza A was modified with a heterologous gene expressing a trimerised SARS-CoV-2 receptor binding domain (RBD) of the spike protein. Using an eight-plasmid reverse genetics platform, we generated replication-competent recombinant PR8 (H1N1) and X31 (H3N2) influenza A viruses. Expression of RBD was confirmed in vitro by infection of MDCK cells and detection of RBD in the cell culture supernatant by ELISA. To assess in vivo infection, C57BL/6 mice were intranasally infected with the recombinant viruses. Immunogenicity was assessed serologically and by detection of B and T cell responses in the lung and lung-draining mediastinal lymph nodes (mLN).

Results:
Recombinant influenza vectors mediate productive infection and elicits elevated serum and lung-localised anti-RBD IgG and IgA antibody titres following a heterologous prime-boost infection with PR8 and X31 recombinant viruses. Concomitant with the serology, mice exhibited robust RBD-specific germinal center and memory B cell responses in both the lungs and mLN.

Conclusion:
Our findings demonstrate recombinant influenza-based vaccines can stably deliver SARS-CoV-2 RBD immunogens. Furthermore, prime-boost vaccination elicits lung-localised RBD-specific antibodies and mucosal B cells. Thus, recombinant influenza-based vaccines provide a novel, tunable platform to seed mucosal immune responses to enhance protection provided by first-generation vaccines against COVID-19.

Regulation of T cell migration by novel CXCR3 chemokine niche formation

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Cell migration is a fundamental process for immune defence. Chemokines are key signalling molecules that guide migration direction by forming concentration gradients, enabling immune cells to efficiently survey tissues and strategically relocate. CXCR3 is an essential chemokine receptor, expressed by activated T cells in eliciting and coordinating inflammatory immune responses. CXCR3 has two ligands CXCL9 and CXCL10 in C57BL/6 mice. Despite being in the same family, the transcriptional sources of CXCL9 and CXCL10 are located in different regions of the lymph node. Here, we use quantitative imaging to characterise CXCR3 chemokines protein distribution in mouse lymph node. We show CXCL9 and CXCL10 exhibit spatially distinct gradient and binding in tissue. In acute LCMV infection, CXCL9 accumulates and binds to the cell surface in the B cell follicle, separate to its cellular source within the T cell paracortex. This suggests a novel interaction between CXCL9 with extracellular matrix components expressed by B cells. In contrast, CXCL10 is concentrated in the interfollicular region in partial colocalisation with its cellular sources. These findings suggest chemokine niche formation within the lymph node underpins T cell migration, interaction, and subsequent differentiation. We further compare chemokine gradients during acute and chronic infections as well as in solid tumour microenvironment. Our work demonstrates a previously unappreciated regulation of T cell migration by CXCR3 chemokines. To deconstruct the chemokine environment, we developed novel microfluidic device and combined with high-resolution Lattice Lightsheet Microscopy. Quantifying dynamic T cell migration in this device will lead us to understand how T cells navigate subcellular content in complex chemokine environment. This will provide new insights in T cell migration and differentiation during disease progression or clearance and generate new avenues for better immunotherapy.

Novel bacteriophage depolymerase DpK2 degrades capsule of hypervirulent *Klebsiella pneumoniae* K2 strains

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Multi-drug resistant strains of *Klebsiella pneumoniae* represent a global health burden. Hypervirulent *K. pneumoniae (hKp)* strains can cause serious disease and infections are difficult to treat. *hKp* are generally characterized by the presence of a thick outer layer of polysaccharide, known as a capsule, that can mediate protection against host innate immunity and environmental hazards.[2] Removal of the capsule can render strains avirulent and susceptible to the environment.[2] Bacteriophages that target *Klebsiella* needs to navigate the capsule to allow access to cell surface receptors for infection of the bacterial cell. Tailed bacteriophages achieve this by producing tail-spike proteins (TSPs) containing depolymerase activity that can specifically degrade target cell capsule. Our team has identified a novel bacteriophage, known as Npat, that can infect K2-type *K. pneumoniae.* Npat encodes a TSP with putative depolymerase activity which was purified by affinity and size exclusion chromatography. The preliminary structural analysis suggest that it is a homotrimer with three distinct domains, a common assembly for phage depolymerases. Using purified protein, we have screened strains encoding four different capsule types (K0, K1, K2 and K3) for capsule loss which shows that the purified protein is specific for a K2-type capsule. We hypothesize that the specificity of Npat_DpK2 for K2 serotype strains arises from its C-terminal carbohydrate binding module that shows structurally homology to other sugar-binding domains. Future studies aim to dissect the protein-substrate interactions using molecular biology approaches. Finally, in conjunction with functional assays, we will be using cryo-electron microscopy to reveal the structure of the enzyme that will elucidate the determinants of specificity.

References

[1] Murray, C. J. L.*, et al.* (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis, *The Lancet.*
[2] Dunstan, R. A.*, et al.* (2021) Mechanistic Insights into the Capsule-Targeting Depolymerase from a Klebsiella pneumoniae Bacteriophage, *Microbiol Spectr.*

The lysine methyltransferase DOT1L is critical for regulatory T cell identity

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Regulatory T (Treg) cells play an indispensable role in maintaining immune homeostasis and preserving tolerance to self and innocuous antigens; however, they can also inhibit anti-tumour immunity. Treg cells are defined by the expression of the transcription factor FOXP3 and can develop in the thymus (tTreg) or be induced (iTreg) from naive $CD4^+$ T cells *in vivo* or *in vitro* in the presence of IL-2 and TGF-β. The establishment of a specific epigenetic landscape, via various epigenetic modifiers, has been shown to be critical for the development, stability and function of Treg cells. However, the precise role of one epigenetic modifier, the lysine methyltransferase Disruptor of telomeric silencing 1-like (DOT1L), in Treg cell biology is unknown. Here, we show that DOT1L is a novel regulator of Treg cell identity. A T cell intrinsic deletion of DOT1L led to a significant reduction of mature Treg cells in the thymus, secondary lymphoid and non-lymphoid tissues. In the absence of DOT1L, iTreg cell differentiation and identity was impaired, resulting in $FOXP3^+$ iTreg cells possessing a transcriptional signature akin to Th1 or $CD8^+$ effector T cells. Strikingly, Treg-specific ablation of DOT1L resulted in severe spontaneous autoimmune and inflammatory disease at 3-4 weeks of age, despite the presence of $FOXP3^+$ tTreg cells. RNA sequencing of DOT1L-deficient tTreg cells also revealed an upregulation of genes associated with a Th1/$CD8^+$ T cell effector response such as *Ifng, Gzmb* and *Tbx21,* with a concomitant downregulation of genes associated with Treg cell identity such as *Foxp1, Ikzf4* and *Bach2*. Taken together, these findings identify DOT1L as a critical regulator of Treg cell identity, potentially serving as a novel target for destabilising or reprogramming Treg cells into Th1/$CD8^+$ effector like T cells during cancer immunotherapy.

Understanding immune responses towards COVID-19 BNT162b2 vaccines in Indigenous people

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Indigenous people are disproportionately affected by infectious diseases, including COVID-19. There are ~798,365 Aboriginal and Torres Strait Islander people in Australia. With high vulnerability to COVID-19, understanding immune responses towards COVID-19 vaccines in Indigenous people is urgently needed.

We evaluated the breadth of immune responses in Indigenous (n=58) and non-Indigenous (n=39) individuals after COVID-19 BNT161b2 vaccination, and in 13 Indigenous hospitalized COVID-19 patients. We assessed receptor binding domain (RBD)-specific antibodies, spike/RBD-probe-specific B-cells, spike-specific T-cells following peptide stimulation using activation-induced marker (AIM) and intracellular cytokine (IFN-γ/TNF) staining, and epitope-specific $CD4^+$/$CD8^+$ T-cell responses via the use of peptide-HLA tetramers directly *ex vivo*.

We found high seroconversion rates for ancestral RBD at 1-mth post-dose 2 of BNT162b2 vaccine for Indigenous (92%) and non-Indigenous individuals (100%). Antibody titres against ancestral and Delta RBD were, however, lower after dose 2 in Indigenous people, and those increased after the booster. Both groups had low Omicron RBD antibodies. Although comparable frequencies of tetramer-specific $CD4^+$ T-cells were found between two groups, dominated by T_{CM}-like phenotype, significantly lower frequencies of SARS-CoV-2-specific $CD8^+$ T-cells were detected in the Indigenous group at 1-mth post-dose 2, however those increased after the booster. T-cell AIM and IFN-γ^+TNF$^+$ production were comparable between Indigenous and non-Indigenous groups at 1-mth and 6-mth post-dose 2.

During hospitalization of Indigenous COVID-19 patients, RBD and nucleoprotein antibodies, antibody-secreting cells, T follicular helper cells, tetramer-specific T-cells and peptide-stimulated AIM^+ T-cells resembled those reported for non-Indigenous COVID-19 hospitalized patients.

Our study provides key insights into immune responses following SARS-CoV-2 infection and vaccination in Indigenous people, and supports recommendations for Indigenous people to receive the COVID-19 booster dose.

ABSTRACTS
POSTER 1

Breast immunity in development and cancer

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Mammary gland development and function are not only controlled by hormones — the immune system is also critical. Duct growth is negatively regulated by T cells, while mast cells and eosinophils promote morphogenesis. Macrophages facilitate the dramatic tissue remodelling during reproduction and plasma cells provide protective IgA to infants. Beyond the basic requirement for these immune cells, their precise functions and mechanisms are largely unknown. Central to this is identifying which breast tissue cells form instructive immune cell niches that determine cell function, and what factors they use to communicate.

We and others have sought to better define the cell interactions in breast immunity by using whole-tissue 3D imaging and intravital imaging. Using these approaches, we discovered a unique population of ‘ductal’ macrophages that survey the entire epithelium with fine dendrites. These techniques have also revealed the exact location of plasma cells that arrive in the breast during lactation. We are now exploring how immune cells are instructed by the breast environment, so that we can learn how to alter cell function to fight infection and cancer. There are three key approaches we are taking to tackle this. Firstly, spatial transcriptomics will allow us to see how neighbouring cells communicate and influence immune cell function. Secondly, gene editing of the mammary epithelium and stromal cells will determine the importance and role of individual niche signals. And thirdly, novel mouse models for targeted cell ablation will help to define their function more accurately. Through these efforts, we hope to discover new avenues for immunotherapeutic treatment of breast diseases like inflammation and cancer.

Revealing neurotoxicity pathways in response to intraventricular polymyxin B in rat

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Abstract

Background:

Intraventricular or intrathecal polymyxins are increasingly used to treat multidrug-resistant (MDR) Gram-negative infections in the central nervous system (CNS). However, our limited knowledge of the mechanisms underpinning polymyxin-induced neurotoxicity significantly hinders the development of safe and efficacious polymyxin dosing regimens.

Methods:

To this end, we conducted transcriptomic analyzes of rat brain and spinal cord 1 h following intracerebroventricular administration of polymyxin B into rat lateral ventricle at a clinically relevant dose (0.5 mg/kg). Mass spectrometry imaging was used to show the distribution of the polymyxin in the rat brain after intraventricular injection and electrophysiology was conducted to record the neuronal activity under polymyxin treatment.

Results:

Following polymyxin B treatment, 66 differentially expressed genes (DEGs) were identified in the brain transcriptome; while none were detected for the spinal cord (FDR ≤ 0.05, fold-change ≥ 1.5). DEGs were enriched in signaling pathways associated with hormones and neurotransmitters, specifically dopamine and (nor)epinephrine. Notably, the expression levels of *Slc6a3* and *Gabra6* were decreased by 20-fold and 4.3-fold, respectively; likely resulting in major perturbations of dopamine and γ-aminobutyric acid signaling in the brain. Mass spectrometry imaging of brain sections revealed a distinct pattern of polymyxin B distribution with the majority accumulating in the injection-side lateral ventricle and subsequently into third and fourth ventricles. Polymyxin B was not detectable in the left lateral ventricle or brain tissue. Electrophysiological measurements on primary cultured rat neurons revealed a large inward current and significant membrane leakage following polymyxin B treatment.

Significance:

Our work demonstrates for the first time, the key CNS signaling pathways associated with polymyxin neurotoxicity. This mechanistic insight combined with pharmacokinetic/pharmacodynamic dosing strategies will help guide the design of safe and effective intraventricular/intrathecal polymyxin treatment regimens for CNS infections caused by MDR Gram-negative pathogens.

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Phage steering on antibiotics: Not always a seesaw effect

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Background
The global threat from antimicrobial resistance requires effective alternative treatment strategies. An increasingly popular approach is bacteriophage (phage) therapy. Phage steering–where phages kill susceptible bacteria and re-sensitise the remaining phage-resistant bacteria to antibiotics–is gaining popularity and would enable phage(s) and antibiotic(s) combination to completely kill bacteria.

Objective
This study investigated the steering effect on antibiotic susceptibility of phage-resistant *Klebsiella pneumoniae* mutants.

Methods
Phage pKMKP103_1-resistant mutants were screened with a transposon mutant library of *K. pneumoniae* MKP103 and confirmed by time-kill studies. Efficiency of plating (EOP) of phage pKMKP103_1 on transposon mutants and wild-type (WT) was compared. One-step profiles and mutation frequencies to pKMKP103_1 were examined. Antibiotic susceptibility was determined using minimum inhibitory concentrations (MIC) and population analysis profile assays. Lipid A profiling was performed using liquid chromatography-mass spectrometry.

Results
Against *rpoN*::Tn (RpoN, RNA polymerase factor sigma-54), pKMKP103_1 was presented with an extended latent period. Although the EOP of pKMKP103_1 on *rpoN*::Tn was unaffected, no bacterial killing activity was observed in time-kills over 24 h. Compared to WT ($7.41\times10^{-6}\pm4.73\times10^{-6}$), mutation frequencies of *mutS*::Tn ($1.42\times10^{-3}\pm1.07\times10^{-3}$) and *mutL*::Tn ($1.55\times10^{-3}\pm1.63\times10^{-3}$) were higher. While *mutL* complementation reduced mutation frequency to the WT level, only partial restoration was seen with that of *mutS*. Intriguingly, a bi-directional shift in colistin susceptibility (WT, MIC=32mg/L) was discovered. An increased susceptibility of *rpoN*::Tn (MIC=4mg/L) was associated with a reduced abundance of L-Ara4N lipid A. The increased resistance of *mutS*::Tn and *mutL*::Tn (MIC=128mg/L) was attributable to their hypermutator phenotype, with ~100-fold increase in subpopulations growing on agar containing 128 mg/L colistin in comparison to WT.

Conclusions
Phage steering does not guarantee increased antibiotic susceptibility. As the mechanisms associated with phage resistance can also potentiate antibiotic resistance, caution is required with phage-antibiotic combination therapy.

Human Respiratory airway model for bacterial infection and drug discovery

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Introduction

Multidrug resistance (MDR) bacterial infections are a major threat to human health and the global economy. However, there are very limited therapeutics available for their treatment. Bacterial infections are the primary cause of morbidity and mortality for chronic lung conditions such as cystic fibrosis, asthma, and chronic obstructive pulmonary disease. While animal models are an important preclinical tool, they have issues with ethics, cost and often do not faithfully recapitulate the human infection and host response. Recent advancements in tissue culture techniques have enabled us to establish an *ex vivo* human lung model from primary human bronchial epithelial cells obtained from normal and diseased donors to mimic the lung environment. In this study, we aim to use these models to examine the pathogenesis and host response to common bacterial pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Methods

We used commercially available primary cells such as normal bronchial epithelial (NHBE) cells and the cystic fibrosis cell line (CuFi-1). Currently, these cells are differentiated at the air-liquid interface (ALI) to form a pseudostratified epithelium and an integral barrier. They will then be challenged with bacteria to characterize the host response and mechanism of pathogenesis. Endpoint analyses will include Hematoxylin and Eosin staining, Transepithelial Electrical Resistance measurements for barrier integrity, cytotoxicity assays, multiplex immunoassays and gene expression analysis.

Results

Optimal differentiation of NHBE and CuFi-1 cells at ALI will be determined using different seeding densities and culture conditions. The disease mechanisms and host response will be evaluated using histology, microscopy, immunoassays and gene expression analysis for different cytokines with and without bacterial challenge.

Conclusion

The establishment of an appropriate airway respiratory model that can recapitulate the *in vivo* lung condition can provide a promising preclinical platform for the understanding of lung infections (or host-pathogen response) and for testing novel therapeutics.

RIPK3 preferentially restrains MAIT cell abundance at steady state

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Programmed cell death encompasses a diverse range of tightly regulated signalling pathways, including apoptosis and necroptosis. Activation of these pathways is balanced by signalling through receptor-interacting protein kinase (RIPK)1 and RIPK3; serving as dual regulators of inflammation and cell death, initiating apoptosis via activation of caspase-8 and necroptosis via the phosphorylation of mixed lineage kinase-like protein (MLKL). The mechanisms regulating cell death in T lymphocytes vary according to developmental stage, subtype, activation state and environmental stimuli. While the regulation of cell death in conventional T cell subsets is well studied, the pathways active in mucosal-associated invariant T (MAIT) cells remain largely uncharacterised.

Considering MAIT cells exhibit an effector-memory phenotype ($CD62L^{lo}$ $CD44^{hi}$) upon thymic egress, here we compare expression of cell death machinery in MAIT cells to that conventional T cell subsets. We report that MAIT cells express high levels of MLKL; similar to conventional effector memory T cells, but distinct from naïve ($CD44^{lo}$ $CD62L^{hi}$) subsets, which lack MLKL expression. We further show that MAIT cells express an abundance of both RIPK1 and RIPK3. Importantly, we demonstrate that the loss of RIPK3 results leads to an increase in MAIT cell frequency in mice. Additionally, we use knockout models to dissect the contribution of caspase-8 dependent apoptosis and MLKL dependent necroptosis to the regulation of MAIT cell accumulation at steady state. Finally, we examine these death pathways in controlling the expansion and contraction of MAIT cells in a murine Francisella tularensis infection model.

Our data demonstrate a role for RIPK3 in restraining MAIT cell numbers at steady state, distinguishing this subset from conventional T cells. Further understanding the pathways regulating MAIT cell survival throughout development, homeostasis, and infection, will be critical to developing the next generation of immune interventions seeking to deplete, expand or otherwise manipulate MAIT cells.

Evolutionary rate of SARS-CoV-2 increases during zoonotic infection of farmed mink

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To investigate genetic signatures of adaptation to the mink host, we characterized the evolutionary rate heterogeneity in mink-associated SARS-CoV-2. In 2020, the first detected anthropozoonotic spillover event of SARS-CoV-2 occurred in mink farms throughout Europe and North America. Both spill-back of mink-associated lineages into the human population and spread into surrounding wildlife was reported, highlighting the potential formation of a zoonotic reservoir. Our findings suggest the evolutionary rate of SARS-CoV-2 underwent an episodic increase upon introduction to the mink host before returning to the normal range observed in humans. Furthermore, SARS-CoV-2 lineages could have circulated in the mink population for a month before detection, and during this period, evolutionary rate estimates of 6.57×10^{-3} could yield an 8-fold increase of mutations compared to the evolutionary rate of SARS-CoV-2 in humans. We suggest that SARS-CoV-2 undergoes a brief, but considerable, increase in evolutionary rate in response to greater selective pressures during species jumps, emphasizing the necessity of monitoring zoonotic SARS-CoV-2 infections.

A High-affinity Gamma-delta T-cell Receptor for Cancer Therapy

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PUBLISH CONSENT WITHHELD

Most peripheral human blood gamma-delta (γδ) T-cells recognise cancer cells by sensing small, phosphorylated metabolites, known as phosphoantigens. An intracellular overabundance of these metabolites, such as the isopentenyl pyrophosphate (IPP), are sequestered by a cell-surface complex composed of butyrophilin (BTN) transmembrane proteins, which triggers a conformational change at the extracellular space, sensed by recombined Vγ9Vδ2+ T-cell receptors (γδTCRs). However, T-cell antitumoral immune response is limited by the threshold of phosphoantigen-mediated activation. As such, we hypothesise that increasing the affinity of this γδTCR to BTN ligands promotes antitumoral activity. Here, we demonstrate how inducing an amino acid change at the CDR2 loop, residue lysine 53, in the TCR δ-chain improves T cell-mediated tumor clearance in vitro. This increased antitumour activity of the modified γδTCR was associated with elevated specific reactivity to BTN member 3A1 (BTN3A1), proving their direct interaction and relation to antitumour action as observed in alanine 53 TCR δ-chain CRISPR/Cas9 modified primary human T-cells. Furthermore, this novel genetically modified γδTCR has been directly assessed in vitro to characterise the efficient cytotoxicity phenotype against human blood and solid cancer cell lines. Hence, improving the antitumour activity of phosphoantigen-reactive γδ T cells is achieved by modulating the reactivity of the γδTCR to BTN family ligands, representing an exciting opportunity to develop advanced γδTCR-based immunotherapeutics.

An Integrative Analysis of RNA, Protein and Metabolite Levels Reveals Distinct Phases of Macrophage Polarization by IFNb

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Type 1 IFNs are important cytokines with key roles in innate immunity. They are produced in response to many different pathogens and can act on multiple cell types to help clear infections. In this study, we investigated the polarization of mouse bone marrow-derived macrophages by IFNb over time and on different molecular levels, using RNA-sequencing, quantitative proteomics and metabolomics. Our combined results show that the IFNb response can be separated into an activation phase, a transcriptional phase and an effector phase.

The activation phase lasts about 1h and initiates the response. IFNb has been shown to regulate different signalling pathways downstream of its receptor through phosphorylation. Subsequently, activated transcription factors enable the well-characterised induction of gene expression. Our data reveals that this activation phase is also characterised by rapid changes to the cellular metabolic profile, which quickly reverts back to baseline levels. Notably, this includes metabolites from the purine metabolism pathway, such as Adenosine and Inosine, which have important immunomodulatory functions.

The transcriptional phase encompasses very few metabolic changes and is instead characterised by a vast number of differentially expressed transcripts, some of which are translated into protein-level changes. Discrepancies observed between RNA- and protein-level changes suggests additional post-transcriptional regulation.

The effector phase starts after 16–24h. A larger number of proteins is differentially expressed, including key mediators of macrophage characteristics and functions. Additionally, a distinct metabolic profile develops, likely influenced by IFNb-induced changes to metabolic enzyme levels. This includes accumulation of Itaconate, an important immunomodulatory metabolite, and elevated intermediates of the Phosphatidylethanolamine (PE) biosynthesis pathway, which generates building blocks for lipid structures. Methylguanine and Crotonate, which can be associated with DNA and histone modifications, are also highly elevated, suggesting an extra layer of regulation at the chromatin level following macrophage polarization by IFNb.

This multi-omics approach shows that IFNb-induced changes in BMDMs are dynamic. Rapid changes to the metabolic profile precede the expression of IFNb-induced transcripts. These transcripts are further regulated by post-transcriptional mechanisms and can then alter metabolite levels, potentially leading to lasting changes to the cellular phenotype.

Characterisation of host cell membrane biophysical changes and cytoskeletal rearrangements during Toxoplasma gondii invasion

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Toxoplasma gondii is an obligate intracellular parasite that is responsible for the human and animal disease toxoplasmosis. They belong to the apicomplexan family including *Plasmodium* species (the agents of malaria) and *Cryptosporidium spp*. (responsible for severe diarrhea). The invasive form of Toxoplasma, known as tachyzoites, can infect any nucleated cell and there complete its life cycle. During invasion, the parasite connects to the host cell by injecting Rhoptry Neck (RON) proteins into the target cell plasma membrane and forms a tight junction (TJ). The TJ acts as anchor point and enables *Toxoplasma* to actively penetrate inside the host cell, forming a protective vesicle known as the parasitophorous vacuole (PV). Once inside the vacuole, the parasite replicates asexually until it egresses from the host cell and invades neighboring cells.

In this study, we used lattice light-sheet microscopy (LLSM) to capture whole cell volumes of *T. gondii* invasion at faster frame rates and lower levels of phototoxicity. To track and characterise parasite interactions with the host cell, we use,tachyzoites expressing mCherry and the calcium sensor GCaMP6. In addition, we apply a combination of live cell dyes or genetically encoded fluorescent reporters to label both membrane and cytoskeletal proteins in the host cell. Using this novel workflow, we are quantitatively investigating biophysical membrane changes and the reorganization of the cytoskeleton during host cell invasion by Toxoplasma. This advanced technology will reveal new aspects of *T. gondii* invasion that could be targeted to prevent severe disease.

Structural mechanism of SARS-CoV-2 entry and activation of spike glycoprotein by engaging unique host factors & potential interventions

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The pandemic COVID19 illness caused by SARS-CoV-2, which causes pneumonia and lower respiratory tract infections, is a serious public health concern, with frightening mutations causing over 6.3 million deaths globally. Whilst effective immunisation shows promise globally, several antiviral treatments are being clinically evaluated to fill the "therapeutic gap" in treating infected people. Understanding the entire repertoire of diverse host factors engaged by SARS-CoV-2 for entry and pathogenicity is required for long-lasting potential therapeutics or vaccines. Here, using a structural and molecular approach, we show multistage processing of SARS-CoV-2 spike-protein for virion activation, infection, and how mutations influence it. We solved the structures of spike-protein in complex with different host cell factors (TMPRSS2, Furin, CD26, and NRP1) with functional activity, and these insights into uncovering how viral spike-protein engages and primed with these multiple host factors, in addition to ACE2, to hijack host cell entry. Furthermore, our COVID19 patient genome sequencing reveals that allele in TMPRSS2 (V160M), and Furin provided protection from COVID19 infection, and its structural mechanism is further addressed and potential drug clinical trials. Additionally, our large-scale retrospective cohort studies proved Arbidol and derivatives as potential therapies for COVID19, using structural studies, we demonstrated the mechanism of action of Arbidol in disrupting spike function. These findings cognize the complete mechanism of viral spike-glycoprotein processing/priming that leads to cascading entry into the host cell, paving the door for future vaccine development and identifying key targets. Our comprehensive, multifaceted research reveals the complexity of the SARS-CoV-2 spike-protein and clinical studies aid in therapies.

Keywords: Host factors, viral entry, antivirals, structure, molecular dynamics, genetic variants

A biparatopic nanobody with broad neutralisation against SARS-CoV-2 variants of concern

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The rapid emergence of the COVID-19 pandemic, and the SARS and MERS epidemics within the past two decades highlight coronaviruses as critically important human pathogenic viruses with pandemic potential. Our strategy is to use therapeutic monoclonal antibodies to disrupt coronavirus entry. Therapeutic monoclonal antibodies against COVID-19 will be critical for preventing or treating infection in vulnerable groups that respond poorly to vaccination such as the immunosuppressed and the elderly, and to provide immediate protection which are important for limiting outbreaks in hotel quarantine and aged care.

Alpacas and llamas have evolved diminutive antibodies called nanobodies, which are the smallest naturally derived single domain antibodies. Nanobodies are of interest due to their small size, high antigen binding affinity, increased stability and their ease of manufacturability. Their special features have driven their increased development as treatments and diagnostics against human diseases, as versatile imaging tools for tracking single molecules to tumours, as crystallization chaperones to high precision discovery research tools. We show that nanobodies block SARS-CoV-2 entry into cells and suppress virus infection in mouse models. Using both cryo-EM and X-ray crystallography approaches, our structural findings reveal that the neutralizing nanobodies bind to distinct sites on SARS-CoV-2. Using these nanobodies, we have designed a biparatopic nanobody that is resilient against existing SARS-CoV-2 variants of concern including Omicron lineages. This biparatopic nanobody has increased affinity, potency and resilience to variants compared to the monovalent nanobody counterparts. Using virus fusion assays, single molecule FRET and structural approaches, we provide insight on the molecular mechanism of neutralization across a diverse collection of SARS-CoV-2 variants of concern.

Post-translational regulation of MHC Class II

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Major histocompatibility complex class II (MHC II) is a transmembrane glycoprotein that displays antigenic peptides to T lymphocytes, stimulating their effector functions. This antigen presentation pathway is tightly regulated by controlling MHC II surface expression and turnover. These events are regulated by ubiquitination, a post-translational modification that directs transmembrane glycoproteins to endocytosis and degradation. In murine and human dendritic cells, MHC II ubiquitination is mediated by an E3 ubiquitin ligase, membrane-associated ring-CH-type finger 1 (MARCH1). Ubiquitin-like protein, ubiquitin-like 3 (UBL3), participates in MARCH1-mediated MHC II ubiquitination. In UBL3-deficient cells, MHC II ubiquitination is reduced, resulting in higher surface MHC II in different immune cells (Liu et al., 2022). Furthermore, prenylation at C114 allows UBL3 recruitment to membrane regions where MARCH1 ubiquitinates MHC II. Here, we address the hypothesis that UBL3 is involved in MHC II ubiquitination.

To investigate UBL3 function, we will utilize a next-generation proximity-dependent biotinylation method, namely BioID2. By fusing a biotin ligase, BirA, to UBL3, endogenous proximal proteins in murine dendritic cells that interact with UBL3 will be biotinylated. The proteins will be enriched using streptavidin beads for immunoprecipitation. Then, mass spectrometry will identify these novel interacting proteins. UBL3 fused to BirA results in lower surface MHC II, meaning that the UBL3 is functioning as normal. We also investigate how preventing UBL3 prenylation affects ubiquitination by comparing with a mutated version, UBL3C114S. UBL3C114S leads to higher surface MHC II, similar to UBL3-deficient cells. This suggests that prenylation is necessary for MHC II ubiquitination.

Hence, this research will bring more clarity to the molecular mechanisms of UBL3 that underlie MHC II ubiquitination, allowing us to further understand post-translational regulation of membrane proteins in immune cells. Moreover, this will help add to the very limited body of knowledge on UBL3.

References

1) Liu, Haiyin, et al. "Ubiquitin-like protein 3 (UBL3) is required for MARCH ubiquitination of major histocompatibility complex class II and CD86." *Nature communications* 13.1 (2022): 1-15.

Extracellular Vesicle Purity Markers Found on Lipid Droplets

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Cell-to-cell communication is essential for effective coordination of antiviral immunity during infection. Small lipid enclosed particles such as extracellular vesicles (EVs) are the dominantly studied extracellular communicators, however, the role of lipid droplets (LDs), which are also lipid-enclosed small particles, remains unexplored in this space. LDs have recently gained attention for their role in antiviral immunity and our laboratory has recently demonstrated that they move between cells and can directly influence the antiviral response of neighboring cells. Mechanisms underpinning this LD movement remain undescribed as does their relationship to EVs; therefore this study aimed to explore this connection.

To understand if LDs are hijacking EV secretion pathways, comparative proteomic analysis of all published LD and EV proteomes was performed. Mining of these databases revealed a significant overlap in the proteomes of EVs and LDs, with both groups sharing proteins involved in biogenesis (*ALIX, AnxA6*), cargo recruitment (*AnxA2, AnxA6*), and transport (*TSG101, EEF2*). Of most interest were the well described EV purity markers; ALIX and TSG101, which are involved in the biogenesis and transport of EVs and have the potential to also be responsible for the transport of LDs between cells. The localisation of ALIX and TSG101 to LDs was confirmed via western blot analysis of isolated pure LD fractions and additionally via super resolution microscopy of cells stained for LDs, ALIX and TSG101. It was recently hypothesised that LDs could be packaged into EVs secreted from adipocytes, therefore we next sought to examine if LDs are packaged into different populations of EVs in alternate cell types. Live cell imaging revealed that LDs were packaged into apoptotic bodies, a group of large sized EVs, and we are now working on developing tools to investigate LD packaging into smaller EVs, such as exosomes, using fluorescently labeled organelles.

To date, there has been little interdisciplinary research investigating the similarities of protein cargo between LDs and EVs and their potential interactions. Here, we have demonstrated for the first time that LDs carry proteins involved in EV biogenesis and secretion which may allude to their transport between cells during infection.

Neutrophil heterogeneity and autoantibody-mediated NETosis in COVID-19 severity risk

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COVID-19 causes a variety of symptoms from asymptomatic, through to mild, moderate or severe. Accumulating evidence shows neutrophil heterogeneity and the generation of Neutrophil Extracellular Traps (NETs) as a hallmark in COVID-19 disease severity. Autoinflammatory diseases in which NETs are associated with inflammatory pathology suggest a role for anti-neutrophil cytoplasmic antibodies in disease exacerbation. Although different neutrophil subpopulations vary in capability to generate NETs, how this relates to their interaction with autoantibodies and whether this contributes to COVID-19 severity remains unexplored. We hypothesize that severe COVID-19 patients are more likely to undergo NETosis in response to circulating autoantibodies, and their neutrophils demonstrate a higher proportion of FcγR and complement receptors.

To address our hypothesis, neutrophils were isolated from peripheral blood, collected from uninfected household contacts (n=12) and convalescent COVID-19 patients showing asymptomatic (n=2), mild (n=6), moderate (n=18), and severe (n=6) symptoms, at ≥ 2 different time points. Neutrophil subpopulations are being immunophenotyped with a 22-colour spectral flow cytometry panel. NETs were generated from neutrophils incubated in different autologous or heterologous serum concentrations, ± FcR block. Immunofluorescence was used to stain NETs, with area and morphology quantified using semi-automated analysis. Serum concentrations of 22 autoantibodies were measured using a custom Luminex panel and C1q circulating immune complex (CIC) measured by ELISA.

Preliminary immunofluorescence data identified distinct NET phenotypes in severe and healthy patients. NET analysis will be continued and correlated with neutrophil immunophenotyping, autoantibodies, and CIC to determine whether NETosis is related to neutrophil heterogeneity, serum factors, or both and how this may impact the outcome of infection. Results will inform whether, differences in neutrophil heterogeneity have the potential as markers toy be applied as screening methods to determine populations more susceptible to severe COVID-19. Additionally, understanding NETosis and its inducing factors may inform therapeutic targets to decrease inflammatory pathology.

Clonal selection defines an atypical human Vδ2⁺ γδ T cell compartment

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Human Vδ2⁺ T cells form the predominant human γδ T cell population in peripheral blood and possess a semi-invariant Vγ9 and Vδ2 T cell receptor (TCR) chain pairing. This TCR repertoire allows Vδ2⁺ T cells to function in an innate-like manner towards pyrophosphate metabolites (PAg) and mount TCR-dependent immune responses to bacterial and parasitic pathogens. Here, we explored the heterogeneity of this Vδ2⁺ T cell immune response in human *Plasmodium falciparum* and cytomegalovirus (HCMV) infection. We find that subpopulations of Vδ2⁺ T cells express alternate and often expanded TCR clonotypes and often these expanded TCRs define a population of PAg unresponsive Granzyme (Gzm) K⁻ CD16⁺ Vγ9⁺Vδ2⁺ T cells (atypical Vγ9⁺Vδ2⁺ T cells). Transcriptional profiling of Vγ9⁺Vδ2⁺ T cells indicated that clonally expanded atypical Vγ9⁺Vδ2⁺ T cells possess a natural killer cell-like transcriptome compared to PAg reactive GzmK⁺ Vγ9⁺Vδ2⁺ T cells. Using a controlled human malaria infection (CHMI) we found that an individual with a predominant clonally expanded atypical Vδ2⁺ T cell population fails to mount a response to *in vivo P. falciparum* infection in humans. Vδ2⁺ T cells are classically responsive to both PAg and inflammatory cytokines, we found that neither stimuli could induce the clonal selection of the Vδ2⁺ TCR. Moreover, four repeated CHMIs over two years in individuals with a predominant PAg reactive GzmK⁺ Vγ9⁺Vδ2⁺ T cell population failed to drive clonal selection. However, cytomegalovirus infection in individuals undergoing lung transplant or hematopoietic stem cell transplantation drove the emergence and selection of atypical Vγ9⁺Vδ2⁺ T cells. Together, we reveal an alternative compartment of human Vγ9⁺Vδ2⁺ T cells that are selected after acute viral infection and that may be an important avenue for harnessing these cells for anti-microbial immunotherapies.

Using CRISPR/Cas9 gene-editing technology to examine CD4+ T cell immune response against malaria infection

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CD4+ T cells play a critical role in inducing long-lived CD8+ T cell response and high-affinity antibody production mediated by B cells during blood-stage Plasmodium infection [1]. T-helper 1 (Th1) and follicular helper T cell (Tfh) cells express several CXC chemokine receptors (CXCR), such as CXCR3, CXCR5, and CXCR6, to circulate within body and interact with other immune cells [2]. To study the role of these chemokine receptors in CD4+ T cell differentiation and response against malaria, I have applied the CRISPR/Cas9 gene-editing technology in naïve TCR transgenic CD4+ T cells specific for Plasmodium epitope (PbT-II cells). The result showed that deleting target genes in naïve PbT-II cells can be achieved by using ribonucleoprotein (RNP)-mediated CRISPR/Css9 method which consists of Cas9 protein and single guide RNA. Secondly, CXCR3 single-knockout and CXCR3/CXCR6 double-knockout did not affect IFN-γ-producing PbT-II cell response in the spleen and liver at 7 days after P. chabaudi chabaudi AS (PcAS) infection. However, the result suggests that knocking out both CXCR3 and CXCR6 impact the expression of T-bet in the liver PbT-II cells. To investigate more about the role of these chemokine receptors in PbT-II cell, I have been studying the CXCR gene-edited PbT-II cell effector and recall response during PcAS infection. To explore the CRISPR/Cas9 gene-editing technique and test different genes, I intend to examine the role of Maf (the transcription factor) and IL2Ra (cytokine receptor) in the PbT-II cell response in the spleen and liver.

1. Kurup, S.P., N.S. Butler, and J.T. Harty, *T cell-mediated immunity to malaria.* Nat Rev Immunol, 2019. **19**(7): p. 457-471.
2. Soon, M.S.F., et al., *Transcriptome dynamics of CD4(+) T cells during malaria maps gradual transit from effector to memory.* Nat Immunol, 2020. **21**(12): p. 1597-1610.

Avian Adenovirus Evolution: What's New?

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Adenoviruses infections are common in a wide range of vertebrates and members of three major genera; *Aviadenoviruses, Atadenovirus*, and *Siadenovirus* predominantly infect avian hosts. Wild birds provide multiple opportunities for viruses to gain infections, but limited studies are available on avian adenovirus in wild birds compared to that poultry. However, some recent findings on avian adenoviruses in wild birds have encouraged us to re-visit previously proposed, decade-old concepts on adenovirus evolution. Here, we extracted available complete genomes and partial DNA polymerase sequences of avian adenoviruses from NCBI and analysed them through different bioinformatic tools. Genomic analyses and phylogenetic reconstructions demonstrated the origin of atadenoviruses from an Australian native passerine bird in contrast to the previously established reptilian origin. In addition, we proved that the theories on higher AT content in atadenoviruses are no longer accurate and cannot be considered as a species demarcation criterion for the genus *Atadenovirus*. Phylogenetic reconstruction further emphasised the need to reconsider genus *siadenovirus* origin. Thus, findings were highly important to understand the adenovirus diversity and their potential to transmit, causing novel infectious threats to birds and other animal species. Further, we strongly recommend extended studies on avian adenoviruses in wild birds to provide finer evolutionary resolution for adenoviruses.

Manufacture of a GMP-like bacterial challenge agent in a research facility for use in humans

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Introduction
Controlled human infection models are a platform for studying host-pathogen interactions, preventive, and therapeutic interventions. A meticulous approach to selection, characterisation, and manufacture of challenge agents is critical to volunteer safety. There is no current Australian framework to inform or review these processes. Here, we describe the requirements to manufacture single-dose vials of *Streptococcus pyogenes* for use in the CHIVAS-M75 pharyngitis human challenge trial.

Methods
We developed processes to manufacture five dose levels of M75 *S. pyogenes* in our research facility, according to the principles of Good Manufacturing Practice (GMP). Manufacture was conducted within a biosafety class II cabinet in a restricted-access room. Bacteria were grown in chemically defined 'animal-free' media, diluted to strength, dispensed into cryovials, and frozen. Following a single freeze-thaw, rigorous quality control testing proceeded in-house and at an independent laboratory.

Results
A detailed Batch Manufacturing Record was developed covering all aspects of the manufacture. All five *S. pyogenes* dose levels complied with specification and were approved for release following an independent review of the documentation, demonstrating the reliability of our workflow.

Conclusion
We successfully manufactured a bacterial challenge agent at a GMP-like standard within a public research facility at a fraction of the prohibitive costs associated with commercial GMP providers. Our workflow and quality assurance processes can be adapted for other bacteria and other purposes, including manufacture of *S. pyogenes* reference strains. Our robust approach has contributed to guidelines developed by an international challenge agent manufacturing consortium funded by the Wellcome Trust.

Cephamycin derivatives as anti-sporulation agents against *Clostridioides difficile*

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Clostridioides difficile, a spore forming bacterium, is a leading cause of nosocomial infections, that range from mild symptoms, diarrhoea, to more severe, inflammation and enlargement of the colon. Spores are crucial mediators of *C. difficile* infection initiation, dissemination, and re-infection. However, current therapeutics do not target sporulation and have no effect on spore formation. Our team has shown that cephamycins, a family of β-lactam antibiotics, can inhibit sporulation by targeting the key sporulation-specific penicillin binding protein *Cd*SpoVD[1]. SpoVD proteins are highly conserved among spore forming bacterium and play an essential role in the synthesis and remodelling of peptidoglycan during sporulation[1]. Specific and selective inhibitors of SpoVD proteins would represent a new drug class that could reduce the burden of spores in hospitals. In our current study, we aimed to map the interactions of the cephamycin, cefotetan, bound to *Cd*SpoVD to better understand why this class of β-lactam was able to inhibit the enzyme. In general, penicillin binding proteins are inhibited by β-lactams when the active site serine residue forms a covalent bond with the lactam ring of the drug. Our analysis of an AlphaFold model of *Cd*SpoVD identified two possible active site serine residues (S311 and S367). We mutated both individually to alanine and assessed for their ability to bind β-lactams. The results show S311A was unable to bind β-lactams while S376A had a decreased affinity for β-lactams, confirming S311 as the active site serine. Computational docking of cefotetan in our *Cd*SpoVD model suggested that the 1,3-dithietan derived substituent could be replaced and derivatives were designed with the aim to maintain potency but improve selectivity of the compounds. A triazole ring was installed in place of the 1,3-dithietan moiety and a small series of derivatives synthesised. Investigation of their *in vitro* binding affinity and anti-sporulation activity showed that compounds possessing phenyl and cyclohexyl groups could potently bind to *Cd*SpoVD and significantly reduce spore numbers in *C. difficile*. Finally, we have assessed our two top performing compounds in an animal model of *C. difficile* infection. This structure activity relationship data will aid in future rational design of selective, novel anti-sporulation agents.

References

[1] Srikhanta, Yogitha N., et al. (2019) “Cephamycins inhibit pathogen sporulation and effectively treat recurrent Clostridioides difficile infection.” *Nat. Microbiol.*4.12: 2237-2245.

Functional assessment of genetic variants affecting the NOD2 signalling pathway in patients with primary immunodeficiency

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Background: Despite the significant advances in genomics, many patients with primary immunodeficiency (PID) remain genetically undiagnosed. Many identified genetic variants are of unknown significance (VUS), requiring functional evidence to determine pathogenicity. Many causative genetic variants function in a limited number of immune signaling pathways, one of which is downstream of the NOD2 receptor. This pathway is activated upon engagement of NOD2 with its ligand: bacterial fragment muramyl dipeptide (MDP). Here, a functional assay was established to assess the impact on NOD2 signaling pathway by novel variants in genes encoding XIAP, A20 and NOD2.

Methods: Whole-exome-sequencing was performed on genomic DNA of PID patients. Patient immune cells were evaluated by flow cytometry for production of intracellular TNF-α in response to L18-MDP stimulation. LPS stimulation and an unstimulated control were used as positive and negative controls, respectively.

Results: Following L18-MDP-stimulation, 27.9-95.9% of monocytes of healthy donors produced TNF-α. Monocytes from a patient with X-linked lymphoproliferative disease due to a *XIAP* mutation did not produce any TNF-α after L18-MDP stimulation. In contrast, for a patient with a heterozygous *A20* VUS, TNF-α production was higher than the median but within the range of the healthy donors with 82.8%. A patient with a heterozygous *XIAP* VUS, and a patient with a heterozygous *NOD2* VUS had lower TNF-α production within the range of healthy controls, with 32.1% and 29.45% respectively.

Conclusion: This established assay assessing NOD2 pathway function is able to identify patients with a complete loss-of-function phenotype but is not yet sensitive enough to detect moderate impairing or enhancing defects. We are currently optimizing the quantitative range of this assay, as well as inclusion of detecting phosphorylation of p38 to improve detection of more subtle functional alterations.

Inhaled dosing regimens of aztreonam and tobramycin against multidrug-resistant hypermutable cystic fibrosis *Pseudomonas aeruginosa* isolates in a dynamic *in vitro* biofilm model

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Pseudomonas aeruginosa (*Pa*) is a biofilm-forming opportunistic pathogen commonly infecting patients with cystic fibrosis (CF). Antibiotic resistance is one of the greatest threats to humans and ensuring effective use of antibiotics is essential. Suboptimal antibiotic regimens can support the rise of multidrug-resistance (MDR), particularly with the hypermutable *Pa* strains that are prevalent in CF. Bacterial lung infections are still a main driver of mortality in patients with CF. Inhaled antibiotics show promising efficacy for patients with CF, however, they have not yet been investigated for the treatment of acute exacerbations. We aimed to evaluate inhalational regimens of aztreonam (AZT) and tobramycin (TOB), alone and combined, against clinical hypermutable *Pa* strains from adults with CF in a dynamic *in vitro* biofilm model.

MDR isolates, CW5 and CW13 with the minimum inhibitory concentrations of ≥32mg/L for AZT and 8mg/L for TOB, from patients with CF were investigated in static-concentration time-kill studies before investigation in a 168h dynamic biofilm model (n=2; inoculum ~10^6 CFU/mL). Inhaled AZT (225mg, 8-hourly) and TOB (600mg, 12-hourly) dosing regimens were examined in monotherapy and combination regimens. The dynamic biofilm model simulated the pharmacokinetics of AZT and TOB in lung fluids based on published pharmacokinetic studies in patients with CF ($t_{1/2}$=3h). Total viable counts were determined for planktonic and biofilm bacteria. Resistant bacteria were quantified at 5 time points. LC-MS/MS confirmed antibiotic exposures.

For both isolates, all monotherapies failed and led to amplification of resistant subpopulations by the end of the study. The combination regimen provided enhanced bacterial killing (>3 $\log_{10}$ CFU/mL more bacterial killing compared to the best monotherapy), thereby performing synergistically against planktonic and biofilm bacteria for both isolates at 168h. Minimal resistant subpopulations were observed in the samples collected from the combination regimen. The results show good reproducibility.

The combination of inhaled AZT and TOB enhanced bacterial killing and suppressed regrowth and resistance of the planktonic and biofilm bacteria of hypermutable multidrug-resistant *Pa* isolates from patients with CF. As this inhaled combination regimen demonstrated promising synergistic activity, further investigation is warranted.

The importance of metabolite antigen attachment to MR1 for MAIT cell development

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MHC Class I-related protein 1 (MR1) is a non-classical antigen presenting molecule which presents vitamin B-related metabolite antigens to mucosal associated invariant T (MAIT) cells. MAIT cells are a subset of innate-like T cells that are abundant in humans and can account for up to 10% of blood T cells and up to 50% of T cells in tissues such as the liver. The MR1-MAIT cell axis is highly conserved among mammals and has been implicated in immunity to pathogens, cancer immunosurveillance, and tissue repair.

MAIT cells require both MR1 and microbial metabolite antigens for their development in the thymus, expansion, and establishment in peripheral tissues. MR1 can present a diverse range of metabolite antigens, with the most potent antigens covalently attaching to MR1 through a lysine residue (K43) in its antigen binding cleft. Antigens which do not bind to MR1 covalently are much weaker in stimulating MAIT cells. It is currently unknown whether covalently bound antigens are essential for MAIT cell development and activation *in vivo*. Previous studies showed that mutating the K43 residue to alanine (K43A) prevents the formation of the covalent bond, leaving only weaker electrostatic interactions between MR1 and its ligand, but allows $MR1^{K43A}$ expression on the cell surface.

We generated a mouse model with the K43A point mutation in MR1 to investigate whether covalent antigen binding is critical for MAIT cell development and expansion. Preliminary investigations revealed that the $MR1^{K43A}$ mice lacked detectable MAIT cells in peripheral tissues although the mutant $MR1^{K43A}$ molecules were expressed in thymocytes at higher levels than wild-type MR1 ($MR1^{WT}$). However, small numbers of cells with mature MAIT cell phenotypes were detected in the thymus of $MR1^{K43A}$ mice that were not found in MR1-knockout mice, and these are currently being characterised. This suggests that the covalent binding of antigens to MR1 is not essential for the early stages of MAIT cell development but is required for the expansion of MAIT cells in the thymus and periphery. This project is uncovering the importance of covalent bond formation between MR1 and its antigen *in vivo*, building insight into MAIT cell development and selection.

Dampening the inflammatory cytokine response in monocytes

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Chronic systemic inflammation increases with age and accounts for initiation and progression of many diseases including cancer, cardiovascular and autoimmune disorders. Acute inflammation is a powerful protective response against infections and is essential for injury repair. Homeostatic processes normally limit this inflammatory response in the process known as ‘tolerance’. This is crucial because acute inflammation contributes to chronic inflammatory diseases, and overactive immune responses like cytokine storms, leading to organ failure in severe cases.

Interleukin-1 receptor-associated kinase-3 (IRAK3) is a critical checkpoint molecule of inflammatory responses in the innate immune system. The pseudokinase domain of IRAK3 contains a guanylate cyclase (GC) centre that generates small amounts of cyclic guanosine monophosphate (cGMP). The mechanisms of IRAK3 actions are poorly understood, with effects of low cGMP levels on inflammation being unknown.

The effects of cGMP on inflammatory markers were assessed in THP-1 monocytes challenged with lipopolysaccharide (LPS). cGMP at sub-nanomolar levels supressed inflammatory markers and this effect was not seen in IRAK3-/- cells, the cGMP effect depends on IRAK3. An inhibitor of soluble GC, ODQ, was examined for its downregulatory effects on cGMP, on NFκB activity and cytokine level. ODQ at sub-nanomolar levels enhanced LPS-induced NFκB activity and LPS-mediated TNF-α protein production. As these findings beg the question that pharmacological agents that modulate cellular cGMP levels may alter cytokine levels, we report initial findings here. Our aim is to identify clinically used compounds that can be used to control cytokine production through IRAK3.

Cross-specific nanobodies against malaria parasite invasion ligands

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Malaria is one of the most life-threatening diseases known to man, caused by the *Plasmodium* species, leading to hundreds of thousands of deaths [1, 2]. Malaria blood stage infection in humans is a critical step in the parasite life cycle and is responsible for all the clinical symptoms caused by the invasion of red blood cells [3]. This occurs through specific ligand-receptor interactions between parasite surface proteins and human red blood cell receptors [4]. Parasite adhesins that function during blood stage infection, are an important target for vaccine development with the potential of protecting against the disease [5]. Two homologous family of proteins sharing a conserved structural scaffold, *P. falciparum* reticulocyte binding protein homolog (PfRh) and *P. vivax* reticulocyte binding proteins (PvRBP) are among the major parasite adhesins involved in red blood cell invasion [6]. Antibodies capable of blocking this parasite invasion have been discovered for these family of proteins highlighting their importance as potential vaccine candidates [6]. We aim to generate cross-specific nanobodies, which are single domain antibodies isolated from camelids, against the PfRh and PvRBP family of proteins via phage display technology and ELISA. The ability of these nanobodies to inhibit receptor engagement and parasite invasion of red blood cells will be determined using red blood cell binding assays, parasite growth assays and FRET competition assays. Furthermore, biophysical and structural approaches including bio-layer interferometry and X-ray crystallography will be used to determine the unique binding epitopes of these cross-specific nanobodies capable of inhibiting parasite invasion against multiple *Plasmodium* species. This will help us identify novel inhibitory epitopes that can guide rational design strategies of future therapeutics against the blood stage parasite adhesins of *Plasmodium falciparum* and *Plasmodium vivax*.

References:

1. Phillips, M.A., et al., *Malaria.* Nature Reviews Disease Primers, 2017. **3**(1): p. 17050.
2. Cowman, A.F., et al., *Malaria: Biology and Disease.* Cell, 2016. **167**(3): p. 610-624.
3. White, N.J., *Determinants of relapse periodicity in Plasmodium vivax malaria.* Malar J, 2011. **10**: p. 297.
4. Paul, A.S., E.S. Egan, and M.T. Duraisingh, *Host-parasite interactions that guide red blood cell invasion by malaria parasites.* Curr Opin Hematol, 2015. **22**(3): p. 220-6.
5. Chotivanich, K., Udomsangpetch, R., Simpson, J.A., Newton, P., Pukrittayakamee, S., Looareesuwan, S., White, N.J., *Parasite multiplication potential and the severity of falciparum malaria.* Journal of Infectious Diseases, 2000. **181**(3): p. 1206-1209.
6. Wright, K.E., et al., *Structure of malaria invasion protein RH5 with erythrocyte basigin and blocking antibodies.* Nature, 2014. **515**(7527): p. 427-430.

Understanding interactions between interleukin-1 receptor associated kinase 3 (IRAK3) and mitochondria in inflammatory responses.

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Interleukin 1 receptor associated kinase 3 (IRAK3) is a cytoplasmic homeostatic checkpoint of inflammation. IRAK3 contains a cryptic guanylate cyclase (GC) centre, capable of producing cyclic guanosine monophosphate (cGMP) from guanosine triphosphate, embedded within its pseduokinase domain, whose role in inflammation is unclear. We hypothesise that the GC centre creates a localised cGMP-enriched nanodomain surrounding IRAK3 and associated proteins and contributes to suppression of immune responses. IRAK3 inhibits signalling cascades downstream of by preventing dissociation of myddosome complexes (containing MyD88, IRAK1 and IRAK4) associated with toll like receptors (TLRs). Therefore, we are investigating the formation of IRAK3-myddosome complexes in the absence and presence of membrane permeable cGMP. We use monocytic THP-1 cells containing IRAK3 or HEK-BLUE cells with components of the TLR4 system and transiently transfect with wild type or mutated IRAK3 as our model cell systems. We established protocols to favour the formation of IRAK3-myddosome complexes following stimulation of TLRs with lipopolysaccharide. We describe our initial antibody testing to visualise Myd88 and IRAK3 in cells. Recently, the IRAK3-myddosome complex has been reported migrating to the inner membrane and matrix of mitochondria. Hence, we have established organelle fractionation protocols to determine if lipopolysaccharide promotes IRAK3 and Myd88 association with mitochondria and how these processes are affected by cGMP levels. Longer term, we will use super-resolution microscopy in conjunction with seahorse assays to probe the interactions between cGMP, IRAK3 and mitochondria. Interestingly, suppression of IRAK3 expression is also associated with high concentrations of reactive oxygen species (ROS) that affects inflammatory responses.

Variation in epigenetic profiles in newborns conceived with assisted reproduction - current evidence and future approaches

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More than 10 million children have been conceived via assisted reproductive technologies (ART) globally. ART is associated with increased risk of adverse obstetric and perinatal outcomes, including low birth weight, preterm birth, being small and large for gestational age, and preeclampsia. The concurrent timing of ART techniques and pre-implantation epigenetic remodeling has led to the theory that ART-induced modulation of the epigenome may be connected to neonatal health risks. Previous studies, including ours (1), have found that newborns born with ART have altered DNA methylation profiles at birth compared to naturally-conceived newborns. This includes several gene loci implicated in complex disease, although the relevance to ART-associated outcomes remains unclear.

Our current study aims at extending the evidence of ART-induced variation in DNA methylation, and to establish an alternative cost-effective method for assessing DNA methylation, compatible with large cohort sample numbers.

We generated genome-wide DNA methylation data covering >850,000 CpG sites in umbilical cord blood of ART (n=63) and naturally-conceived newborns (n=836) from a longitudinal birth cohort (Barwon Infant Study). We found significant differences in DNA methylation between groups at 1700 genomic sites, including in genes *CHRNE*, *NECAB3* and *WRB/GET1* that have been previously reported as showing ART-associated variation in methylation levels (1-3). Next, we designed a set of targeted bisulfite-based sequencing assays with utility to validate candidate genes, using bisulfite-specific primers that can be combined in a single amplification reaction to interrogate genomic regions of interest. This bisulfite-sequencing-based method can be used in future studies to investigate how widespread ART-associated epigenetic variation is across different tissues, the temporal stability of epigenetic variability and the extent which different ART components contribute to epigenetic differences.

1. Novakovic B, et al. (2019) Assisted reproductive technologies are associated with limited epigenetic variation at birth that largely resolves by adulthood. Nature Communications. 10(1):3922.
2. Håberg S, et al. (2022) DNA methylation in newborns conceived by assisted reproductive technology. Nature Communications. 13(1):1896.
3. Yeung E, et al. (2021) Conception by fertility treatment and offspring deoxyribonucleic acid methylation. Fertility and Sterility. 116(2):493-504.

Killer MAITs: Investigating the cytotoxic capacity of MAIT cells towards tumours

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Mucosal-associated invariant T (MAIT) cells are a subset of innate-like T cells that recognise small metabolite antigens (eg. 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU), a riboflavin pathway intermediate synthesised by microbes) presented by MHC class I-related protein 1 (MR1), a ubiquitous and highly conserved protein expressed by mammalian cells[1]. Activated MAIT cells engage several inflammatory and cytotoxic pathways and have a well-defined protective role in bacterial infection[2].
Recent research into the role of MAIT cells in other pathologies has demonstrated correlation between MAIT infiltration into the tumour microenvironment and poor clinical outcomes in a range of cancer types[3]. Conversely, *in vitro* data suggest a protective role for MAIT cells in cancer[4]. Overall, many basic questions remain regarding MAIT cell activity in the tumour microenvironment.

Using *in vitro* techniques, we define the activation and cytotoxic capacity of primary human MAIT cells in response to tumour cells. Here, we show that MAIT cells possess an innate cytotoxic capacity towards tumour cell lines; however, the MAIT killing response is variable between individuals.

Future directions involve investigation into enhancing the observed cytotoxic response with the addition of stimulating signals, and determining the MR1 dependence of this response. Overall, exploiting the highly conserved nature of the MAIT TCR and MR1 to elicit MAIT cell responses to tumours presents an attractive avenue for development of “off the shelf” immune-based therapeutics that are broadly applicable to all individuals[5].

1. Godfrey, D. I., Koay, H.-F., McCluskey, J. & Gherardin, N. A. The biology and functional importance of MAIT cells. *Nat. Immunol.* **20**, 1110–1128 (2019).
2. Wang, H. *et al.* MAIT cells protect against pulmonary Legionella longbeachae infection. *Nat. Commun.* **9**, 3350 (2018).
3. Duan, M. *et al.* Activated and Exhausted MAIT Cells Foster Disease Progression and Indicate Poor Outcome in Hepatocellular Carcinoma. *Clin. Cancer Res.* **25**, 3304–3316 (2019)
4. Gherardin, N. A. *et al.* Enumeration, functional responses and cytotoxic capacity of MAIT cells in newly diagnosed and relapsed multiple myeloma. *Sci. Rep.* **8**, 4159 (2018).
5. Cogswell, D. *et al.* MAIT cells: Partners or enemies in cancer immunotherapy? *Cancers* **13,** 1502 (2021).

Targeting human coronaviruses using nanobodies

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Coronaviruses are a diverse family of enveloped single-stranded RNA viruses that infect humans as well as a broad variety of other species. To date, seven human coronaviruses (hCoVs) have been identified. HCoV-OC43, HCoV-HKU1, HCoV-NL63 and HCoV-229E cause endemic, mild respiratory disease associated with the "common cold". In contrast, SARS-CoV, MERS-CoV and SARS-CoV2 are highly pathogenic, having caused two epidemics in 2003 and 2012, as well as the ongoing COVID-19 pandemic. Outside of SARS-Cov2, there are currently no approved prophylactic or therapeutic treatments for hCoVs.

The rapid emergence of the COVID-19 pandemic and its widespread impact demonstrate the need for effective hCoV therapeutics going into the future. Our lab utilises a nanobody platform to develop targeted therapeutics against infectious diseases. Nanobodies are small antigen-recognising domains derived from heavy-chain-only antibodies produced by camelids. Nanobodies have many characteristics which lend them favourably to therapeutic development such as their small size, stability across temperature and pH, high antigen binding affinities and high expression across a variety of expression systems.

Nanobody libraries for MERS-CoV, HCoV-OC43, HCoV-HKU1, HCoV-NL63 and HCoV-229E were generated through immunisation of alpacas. Nanobodies that bind with high affinity to their respective recombinant receptor binding domains were isolated and characterised. Of note, we identified nanobodies that potently neutralised hCoV-OC43 *in vitro* using a microneutralization assay. The ability of these nanobodies to confer *In vivo* protection against hCoV-OC43 was tested using a mouse model of infection. The pipeline established here for the generation of nanobodies against human coronaviruses will aid in the development of novel therapeutics against current and future coronavirus outbreaks.

Novel approaches to the detection and surveillance of *Neisseria gonorrhoeae*

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In 2020, the sexually transmitted bacterial pathogen, *Neisseria gonorrhoeae*, caused over 80 million global cases of gonorrhoea (1). Concerningly, the number of circulating *N. gonorrhoeae* strains resistant to antibiotic treatments has risen over the past century. As such, widespread and accurate diagnostic testing is essential.

To improve access to diagnostic testing for gonorrhoea, sample self-collection approaches have been introduced. Essentially, individuals can collect their own samples, and then send these to a laboratory or clinic for analysis. Recent studies show that self-collection has increased the usage of testing services as well as detection rates of gonorrhoea (2). However, relatively little is known about the conditions required for maintaining *N. gonorrhoeae* sample integrity between sample collection, and laboratory detection. Accordingly, we investigated the effect of four sample storage variables – time, temperature, storage buffer and sample concentration across multiple diagnostic platforms to determine the ideal parameters for sample storage and transport. For *N. gonorrhoeae* samples spiked into differing storage buffers, our preliminary findings showed a reduction in *N. gonorrhoeae* detection when stored for longer than 4 days, particularly at or above 25°C. These findings were substantiated by ANOVA statistical analysis.

Furthermore, to facilitate improved medical intervention for individuals infected with gonorrhoea, a more informative analysis of samples is urgently needed. Thus, we investigated whether samples collected for nucleic acid testing could yield high-quality genomic data, suitable for in silico determination of antimicrobial resistance and epidemiological typing of *N. gonorrhoeae*. To achieve this, we optimised a novel metagenomic sequencing approach, known as target capture enrichment sequencing which is utilised to increase the abundance of *N. gonorrhoeae* DNA relative to host/human DNA in any given clinical sample. Currently, we have successfully applied this protocol on a range of clinical samples containing *N. gonorrhoeae*. Taken together, our findings could lay the foundation for 'precision medicine' approaches in diagnosing and treating gonorrhoea in the near future.

References

1. WHO. Multi-drug resistant gonorrhoea [Internet]. Geneva: WHO Press; 2021 Nov 10. Available from: https://www.who.int/news-room/fact-sheets/detail/multi-drug-resistant-gonorrhoea
2. Kersh EN, Shukla M, Raphael BH, Habel M, Park I. At-Home Specimen Self-Collection and Self-Testing for Sexually Transmitted Infection Screening Demand Accelerated by the COVID-19 Pandemic: A Review of Laboratory Implementation Issues. Journal of Clinical Microbiology. 2021;59(11):e02646-20.

Perturbed *S*-palmitoylation cycles alters protein trafficking in dendritic cells

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Protein *S*-palmitoylation is the post-translational modification of proteins by the addition of the lipid molecule, palmitic acid. Like other post-translational modifications, *S*-palmitoylation can influence protein-protein and protein-lipid interactions. It can also determine protein trafficking to membranes, such as the cell surface. These functions are crucial for effective cell-to-cell communication, via receptors and membrane-bound or soluble ligands.

Cellular communication is especially important in the immune system, where specific interactions between receptors and ligands on antigen-presenting cells and effector cells can result in either activation or inhibition of an immune response. It is predicted that many key immune proteins are *S*-palmitoylated, and therefore that *S*-palmitoylation plays an essential role in the immune response. Previous studies have revealed that dendritic cells, a type of antigen-presenting cell, have an abundance of *S*-palmitoylated proteins. However, elucidating these proteins and the purpose of their modification has been a challenge.

In this project, broad inhibition of palmitoylation or depalmitoylation (the removal of the *S*-palmitoyl moiety) using enzyme-inhibiting drugs has shown a perturbed phenotype of dendritic cells. When palmitoylation is dysregulated, the expression of a broad array of receptors are both increased and decreased at the cell surface. To further study aberrant palmitoylation conditions in dendritic cells, I have produced a CRISPR/Cas9 gene knockout of the depalmitoylation enzyme, palmitoyl-protein thioesterase 1 (PPT1) in the MuTu DC1940 cell culture line. In the absence of PPT1, dendritic cells have a decreased cell surface expression the co-stimulatory molecule B7-1, as well as the inhibitory molecule programmed death-ligand 1 (PD-L1). These knockout cells also show an increased cell surface expression of B7-2, the scavenger receptor C-type lectin 9A (Clec9A) and the chemokine receptor XCR1. Future research targeting protein internalisation and localisation will aim to determine exactly how the trafficking of these receptors is being altered. Additionally, *in vitro* experiments studying antigen presentation and cellular activation by dendritic cells will ultimately decode the effect that the loss of depalmitoylation has on the immune response.

The capacity of B-cell memory to recognise Omicron BA.2 and BA.4/5 following COVID-19 adenoviral vector vaccination

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Background: Two novel vaccine formulations have been developed to combat the SARS-CoV-2 pandemic: mRNA and adenoviral vector SARS-CoV-2 vaccines, with distinct efficacy rates and capacities to elicit humoral immune responses. SARS-CoV-2 variants of concern (VoC) escape vaccine-elicited neutralising antibodies to varying degrees. Memory B cells (Bmem) may represent a better marker of protection against severe disease than serum antibodies in the long term, as they generate rapid recall responses to secondary infections. Both vaccine types generate Bmem recognising the SARS-CoV-2 Spike receptor-binding domain (RBD). However, the phenotype and kinetics of the Bmem response elicited by the adenoviral vector vaccine ChAdOx1 (AstraZeneca) is largely unknown, especially with regards to the capacity to recognise Omicron variants.

Objectives: 1) Characterise the RBD-specific IgG and Bmem response to the first and second doses of the ChAdOx1 vaccine; and 2) determine their capacity to recognise the two predominant Omicron VoC sublineages, BA.2 and BA.4/5.

Methods: Recombinant RBDs of the Wuhan, Delta, Omicron BA.2, and BA.4/5 variants were produced for ELISA-based serology, and biotinylated for fluorescent tetramer formation to identify RBD-specific Bmem by flow cytometry. Peripheral blood was sampled from 35 healthy ChAdOx1 vaccine recipients pre-vaccination, and 4 weeks after dose 1 and dose 2. Plasma IgG specific for each variant were quantified by ELISA, and absolute numbers and immunophenotypes of Bmem recognising Wuhan, Omicron BA.2, and BA.4/5 were measured using flow cytometry.

Preliminary results and expected outcomes: Double dose vaccination with ChAdOx1 elicited a gradual increase in IgG levels against Wuhan RBD. The capacity of IgG to bind Delta was not affected, but recognition of both Omicron sublineages was below 50% of the Wuhan response in all 20 donors analysed by ELISA. All 8 donors analysed by flow cytometry generated RBD-specific Bmem after the first dose, and these were generally boosted in number after the second. A substantial fraction of Wuhan-specific Bmem recognised one or both Omicron variants. Further ELISA and flow cytometry experiments are currently ongoing to examine the whole cohort. This investigation will give insight into the extent of humoral immune memory elicited by primary ChAdOx1 vaccination, to understand the protection it provides against breakthrough infections with VoC.

Differential immune detection of *Bacteroides fragilis* and their outer membrane vesicles

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Outer membrane vesicles (OMVs) are secreted by Gram-negative bacteria and package bacterial cargo including proteins, peptidoglycan, lipids and nucleic acids, enabling the delivery of immunogenic cargo to host cells. The cargo composition of OMVs can determine their detection by host innate immune receptors and their biological functions. Recently, the release of OMVs by the intestinal microbiota, including the commensal *Bacteroides fragilis*, have emerged as a novel mechanism to mediate immune responses in the host. In this study, we aimed to delineate the immune receptors activated by *B. fragilis* OMVs to mediate host innate immune responses compared to their parent bacteria.

First, the size and composition of purified *B. fragilis* OMVs was characterised, revealing that immunostimulatory bacterial cargo including proteins, LPS, peptidoglycan and nucleic acids were associated with *B. fragilis* OMVs. Additionally, we observed the enrichment of protein, LPS and peptidoglycan cargo into OMVs. Using confocal microscopy, we saw that *B. fragilis* OMVs enter host epithelial cells to mediate the intracellular delivery of bacterial RNA and peptidoglycan. Furthermore, the potential of OMVs to activate innate immune receptors was compared to their parent bacteria using HEK-Blue reporter cell lines. We identified that OMVs as well as live and heat-killed *B. fragilis* bacteria could all activate Toll-like receptor (TLR)-2. However, only *B. fragilis* OMVs induced the activation of TLR4, TLR7 and NOD1 that detect bacterial LPS, RNA and peptidoglycan, respectively.

Collectively, our results demonstrate that *B. fragilis* OMVs activate distinct innate immune receptors compared to their parent bacteria, revealing that OMV secretion by the intestinal microbiota directly facilitates the activation of host immunity. Our findings contribute to accumulating evidence highlighting the importance of OMV secretion by the microbiota at the gastrointestinal mucosa.

p-cresol sulfate acts on epithelial cells to reduce allergic airway inflammation

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p-cresol sulfate is a microbial metabolite derived from L-tyrosine and was recently discovered to have immunoregulatory influences in the context of allergic airway inflammation (Wypych et al. 2021). Administration of *p*-cresol sulfate to mice led to reduced house dust mite-induced production of CCL20, a chemokine responsible for the recruitment of lymphocytes and dendritic cells. We are using *p*-cresol sulfate as a small molecule template to develop novel therapeutic agents against allergic asthma. Our aim is to first determine the molecular mechanism of action of *p*-cresol sulfate in alleviating allergic airway inflammation. We have isolated primary mouse lung cells and stimulated them with lipopolysaccharide, a stronger inducer of CCL20, which led to the identification of type II epithelial cells as the main cell type affected by *p*-cresol sulfate. This data was confirmed utilising A549 human alveolar epithelial cells. RNA sequencing on *ex vivo* derived mouse epithelial cells revealed that *p*-cresol sulfate influenced heat shock protein 90 (HSP90) gene expression, and indeed, blockade of HSP90 was also shown to reduce CCL20 production, suggesting it may be involved in *p*-cresol sulfate's mechanism of action. Furthermore, molecular modelling has indicated that *p*-cresol sulfate binds to an inter-domain pocket of epidermal growth factor receptor (EGFR). We are currently investigating EGFR-signalling by western blotting and *in silico* analysis. In conclusion, *p*-cresol sulfate acts on airway epithelial cells to reduce CCL20 production and consequently allergic airway inflammation. Elucidation of the molecule's mechanism of action may provide the impetus for development of new chemical entities based upon *p*-cresol sulfate, which could lead to novel therapeutics against allergic asthma and other atopic diseases.

Reference

Wypych, Tomasz P, Céline Pattaroni, Olaf Perdijk, Carmen Yap, Aurélien Trompette, Dovile Anderson, Darren J Creek, Nicola L Harris, and Benjamin J Marsland. 2021. "Microbial Metabolism of L-Tyrosine Protects against Allergic Airway Inflammation." *Nature Immunology*. https://doi.org/10.1038/s41590-020-00856-3.

Similar antibody and higher T-cell responses after vaccination against SARS-CoV-2 in middle-aged people with well-controlled HIV-1 compared to demographically and lifestyle-comparable controls

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Background: Vaccination against SARS-CoV-2 reduces the risk of severe and/or symptomatic COVID-19. However, studies comparing humoral and cellular SARS-CoV-2 vaccine responses in people living with HIV-1 (PWH) and comparable HIV-negative controls are scarce.

Method: SARS-CoV-2 Spike (S)-specific IgG was measured by Luminex immunoassay and T-cell responses by IFNγ release after S and N-peptide-pool stimulation in samples from PWH and HIV-negative controls of the Amsterdam AGEhIV COVID-19 substudy, collected before vaccination and 4-13 weeks after completing the initial SARS-CoV-2 vaccination course. Prior SARS-CoV-2 infection was determined with a positive INgezim® nucleocapsid IgA/IgM/IgG test or self-reported positive PCR. Factors associated with anti-S IgG titers and T-cell responses were assessed by multivariable linear and tobit regression, respectively. Ancestral and Delta SARS-CoV-2 neutralization was determined in a subgroup of 2 x 40 age-, gender- and vaccine-matched PWH and controls on VeroE6 cells with a MTT-assay.

Results: Anti-S IgG titers and neutralization were not significantly different between 195 PWH and 246 controls. Prior SARS-CoV-2 infection (β=0.77), mRNA vaccine (β=0.56), female gender (β=0.24) and fewer days between last vaccination and sampling (β=0.07) were associated with higher, and a CD4/8 ratio<1.0 with lower (β=-0.39) anti-S IgG titers, without significant interactions between HIV-status and any of these factors. T-cell responses were higher in PWH before and after vaccination. Prior SARS-CoV-2 infection (β=0.97), HIV-positive status (β=0.63) and fewer days between last vaccination and sampling (β=0.10) were associated with higher T-cell responses, after adjusting for pre-vaccination levels.

Conclusions: At 4-13 weeks after SARS-CoV-2 vaccination, PWH have similar IgG and neutralizing antibody but higher T-cell responses compared to demographically and lifestyle-comparable controls without HIV. Several factors affecting the height of the responses were identified, which were similar in both groups. Future analyses will explore potential relationships with immune senescence, functionality of the T-cell response and durability of immunity in PWH.

Lipid Droplets are Metabolic Hubs During Viral Infection

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During viral infection, many cellular and metabolic alterations occur within cells, however, it is unclear whether initial metabolic changes are host driven to facilitate effective antiviral immunity, in contrast to being viral driven. We have previously shown that lipid droplets (LDs) are upregulated by the host during the initial stages of viral infection, enhancing the cellular antiviral state. During the later stages of viral infection however, LD production is significantly downregulated, potentially as a viral immune evasion strategy. Little is known about the contribution of LDs to the metabolic shift seen during infection therefore, this study sought to investigate this.

To investigate important host driven lipid enzymes, western blot analysis was performed on six key regulators of cellular metabolism (PPARα, PPARγ, FADS1, FADS2, ALOX5, ALOX15) during dsRNA activation of early antiviral innate immune pathways. Of these six enzymes, only PPARα and FADS1 were upregulated at 4 hours post dsRNA simulation. Interestingly, emerging evidence indicates that the expression of PPARα and FADS1 is downregulated by several viruses, impairing innate anti-viral immune responses, thus indicating that they could be a target. Proteomic analysis of isolated pure LDs from zika virus (ZIKV) infected cells (dsRNA virus) revealed the presence of both FADS1, and FAM12OA (constitutive activator of PPARα); with FADS1 being upregulated in these fractions early at 8 hrs post ZIKV infection and downregulated during active infection (24 hpi).

Our laboratory has shown that LD upregulation involves activation of EGFR Therefore, to understand if LDs are linked to the upregulation of these enzymes following innate immune stimulation of cells, we plan to inhibit EGFR to observe expression of PPARα and FADS1 during infection. These studies in addition to metabolic assays will provide a greater understanding of the role LDs have in metabolism during viral infection.

Overall, this work has provided insights into the cellular metabolic pathways upregulated by the host during viral infection and is the first step to understanding the antiviral role of LDs during infection.

The role of the tetraspanin CD53 in type 2 asthma responses

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Asthma is a chronic lung disease affecting millions of people worldwide. Many patients have a form of asthma characterised by type 2 inflammation, which is driven by eosinophils and Th2 cytokines. To further understand the molecular mechanisms underlying type 2 asthma pathogenesis, tetraspanins were investigated. Tetraspanins function to organise plasma membrane molecules into highly structured microdomains and have important roles in cell signalling and cell-cell contact. Previous studies on tetraspanins have found that they are involved in a variety of processes including cell migration, development, reproduction and immunity (1); however, the role of CD53, which is expressed on most leukocytes, is less well understood. CD53 regulates lymphocyte recirculation through L-selectin (2) as well as early B cell development (3) and we hypothesised it could be important in type 2 asthma. Using CD53 knockout mice, we assessed the role of CD53 in the classical ovalbumin sensitisation and challenge model of type 2 asthma. We found that CD53-/- mice had reduced leukocyte infiltration into the bronchoalveolar lavage and lung tissue after asthma challenge compared to C57BL/6 controls. Furthermore, asthma-challenged CD53-/- mice had markedly reduced serum IgE levels, a hallmark of the allergic asthma response. Additionally, the lung-draining mediastinal lymph nodes had a larger proportion of CD4+ and CD8+ T cells compared to control asthma-challenged mice, suggesting impaired T cell migration from the lymph nodes to the lungs. However, PAS/AB staining of the lungs showed a similar degree of mucus cell metaplasia and inflammation in bronchial regions, which will be further assessed. In conclusion, we have found a potential role of CD53 in mediating cellular trafficking that influences type 2 asthma pathogenesis.

1. Termini CM, Gillette JM. Tetraspanins Function as Regulators of Cellular Signaling. Frontiers in Cell and Developmental Biology. 2017;5.

2. Demaria MC, Yeung L, Peeters R, Wee JL, Mihaljcic M, Jones EL, et al. Tetraspanin CD53 Promotes Lymphocyte Recirculation by Stabilizing L-Selectin Surface Expression. iScience. 2020;23(5):101104.

3. Greenberg ZJ, Monlish DA, Bartnett RL, Yang Y, Shen G, Li W, et al. The Tetraspanin CD53 Regulates Early B Cell Development by Promoting IL-7R Signaling. J Immunol. 2020;204(1):58-67.

Antibody Responses To Polymorphic Regions Of The Lead Malaria Vaccine Antigen

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Malaria caused over 600,000 deaths in 2020, predominantly in young children. The first malaria vaccine, known as RTS,S, was recently recommended for widespread use in at-risk children. However, vaccine efficacy is modest (30-50%) and thus more efficacious vaccines are needed. The mechanistic basis of vaccine-induced immunity is unclear, but antibodies are known to play a main role in mediating protection.

RTS,S is a virus-like particle expressing a truncated form of the major parasite surface protein, circumsporozoite protein (CSP). It includes the major NANP-repeat epitope, a predominant antibody target, but excludes minor repeat sequences and other recently identified protective epitopes. The vaccine also includes the C-terminal domain of CSP. Within the malaria parasite population, this domain is highly polymorphic, however RTS,S is based on only one parasite strain, which occurs in less than 10% of infections in malaria-endemic regions. Polymorphisms may contribute to suboptimal vaccine efficacy, but it remains unclear how this may impact vaccine-induced antibodies.

We investigated the ability of vaccine-induced antibodies to recognise key epitopes and polymorphic regions of CSP to understand how antibody specificity may impact vaccine-induced immunity and efficacy. Using animal models, we showed that vaccination with full-length CSP induced broader reactivity to different protective epitopes (major and minor repeat epitopes) compared to vaccination with the RTS,S construct. Analysis of responses in a longitudinal cohort of young children vaccinated with RTS,S (n=737) demonstrated significant variability in antibody recognition of NANP-repeat epitopes, which correlated with vaccine efficacy. Surprisingly, a proportion of children had promiscuous antibodies that recognised related epitopes within CSP that were not present in the vaccine construct. Furthermore, RTS,S-induced antibodies appear to have reduced binding to diverse alleles within the C-terminal region that are prevalent in African populations. Our findings reveal differential antibody recognition to polymorphic regions within the RTS,S vaccine antigen, and provide insight into how targeting conserved epitopes may be an approach to enhance vaccine efficacy.

Investigating the role of serotonin in inflammatory responses induced by *Helicobacter pylori* infection

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Helicobacter pylori is a significant cause of chronic gastric diseases, such as ulcers and gastric cancer. The host's natural immune response to this infection is ineffective and indeed causes many of the symptoms of the diseases associated with infection. Antibiotics are currently the mainstay of treatment for the pathogen, but there is an increasing problem of resistance globally. Therefore, it is desirable to have an effective vaccine against *H. pylori.* Despite encouraging results in animal models, little success has been achieved in humans however. To develop a safe and effective vaccine, a better understanding of the molecular interactions between the host's immune system and the bacterium is required.

Inflammatory Th1/17 responses are associated with both inflammatory gastritis which does not clear infection, and vaccine- induced reductions in colonization in animal models. An influx of CD4+ T cells is correlated with vaccine- induced protection. There is also evidence that gastric hormones play a role in the inflammatory response to *H. pylori* infection. Here we investigated the role of serotonin, also known as 5-Hydroxytryptamine (5-HT) in the response of gastric epithelia in and *in vitro* and a mouse model. In an *in vitro* model of AGS cells, qPCR analysis showed that 5-HT receptors HTR1A, HTR2A and the pro inflammatory chemokines IL-8 and CXCL8 were all upregulated after co culture with *H. pylori* for 6h. In a mouse model, only HTRB1 was upregulated after 3 days, at 3 weeks post infection Mip-2, Mip-2 and HTR1A were upregulated. Further we showed that vaccinated mice secreted higher levels of 5-HT in the antrum than *H. pylori* challenged controls. Flow cytometry analysis revealed that the expression of HTR1A was reduced on CD4+ T cells, Neutrophils and macrophages in vaccinated compared to control mice, suggesting that 5-HT promotes inflammation rather then protective responses. Together these data support a role for 5-HT signaling in the inflammatory response to *H. pylori* infection, and that it may play a role in the regulation of protective response in vaccinated mice.

In Vivo Assembly of Epitope-coated biopolymer particles that induce anti-tumor responses

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Biopolymers have been investigated for their use as vaccine carriers due to their flexibility and effectiveness in inducing immune response (1). We explored the potential of bacterially assembled biopolymer particles (BP) made of polyhydroxybutyrate (PHB) as vaccine (2). After subcutaneous BP vaccination, C57BL/6 mice show no change in the number of innate and adaptive immune cells in the draining lymph nodes, but a temporary increase in TNF-α, MCP-1 and IL-6 is observed 16-hours post-injection. Next, we genetically engineered BP to express model antigen ovalbumin (BP-OVA) to further assess its antigen presenting capacity. Primary DCs are shown to present OVA-associated antigens to OVA-specific $CD8^+$ and $CD4^+$ T cells *in vitro* and *in vivo*. Subsequently, we found that subcutaneous vaccination of BP-OVA induces potent cytotoxic T cell (CTL) response in absence of $CD4^+$ T cells help, as shown after detection of non-significant change of cytotoxic activity in vaccinated I-$Ab^{-/-}$ and $CD40^{-/-}$ mice compared to wild-type mice. Neither is conventional DCs type I (cDC1) required for BP-OVA CTL response, as shown by no change of CTL response in $Batf3^{-/-}$ mice. Subsequently, PolyI:C improves BP-OVA generated CTL response significantly, where CpG and lipopolysaccharide have failed to do so. Lastly, vaccination with BP-OVA show protective responses against B16-OVA melanoma and Eμ-myc-GFP lymphoma inoculation in vivo as shown by reduction of tumour nodules and tumour number respectively. In summary, we have characterised the immunogenicity of BP as vaccine carrier and demonstrated its effectiveness against tumour cells.

1. M. F. Moradali, B. H. A. Rehm, Bacterial biopolymers: from pathogenesis to advanced materials. *Nat. Rev. Microbiol.* **18**, 195–210 (2020).
2. N. A. Parlane, S. K. Gupta, P. Rubio-Reyes, S. Chen, M. Gonzalez-Miro, D. N. Wedlock, B. H. A. Rehm, Self-Assembled Protein-Coated Polyhydroxyalkanoate Beads: Properties and Biomedical Applications. *ACS Biomater. Sci. Eng.* **3**, 3043–3057 (2017).

The role of Type III interferon signalling in cDC1-targeting vaccination

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Type 1 conventional dendritic cells (cDC1) are the key antigen-presenting cells (APCs) responsible for efficient cross-presentation and CD8 T cell activation. For this reason, a cDC1- targeting cancer vaccine has been intensively studied *in vivo* and demonstrated great efficacy in generating antigen-specific, endogenous CD8 T cell responses, particularly when co-administered with adjuvants, such as polyinosinic: polycytidylic acid (poly I:C). Poly I:C is a synthetic analog of double-stranded RNA that is recognised by toll-like receptor 3 (TLR3). And type III interferon (IFN-III or IFN -λ), is one of the major cytokines selectively produced by cDC1s upon poly I:C-mediated TLR3 activation.

A recent study showed a strong correlation between positive clinical outcomes and IFN-λ producing cDC1s in patients with breast cancer (1). It further correlated IFN-λ production with a favourable tumour microenvironment for recruiting and activating cytotoxic immune cells, indicated by increases in TNF-α, IFN-γ and CXCL10 (1).

It remains unclear how these cDC1-driven, IFN-λ-mediated innate signals play a role in antigen presentation and CD8 T cell priming. Our preliminary *in vitro* data reveals that there is no change in IFN-λ production by cDC1 when targeted with the cDC1-targeting vaccine. Therefore, we sought to investigate the impact of IFN- λ signalling in *in vivo* cDC1-targeting vaccination using IFN- λR1 KO mice. We hypothesised that lacking IFN-λ will have a negative impact on vaccine-induced endogenous CD8 T cell responses.

1. Hubert M, Gobbini E, Couillault C, Manh T-PV, Doffin A-C, Berthet J, et al. IFN-III is selectively produced by cDC1 and predicts good clinical outcome in breast cancer. Sci Immunol. 2020;5(46).

Immunity to malaria: association between antibodies and clinical protection in *Plasmodium vivax* low-endemic areas

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Malaria is one of the top ten causes of death in low-income countries. Immunity to *Plasmodium spp.*, the causative agent of malaria, is difficult to achieve. A single infection with *P. vivax* does not confer long-lasting protection, and multiple exposures are required to build up immunity. Furthermore, naturally acquired immunity only offers protection against symptomatic disease, leaving the individual susceptible to asymptomatic infection. One existing malaria vaccine has been approved for use but is only recommended for children living in moderate-to-high burden areas of *Plasmodium falciparum*. As *P. vivax* is the predominant *Plasmodium* species in many countries, a clear vaccine gap exists. The search for a *P. vivax* vaccine is notably difficult due to the complex lifecycle of the parasite and the sheer number of proteins it expresses, providing thousands of potential protein targets.

This research aims to determine the association between antibodies to 22 mostly blood-stage *P. vivax* antigens and protection against future clinical infections. To do this, we have analysed previously collected data (Longley et al, 2020) consisting of epidemiological variables and antibody data from a yearlong observational cohort in Thailand, a malaria-endemic area. Total IgG antibody levels were quantified at the beginning of the study using a Luminex multiplex system, with subsequent malaria infections identified through monthly active case detection. We then performed a survival analysis to determine the association between antibody levels and protection from clinical malaria, as well as the impact of epidemiological factors such as age and gender. The results of the Thai cohort identified total IgG antibodies against 10 antigens are important for protection against clinical malaria. We are currently performing this analysis on a similar dataset collected in Brazil to corroborate the results in a second malaria-endemic area.

By identifying the biological basis of naturally acquired immunity, we can provide the foundation for future immune-based therapies with the ultimate goal of reducing the malaria burden in *P. vivax*-endemic areas.

Developing phage depolymerase enzymes to disarm *Klebsiella pneumoniae*

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In Australia, hospital acquired infections (HAIs) result in 2 million extra days of hospitalisation each year (1). HAIs with multi-drug resistant (MDR) *Klebsiella pneumoniae (Kp)* present a serious risk for patients due to limited effective treatment options. Despite rigorous cleaning protocols, the most common cause of HAIs are from bacteria present in the hospital environment (equipment, surfaces, patients, and staff) (2). Novel strategies are needed to improve hospital cleaning outcomes. One such approach involves examining bacteriophages, which produce a multiplicity of enzymes that have anti-bacterial properties. One such enzyme family, known as the depolymerases, can degrade both the protective sticky capsule that covers the *Kp* cell as well as bacterial biofilms, rendering the bacteria avirulent. Depolymerase activity could be exploited to provide a solution to HAIs by disarming pathogenic bacteria before they reach the patient as part of a disinfectant regimen. However, presently depolymerases are poorly characterised in the literature and are highly variable in sequence and enzymatic mechanism. More research is needed to understand the molecular determinants of their structure, specificity, and function.

We have mined our extensive *Kp*-targeting phage collection and identified putative depolymerases. In this study, we are building a knowledge base of the depolymerases' biological activity, mechanism of action and structure. To date, we have purified seven recombinant depolymerase enzymes and have shown them to be active against multiple *Kp* strains (including hypervirulent strains). These enzymes additionally demonstrate remarkable shelf-life and stability in harsh chemical and thermal environments. The data gained is transformative to the knowledgebase of these underappreciated enzymes. Additionally, given these highly advantageous biochemical properties, we expect these enzymes to serve as an excellent platform to prevent *Kp* contamination via a disinfectant strategy as part of surface decontamination formulations or as an anti-fouling coating on hospital surfaces. These future bio-agents can be engineered in order to generate candidates with superior properties to confront the emergence of MDR *Kp.*

References

[1] Australian Safety and Quality Goals for Healthcare: Goal 1.2: Healthcare-Associated Infection Action Guide 2012 20 May 2020:[12p.]. Available from: https://www.safetyandquality.gov.au/sites/default/files/migrated/1.2-Healthcare-Associated-Infection.pdf.
[2] Hughes R. Patient Safety and Quality: An Evidence-Based Handbook for Nurses. 2008

ABSTRACTS
POSTER 2

Risk factors for SARS-CoV-2 infection, by variant of concern: a systematic literature review

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Background:
Identifying how risk changes as new SARS-CoV-2 variants of concern (VoC) emerge and how infection risk may change as public health and social measures (PHSM) are adjusted, allows for a better understanding of who is at greater risk, especially early in the pandemic. As new, more transmissible VoC emerge, vaccination rates increase and PHSM are eased over the course of the pandemic, it is important to understand risk factors for SARS-CoV-2 infection and whether they change over time. This systematic literature review aims to identify the risk factors for SARS-CoV-2 infection and describe these by VoC.

Methods:
Searches were performed in MEDLINE, PubMed and Embase databases on 5 May 2022. Eligibility included: observational studies published in English, after 1 January 2020; any age group; the outcome of SARS-CoV-2 infection; and described exposure risk factors (e.g., socioeconomic status, employment type/status, household size, ethnicity). Results were synthesized into a narrative summary with respect to measures of association, by VoC. ROBINS-E tool was utilised for risk of bias assessment.

Results:
From the database searches, 6197 studies were retrieved. After duplicate removal, title/abstract and full text screening, 43 studies were eligible for inclusion. Common risk factors included older age, ethnicity, low socioeconomic status, male gender, increased household size, occupation/lower income level, inability to work from home/public transport use, and lower education level. Most studies were undertaken when the ancestral strain was predominant. Many studies had some selection bias due to testing criteria and limited laboratory capacity.

Conclusion:
Understanding who is at risk enables the development of strategies that target priority groups at each of the different stages of the pandemic and helps inform vaccination strategies and other interventions which may also inform public health responses to future respiratory infection outbreaks.

Deciphering the molecular mechanisms behind trans-synaptic transmission of rabies virus.

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Neural junctions known as synapses are responsible for the transfer of communication signals between neurons and enable all aspects of brain function. Rabies (*lyssavirus*) is a deadly zoonotic virus that has the unique ability to infect the host nervous system and exclusively transfer across synapses. This trans-synaptic transmission allows rabies virus to largely evade detection from the immune system and leads to irreversible damage to the nervous system. Although the virus has existed over several centuries, a lot is still unknown about the pathogenesis of the virus and the signaling pathways responsible for its trans-synaptic transmission. It is known that the rabies viral glycoprotein is essential for its movement across the synapse, but the mechanisms involved are still unknown.

In this study we developed a lentiviral expression system to individually express rabies glycoprotein in neurons and study how it mediates trans-synaptic transmission. We used advanced confocal microscopy to analyse the trans-synaptic transfer of the virus in high and low neuroinvasive strains. The highly invasive strain showed a more efficient trans-synaptic transmission of the virus, and the glycoprotein derived from this strain increased dendritic filipodia-like structures leading to increased synapse formation between neurons. We are now performing proteomic analyses to identify the neuronal proteins influenced by rabies glycoprotein to hijack synapse formation. We will then perform super-resolution microscopy to investigate this novel role of rabies glycoprotein in synapse formation at nanoscale resolution.

The identification of neuronal host proteins and mechanisms involved in trans-synaptic transmission of the virus could provide valuable knowledge to develop future therapeutics for rabies treatment. Additionally, these studies are expected to identify novel neuronal molecules involved in synapse formation guided by the rabies glycoprotein, increasing our basic understanding of synaptogenesis. This information could also be used to design next generation rabies inspired therapeutics to increase synapse formation in neurodegenerative and neurodevelopmental diseases that occur as a result of synapse degeneration or deformation.

Using super resolution microscopy to visualise epigenetic control of T cell fate and function.

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T cells are key immune cells which provide immunity against bacteria, viruses and cancers. Upon detecting cognate antigen, naive T cells proliferate and differentiate, spawning activated effector cells and long-lived memory cells. Recent work has shown that epigenetic marks such as histone modifications play a critical role in controlling T cell differentiation and their ability to combat pathogens (1-2). Histone post translational modifications (PTMs) are speculated to regulate gene expression through changes in the spatial organisation of chromatin (3). However, most epigenetic research involves using biochemical assays which overlook the spatial organisation of histone PTMs. Therefore, visualising the T cell nuclei would provide information on the distribution, clustering and co-localisations of histone modifications. We utilise direct stochastic optical reconstruction microscopy (dSTORM) to image single molecules in 2D and 3D down to a resolution of 20 nm (4).

Visualising T cells with dSTORM, we identified active histone mark H3K4me3 to be localised in the centre of the nucleus, whilst repressive histone mark H3K27me3 was found towards the nuclear periphery. This indicates that genes that are active and repressed occupy different parts of the nucleus, and as such, suggest that movement between these different compartments may choreograph T cell differentiation. Effector T cells that lacked the methyltransferase DOT1L, which methylates H3K79me2, resulted in an altered spatial organisation of the histone PTM H3K4me3. This suggests that there is a potential cross-regulation of histone PTMs and spatial organisation as has been described for other pairs of modifications (5). Using dSTORM imaging, we are interested in understanding if and how histone PTMs determine nuclear positioning and whether this in turn regulates T cell differentiation and function. Understanding what drives T cell differentiation and function would be crucial to designing T cell-based immunotherapies, vaccines and preventing autoimmunities.

1. Tumes, DJ., Onodera, A., Suzuki, A., Shinoda, K., Endo, Y., Iwamura, C., Hosokawa, H., Koseki, H., Tokoyoda, K., Suzuki, Y., Motohashi, S and Nakayama, T. 2013. The Polycomb Protein Ezh2 Regulates Differentiation and Plasticity of CD4+ T Helper Type 1 and Type 2 Cells. *Immunity* 39: 819-832.
2. Scheer, S., Runting, J., Bramhall, M., Russ, B., Zaini, A., Ellemor. J., Rodrigues, G., Ng, J and Zaph, C. 2020. The Methyltransferase DOT1L controls activation and lineage integrity in CD4+ T cells during infection and inflammation. *Cell Reports* 33: 108505.
3. Cheung, P and Lau, P. 2005. Epigenetic Regulation by Histone Methylation and Histone Variants. *Molecular Endocrinology*. 19: 563-573.
4. Rozario AM., Morey, A., Elliott, C., Russ, B., Whelan, DR., Turner, SJ and Bell, TDM. 2022. 3D Single Molecule Super-Resolution Microscopy of Whole Nuclear Lamina. *Frontiers in Chemistry*. doi: 10.3389/fchem.2022.863610
5. Russ, BE., Olshanksy, M., Smallwood, HS., Li, J., Denton, AE., Prier, JE., Stock, AT., Croom, HA., Cullen, JG., Nguyen, MLT., et. al. 2014. Distinct epigenetic signatures delineate transcriptional programs during virus-specific CD8+ T cell differentiation. *Immunity* 41: 853-865.

Lipid droplets act as platforms for innate immune signalling proteins

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Lipid Droplets (LDs) were initially considered simply as a cellular energy source but are now recognised as critical organelles in signalling events, transient protein sequestration and inter-organelle interactions. Recently, our laboratory has demonstrated that LDs are upregulated during viral infection and contribute to an enhanced interferon driven antiviral response. However, the mechanisms utilized by LDs to enhance antiviral immunity remain unknown.

Here, we describe for the first time that there are several key antiviral signalling molecules that localise to LDs during this response. Proteomic analysis of isolated LDs from mock, or infected cells has revealed there was 92 significantly upregulated proteins on LDs 24 hr post viral mimic infection with 13% of the significantly enriched proteins being associated with the interferon response. Of these, viperin, RIG-I, STAT1, MX1 and ISG15 were all significantly upregulated at 24hpi, with MX1 and ISG15 upregulated as early as 8 hpi. As many of the significantly upregulated proteins detected are thought to be cytoplasmic, we designed a novel technique to confirm the localisation of these signalling proteins to the LD. Cells were transfected with fluorescently labelled proteins of interest, LDs isolated, and confocal microscopy was utilised to detect our targets. All targets were observed on a subset of LDs ranging from 80% for a LD resident protein (Viperin) and ~10% for novel antiviral LD proteins, including STAT1, STAT2 and MX1. Western blotting also confirmed the localisation of these novel proteins to LDs. In addition to key antiviral proteins, proteomic analysis also revealed that approximately 10% of LD proteins are involved in regulating post translational modifications (PTMs) including ubiquitylation, phosphorylation and ISGylation, with ISGylation regulatory proteins also upregulated following viral mimic infection. The mechanism by which antiviral proteins localise to LDs remains elusive, and PTMs of these proteins may contribute to transient protein localisation following infection.

Here, we demonstrate that there are important antiviral immune signalling proteins that localise to the LD following viral mimic stimulation, indicating that the LD may act as a signalling platform for signalosome formation to aid host immunity for the first time.

Investigating the role of γδ T cells and IL-4 in radiation-attenuated sporozoite vaccination

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Studying immune responses to pathogens has often provided mechanisms to understand the basic biology of complex immunological systems. One such example of this is intravenous vaccination with radiation-attenuated sporozoites (RAS), the current gold-standard vaccination strategy against liver-stage malaria. In mice, RAS vaccination confers sterile protection against challenge infection through expansion and proliferation of antigen specific $CD8^+$ and $CD4^+$ T cells in the spleen. Expanded cells then migrate to the liver wherein a small subset will remain as resident-memory T cells (T_{RM}) and provide long-lived sterile protection from reinfection. In the absence of γδ T cells, an effective immune response is not generated, leaving mice susceptible to reinfection. The exact cellular mechanisms driving the effective response is currently unknown, though previous work in the lab suggests a role for early priming of $CD8^+$ and $CD4^+$ T responses in the spleen. Using *Plasmodium*-specific T cells as a readout for effective immunity, we have identified a role for γδ T cells in the early expansion of antigen-specific $CD4^+$ and $CD8^+$ T cells. To interrogate the role of γδ T cells following RAS, an antibody-mediated blockade against the γδ TCR confirmed a role for these cells in the first 24 hours after antigen exposure. Further investigation of molecules involved in the γδ T cell response revealed a requirement for IL-4 in the early cellular response to RAS and the expansion of antigen-specific T cells. By implementing novel techniques and biological tools, we reveal insights on the role of γδ T cells in an infection model and begin to elucidate their complex functions.

The PTRAMP-CSS heterodimer plays an essential role in *P. falciparum* invasion of human erythrocytes

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The most severe form of malaria is caused by *Plasmodium falciparum*.[1] Despite previous control measures, an effective vaccine against malaria is of high priority.[2] Currently, *P. falciparum* Rh5 is a leading blood-stage vaccine candidate.[3] During erythrocyte invasion by parasites, the ligand PfRh5 forms a complex with PfCyRPA and PfRipr (RCR complex) and binds to basigin on the host cell.[4, 5] PfPTRAMP and PfCSS have been found to interact with PfRipr as a disulphide-linked heterodimer to form a pentameric complex, PCRCR. Using invasion inhibitory nanobodies raised against PfPTRAMP and PfCSS, we utilised lattice light-sheet microscopy to demonstrate that the heterodimer is essential for merozoite invasion. The PCRCR complex functions to anchor the merozoite and erythrocyte membrane brought together by strong parasite membrane deformations. The structure of PfCSS in complex with nanobodies was solved to identify an inhibitory epitope. These findings define the function of the PCRCR complex, and elucidation of invasion neutralising epitopes provides a roadmap towards structure-based immunogen design of these antigens for a blood-stage malaria vaccine.

References

1. Weiss GE, Gilson PR, Taechalertpaisarn T, Tham WH, de Jong NW, Harvey KL, Fowkes FJ, Barlow PN, Rayner JC, Wright GJ *et al*. (2015) Revealing the sequence and resulting cellular morphology of receptor-ligand interactions during Plasmodium falciparum invasion of erythrocytes. *PLoS Pathog*. 11(2):e1004670.
2. World Health Organisation (WHO) (2020) World Malaria Report 2020.
3. Minassian AM, Silk SE, Barrett JR, Nielsen CM, Miura K, Diouf A, Loos C, Fallon JK, Michell AR, White MT *et al*. Reduced blood-stage malaria growth and immune correlates in humans following RH5 vaccination. *Med*.
4. Wong W, Huang R, Menant S, Hong C, Sandow JJ, Birkinshaw RW, Healer J, Hodder AN, Kanjee U, Tonkin CJ *et al*. (2019) Structure of Plasmodium falciparum Rh5-CyRPA-Ripr invasion complex. *Nature*. 565(7737):118-121.
5. Volz JC, Yap A, Sisquella X, Thompson JK, Lim NT, Whitehead LW, Chen L, Lampe M, Tham WH, Wilson D *et al*. (2016) Essential Role of the PfRh5/PfRipr/CyRPA Complex during Plasmodium falciparum Invasion of Erythrocytes. *Cell Host Microbe*. 20(1):60-71.

Nanobodies targeting malaria transmission-blocking candidate

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Malaria parasites develop both in humans and the Anopheles mosquito. Malaria parasite transmission occurs when an infected female *Anopheles* mosquito injects malaria parasites into a human host. Within mosquito, the sexual stages of the malaria parasites, which are called gametocytes, undergo sexual reproduction and several developmental stages to finally produce sporozoites that migrate to the mosquito's salivary glands, ready to infect humans. Parasite numbers are very low in the mosquito stages, representing a promising opportunity for intervention whereby malaria transmission can be blocked by inhibiting the function of essential sexual stage antigens. A major target of transmission-blocking interventions is the 6-cysteine protein P48/45. The 6-cysteine protein family is a family of abundant, highly conserved and surface-exposed proteins that are expressed by malaria parasites throughout their life cycle and play critical roles in their development (1). P48/45 is expressed on the surface of gametocytes and is essential for male fertility, with knock-out males unable to attach to and fertilize female gametes. Recognition of P48/45 by human sera correlates with the ability of sera to block parasite transmission and antibodies against P48/45 have transmission-blocking activity. We have generated nanobodies (2, 3), against *Plasmodium falciparum* P48/45 and demonstrated their specificity using western blotting, ELISA and bio-layer interferometry. We will characterize the P48/45 nanobodies for their ability to block parasite transmission using exflagellation assays and standard membrane feeding assays. For nanobodies with transmission blocking capabilities, we will determine the inhibitory epitopes using structural approaches. Collectively, our results will provide the first collection of nanobodies against Pfs48/45 and insights into the domains of P48/45 that are involved in parasite transmission.

References

1. Lyons FMT, Gabriela M, Tham W-H, Dietrich MH. Plasmodium 6-Cysteine Proteins: Functional Diversity, Transmission-Blocking Antibodies and Structural Scaffolds. Frontiers in Cellular and Infection Microbiology. 2022;12.
2. Muyldermans S. Nanobodies: natural single-domain antibodies. Annu Rev Biochem. 2013;82:775-97.
3. Pardon E, Laeremans T, Triest S, Rasmussen SG, Wohlkonig A, Ruf A, et al. A general protocol for the generation of Nanobodies for structural biology. Nat Protoc. 2014;9(3):674-93.

The off-target effects of BRAF inhibitors on murine dendritic cell activation

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Many aggressive and resistance cancers, such as melanoma, have a *BRAF*^{V600E} mutation, which promotes over 500-fold increased activation of the mitogen-activated protein kinase-extracellular signal-regulated kinase (MAPK-ERK) signalling pathway. This can result in uncontrolled proliferation that transforms regular cells into cancer cells. Therefore, BRAF kinase inhibitors have been developed to specifically target the mutated BRAF^{V600E} protein and block this uncontrolled proliferation. While BRAF inhibitors have shown some clinical benefit, there are a high proportion of non-responders and many patients acquire resistance over the course of treatment. Additionally, there are many side effects and toxicities associated with these therapies that are thought to arise from off-target effects of the treatment.

Although dendritic cells (DCs) are integral to strong anti-tumour responses, the effects of BRAF inhibitors on DC function are not understood. Thus, the aim of this work is to determine whether the BRAF inhibitor Dabrafenib has off-target effects impacting DC function and phenotype and whether these effects are dependent on MAPK-ERK signalling. Using *ex vivo* murine splenic DCs, we show that Dabrafenib affects proinflammatory cytokine production and surface activation marker expression. Additionally, we find that these effects are not mediated through canonical MAPK-ERK signalling pathways. This work may inform future melanoma treatment strategies by enhancing our understanding of off-target effects on immune cells and how these may contribute to treatment-related toxicities.

Establishing Australia's Vaccine Safety Health Link

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Background: Post-licensure vaccine safety surveillance of Adverse Events Following Immunisation (AEFI) is critical to maintain public safety and confidence in vaccines. In most countries around the world, including Australia, surveillance predominantly relies on spontaneous reporting whereby healthcare providers and consumers report AEFI to their jurisdictional safety service. However, spontaneous surveillance is prone to underreporting and reporting bias, making rapid signal verification (or refuting) difficult. Thus, we are creating the Vaccine Safety Health Link (VSHL), a large linked database of routinely collected datasets for improved vaccine safety signal investigation and verification.

Methods: VSHL links records from Australia's nationwide records of vaccination (Australian Immunisation Register) with administrative records from hospital, primary care, perinatal care, disease and mortality datasets to assess vaccinee outcomes in near real-time. Linkage will occur at the Centre for Victorian Data Linkage and the resulting linked dataset will be analysed by participating state vaccine safety services. Results will be communicated to local, state, and national authorities to inform policy, consumers, and healthcare professional action.

Discussion: VSHL will be Australia's largest vaccine safety database and the only one that incorporates primary health and perinatal outcomes. The routinely collected datasets will allow analysis of a broad spectrum of suspected AEFI, including those that may have gone unreported due to minor symptoms, a perceived lack of association with vaccination, lack of understanding around reporting or inability to report. The detailed demographic information available in these datasets allows disaggregated data analyses of AEFI in vulnerable and under-represented populations. VSHL will also provide epidemiologists ability to refute spurious concerns where no associations are found with greater confidence. Ultimately, improved sensitive and rapid signal investigation and validation will ensure the continued safety of all vaccines administered in Australia and maintain provider and community vaccine confidence.

Differential contribution of resident and infiltrating phagocytes in the defense against *L. longbeachae*

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L. longbeachae is the second most prevalent cause of Legionnaire‘s disease, a severe form of pneumonia. Within the lung, alveolar macrophages are the first cells encountering the bacteria. It is largely unknown whether these resident phagocytes serve as a replicative niche for *L. longbeachae* and how infiltrating phagocytes might contribute to the defense. Therefore, the main aim of this study was to use newly generated fluorescent reporter bacteria to track *L. longbeachae* during infection. Our results showed that during the early phase mainly resident alveolar macrophages contained bacteria and that with the progress of infection those cells significantly decreased. This decrease was accompanied with a massive influx of inflammatory phagocytes, mainly neutrophils. Analysis of bacterial viability revealed that neutrophils were apparently most efficient in killing internalized bacteria whereas alveolar macrophages contained the highest number of viable bacteria per cell. Restriction of those cell types in the lung during infection with *L. longbeachae* unveiled that infiltrating neutrophils are required for efficient bacterial clearance. In striking contrast lung-resident alveolar macrophages promote progress of infection. The presence of NK or T cells as well as the production of IL-18 and ROS were required for efficient microbiocidal activity of neutrophils. Our results highly suggest resident alveolar macrophages to be a replicative niche for *L. longbeachae* while infiltrating neutrophils confer protection. We are currently investigating if and how alveolar macrophages produce IL-18 in order to stimulate IFN-γ secretion by T and NK.

Mapping the commitment of haematopoietic progenitors to the T cell lineage

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T cells develop in the thymus from bone marrow-derived haematopoietic progenitors that migrate via the bloodstream. Previous studies have identified multiple haematopoietic progenitors with T cell lineage potential in the bone marrow. However, the identity of the populations that actually colonise the thymus remains unclear. The characterisation of these thymus seeding progenitors and their immediate progeny, the early thymic progenitors, has been challenging due to their rarity. Moreover, at least at a population level, these progenitors are thought to remain multipotent after entering the thymus. Thus, they can differentiate into non-T cell lineages until the thymic microenvironment drives their commitment toward the T cell lineage. However, whether the development of non-T cell lineages occurs in the thymus in physiological settings is unclear.

To characterise the earliest stages of T cell development in the thymus, $CD4^{-}CD8^{-}$ double negative thymocytes were profiled in depth by single-cell RNA-sequencing, which allows for the identification of distinct and potentially rare populations based on their transcriptional profiles. Within double negative thymocytes, we identified a novel and transcriptionally discrete population that expressed multiple multipotency-associated markers. However, the overall transcriptional profile of this population did not align with the previously identified "traditional" early thymic progenitor. This novel population also differed in cell surface marker expression. Still, the two populations exhibited similar T cell lineage potential in OP9-DL1 *in vitro* cultures. These findings suggest that there may be more than one population of early thymic progenitors. Future work will determine the non-T cell lineage potential and bone marrow-derived precursors of these thymic progenitors.

A potential role for ADAMTS versicanases in influenza virus infection

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Influenza viruses cause significant global public health concern, with upwards of 600,000 people dying from influenza virus infection and associated complications each year. The SARS-CoV-2 public health measures put in place to stop the spread of SARS-CoV-2 also temporarily kept influenza virus infections suppressed. Removal of restrictions in 2022 has seen a surge in laboratory confirmed cases of influenza virus infection, adding pressure on overburdened healthcare systems. While it is difficult to completely prevent influenza virus infections due to high mutation rates, it may be possible to improve interventions to prevent severe infection. One approach currently being investigated is targeting of host factors not typically associated with immunity. These host factors are not virus-specific and are therefore not vulnerable to viral mutations. Previous studies in our group have highlighted a potential role for extracellular matrix proteinases in viral immunity. Specifically, the A Disintegrin-like and Metalloproteinase with Thrombospondin-1 repeats - 5 (ADAMTS5) enzyme has been shown be involved in efficient migration of influenza virus-specific CD8+ T cells from the lymph node to the periphery, where it contributes to the resolution of infection. Using an ADAMTS15 reduction mouse model we herein present data from a closely related family member, ADAMTS15. This data, coupled with *in vitro* ADAMTS manipulation studies indicate that ADAMTS proteoglycanases impact cytokine expression and virus replication, and are playing an important role in the immune response to influenza virus infection. We conclude that manipulation of ADAMTS enzyme expression may improve management of influenza virus infections and reduce the overall burden of disease.

Eosinophil Hyperactivity in Early-Life Induces Lung Inflammation and Long-term Lung Defects

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Type 2 immune responses have been closely associated with the final stage of lung development, known as the alveolarization stage, which occurs postnatally. In the neonatal lung, IL-33 secretion by alveolar epithelial cells promotes mass expansion of ILC2s, alternatively-activated macrophages, mast cells, basophils, and eosinophils. Overexpression of IL-33 during alveolarization alters lung development and induces inflammation, which are hallmarks of the neonatal lung disease bronchopulmonary dysplasia; however, the role of eosinophils in lung development is largely unelucidated. We generated a mouse model of eosinophil hyperactivity and discovered that lung development was significantly impaired, which induced life-long changes to the respiratory system. These mice had activated eosinophils with increased Siglec-F and CD11c expression and were greatly expanded in the developing lung, peaking at postnatal day 14, compared to control mice. This coincided with increased pulmonary levels of eotaxin, GM-CSF and IL-5, all involved in eosinophil recruitment and activation. The resulting eosinophilia was associated with an expansion of activated alveolar macrophages (AMφs), development of multinucleated giant cells, the presence of chitinase crystals both within AMφs and the airspaces, and airspace enlargement. Despite regression of eosinophils following completion of lung development, AMφ-dominated inflammation persisted into adulthood, alongside emphysema, fibrosis, chitinase crystal deposition, and lung function deficits. These results show a mechanism by which excessive type 2 polarisation, in the form of eosinophilia and eosinophil hyper-reactivity, during the alveolarization stage of postnatal lung development promotes sustained inflammation and chronic lung damage.

Role of urokinase plasminogen activator protein in *Helicobacter pylori* pathogenesis

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Infection with *Helicobacter pylori* is associated with an enhanced risk of gastric cancer. Upon *H. pylori* infection, gastric epithelial cells (AGS) develop an elongated shape, called the hummingbird phenotype. Hummingbird induction of AGS cells is triggered by *H. pylori* oncoprotein CagA. However, the molecular mechanism of how *H. pylori* CagA induces hummingbird phenotype remains unclear. *H. pylori* infection of gastric epithelial cells upregulates the level of urokinase plasminogen activator (uPA) protein compared to the uninfected cells. Moreover, uPA protein is associated with cell motility and migration in multiple cancers. Therefore, we were interested to know whether *H. pylori* exploits the uPA catalytic pathway for triggering hummingbird induction of AGS cells. We observed that the pre-treatment of AGS cells with Amiloride (a diuretic drug and uPA inhibitor) resulted in a significant attenuation of hummingbird induction level by approximately 50% in *H. pylori*-infected cells than the inhibitor-untreated cells. The level of attenuation of hummingbird induction of AGS cells by uPA inhibitors was verified using a novel uPA-specific small molecule inhibitor (BB2-30F). uPA inhibitors influence the level of hummingbird induction without inhibiting *H. pylori* adhesion to host cells and the type IV secretion system (cagT4SS) activity, for example, interleukin (IL)-8 induction of AGS cells and total CagA level. Furthermore, uPA inhibitors did not inhibit the level of CagA phosphorylation in AGS cells, which is indispensable for hummingbird induction. Interestingly, the expression of *PLAU* gene (encodes uPA protein) is upregulated in AGS cells in response to *H. pylori* infection in a partly CagA-dependent manner. Therefore, our findings infer that *H. pylori* infection of gastric epithelial cells exploits the uPA catalytic pathway to trigger the induction of the hummingbird phenotype. This study recommends trials using BB2-30F as a potential therapeutic drug against *H. pylori*-induced gastric cancer.

The role of butyrophilins in regulating αβ T cell responses

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Butyrophilins (BTNs) are members of the immunoglobulin superfamily commonly expressed on peripheral blood mononuclear cells. Their role in inducing human γδ T cell activation is well-understood. However, several murine studies suggest that BTNs can also negatively regulate αβ T cell function. A recent study reported that BTN member 3A1 (BTN3A1) attenuated $CD4^+$ and $CD8^+$ T cell responses in humans through interaction with CD45RO. To further investigate the potential role of BTN3A1 in regulating $CD4^+$ and $CD8^+$ T cell function, we engineered BTN3A1-overexpressing K562 antigen presenting cells. We also explored the potential role of BTN2A1, another BTN molecule involved in activating human $Vγ9Vδ2^+$ T cells, in the same manner. Furthermore, we investigated whether BTNs interact with CD45RO by generating tetramers expressing CD45RO, truncated CD45RO D1-D4 domains and BTN3A1 extracellular domain. We demonstrated that the presence of either BTN2A1 or BTN3A1 did not alter T cell activation, proliferation, cytokine production or K562 cell cytotoxicity. Tetramers expressing CD45RO and truncated CD45RO D1-D4 domains did not bind to HEK293T cells overexpressing BTN proteins. Likewise, the BTN3A1 extracellular domain tetramer did not bind human $CD45RO^+$ PBMCs. Hence, BTN2A1 and BTN3A1 do not appear to modulate $CD4^+$ and $CD8^+$ T cell function, nor do they appear to interact with CD45RO which contradict published findings. Therefore, future studies are required to confirm if these BTN molecules indeed exert immunoregulatory activity on αβ T cells.

Effect of childhood vaccination on antimicrobial resistance and pneumococcal populations among children in Mongolia.

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Streptococcus pneumoniae (the pneumococcus) is the leading cause of bacterial pneumonia globally. Nasopharyngeal colonisation is a prerequisite for pneumococcal disease. Pneumococcal conjugate vaccines (PCVs; covering 7-13 serotypes) have been effective at reducing disease, however, the uptake in Asia is slow and serotype replacement often occurs. Global observational studies of PCV impact have shown a reduction in antimicrobial resistance following vaccine introduction. However, patterns of resistance remain unknown in Asia, a region with high antimicrobial use. We aim to investigate both pneumococcal population changes and changes in antimicrobial resistance following PCV introduction in Mongolia.

Nasopharyngeal swab samples (n=254) were obtained from children under the age of five from Songinokhairkhan, a district of Mongolia's capital Ulaanbaatar between 2015 and 2021. After culture on selective agar, n=228 pneumococci were isolated from pre-vaccine (98/228, 42.98%) and post-vaccine (130/228, 57.02%) introduction periods. Following DNA extraction, whole genome sequencing was performed using 2 x 150 base paired end sequencing on the NovaSeq6000 platform. PopPUNK was used to assign global pneumococcal sequencing clusters (GPSCs) and PneumoCaT was used to determine serotype. Antimicrobial resistance was predicted using bioinformatic pipelines and validated using phenotypic resistance testing (n=16 antimicrobials) using CLSI methods and breakpoints.

Preliminary data from 132 isolates shows expansions of non-vaccine serotypes 15A (GPSC904;9; 0/72, 0% vs. 16/60, 26.67%) and 34 (GPSC45; 4/72, 5.56% vs. 7/60, 11.67%) in the post-vaccine era and an overall reduction in vaccine serotypes when comparing isolates collected before (49/72, 68.06%) and after (19/60, 31.67%) vaccine introduction. Preliminary data suggests reductions in antimicrobial resistance following vaccine introduction to clinically relevant antibiotics – erythromycin (62/72, 86.11% vs. 50/60, 83.33%), co-trimoxazole (63/72, 87.5% vs. 33/60, 55%), chloramphenicol (20/72, 27.78% vs. 11/60, 18.33%) and clindamycin (47/72, 65.28% vs. 37/60, 61.67%).

Results so far suggest PCV has reduced both vaccine-type carriage and antimicrobial resistance. Expansion of non-vaccine type lineages has been observed highlighting the importance of continued genomic surveillance in the region.

Immunological memory response following reduced-dose pneumococcal vaccination schedule

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Streptococcus pneumoniae is the most common cause of pneumonia in children < 5 years, the immunocompromised and the elderly. At the time of this study, two pneumococcal conjugate vaccines (PCV) were available, PCV10 and PCV13 recommended as a 3-dose schedule but this is very costly for low-to-middle-income countries (LMIC). Reduced-dose PCV schedules (1 or 2 doses) is one strategy to reduce cost, however, limited evidence on cellular immune memory is available. This study aimed to examine cellular immune memory in infants given 1 or 2 doses of PCV10 or PCV13 in Vietnam as part of a RCT. Infants received either 1 dose (at 12m of age) or 2 doses (at 2m and 12m of age) of either PCV10 or PCV13 and blood samples were collected before (baseline) and one-month post-vaccination at 12m of age. A total of 149 Peripheral Blood Mononuclear Cell (PBMCs) samples were analysed using high-dimensional flow cytometry to examine 14 B-cell and 20 T-cell populations. Interim findings from this analysis showed that for B cell populations, total B memory, IgD memory, IgM memory were significantly higher compared with baseline ($P<0.05$). For T cells, CD4+ Effector Memory, Th17, Treg memory, Tfh cells, CD8+ Central Memory and CD8+ Effector Memory were significantly higher compared with baseline ($P<0.05$). Analysis is ongoing, with unblinding of the study groups planned by August and will allow detailed comparison between the two PCVs and schedules used. Demonstration of cellular immune responses following 1 or 2 doses of PCV would provide confidence for the use of these schedules in LMICs to maintain long-term protection into the second year of life when disease burden is highest.

Impact of inflammasome-induced extracellular vesicles on different bystander cells

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Inflammasomes are multimeric complexes whose activation triggers caspase-1 cleavage resulting in processing of the cytokines IL-1β and IL-18, and the pore-forming protein gasdermin D. This, in turn, initiates programmed inflammatory cell death (pyroptosis). However, even in the absence of IL-1β and IL-18 signalling, inflammation develops, pointing towards the importance of other factors released upon inflammasome activation, such as activated inflammasome itself or extracellular vesicles (EVs). We therefore hypothesised that EVs enhance the paracrine inflammatory effects of inflammasome-activated cells.

To investigate this, we isolated EVs from inflammasome-activated cells using differential centrifugation and size-exclusion chromatography. We next analysed the RNA and protein content of EVs and investigated their uptake by and their effect on different bystander cells.

Our results show that EV secretion is increased in macrophages stimulated with inflammasome activators relative to controls. Inflammasome-elicited EVs show specific RNA signatures and are taken up by diverse recipient cells, including macrophages, endothelial cells, epithelial cells, fibroblasts, and T cells (primarily activated T cells). In macrophages, EV-associated interferon β induces an interferon signature response and if the recipient cells are subsequently exposed to inflammasome stimuli, the NLRP3 inflammasome response is dampened, suggesting a negative-feedback loop.

Taken together, these findings indicate that EVs may not only serve as diagnostic markers for inflammatory disease, but also play an important role in the systemic response towards inflammasome activation.

The more the merrier: bacteriophage cocktails provide superior suppression of *Serratia marcescens* compared to individual phages

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With the rise of antimicrobial resistance, there has been a resurgence of bacteriophage research as alternative treatment options for bacterial infections. While phages are seen to be effective against reducing bacterial populations, spontaneous resistant mutants often form and decrease phage efficacy over time. Therefore, a more suitable option to overcome this problem is the application of multiple phages as a 'cocktail'; containing multiple phage species that preferably use different modes of infection. In this study, we isolated and characterised four *Serratia marcescens* phages (Smarc1 – 4) to determine their suitability for potential phage treatment. All four phages were found to be genetically distinct from each other, and so their modes of infection through the bacterial cell wall were investigated. *S. marcescens* mutants that gained resistance to each phage were isolated and sequenced. We found that each *S. marcescens* mutant strain contained different mutations, either within genes or promoter regions that relate to cell wall formation and structure. Using both the wild-type *S. marcescens* strains and mutants, liquid infection assays of either the individual phages or as a cocktail were tested for their killing efficacy. While the individual phages worked well to suppress bacterial growth, the combination of all four phages together in most cases created a better killing efficacy. Some bacterial growth was completely suppressed by the phage cocktails over an 18-hour period, while others were suppressed for a minimum of five hours with bacterial growth stunted, unable to recover to wild-type levels. While there are some drawbacks in developing cocktails such as the requirement of multiple well characterized phages species, it should be considered as a highly desirable treatment method for bacterial suppression and killing in the future.

COVID-19 mitigation measures in early childhood education and care and schools: a systematic review

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INTRODUCTION: School closures due to the COVID-19 pandemic have caused widespread negative impacts on academic progress, social development, and physical and mental health. Early childhood education and care (ECEC) and schools are important for learning, social and emotional development, wellbeing and in some settings, nutrition and identification of vulnerable students. To ensure that ECEC and schools remain a safe working and learning environment, many countries introduced mitigation measures to minimise the risk of SARS-CoV-2 transmission with in-person learning. However, there are few studies that assessed the impact of mitigation measures on transmission in ECEC and schools. We performed a systematic review on the evidence of impact of ECEC and school mitigation measures on reducing SARS-CoV-2 transmission within these settings.

METHODS: Literature searches were conducted in PubMed, Medline, Embase and secondary hand search. Terms searched included: mask-wearing, ventilation, cohorting, physical distancing, screening tests and vaccination. Database results were restricted from 1 December 2019 to 10 December 2021 and published in English. Studies unrelated to transmission, outbreaks, infections, hospitalisations or mortality, or ECEC and schools were excluded. Studies were assessed for risk of bias and results were summarised as a narrative review.

RESULTS: The initial literature search resulted in 1542 studies, title screening 223 and abstract screening 48 studies. Secondary hand search resulted in 21 additional studies. After review of full text, 36 studies were included. All studies were conducted in high-income countries and prior to the global spread of the Omicron variant, and nine assessed multiple mitigation measures combined. Masks have low quality evidence and screening tests have some evidence of reducing transmission in schools. We found few studies assessing the impact of ventilation, cohorting, physical distancing and vaccination in isolation from other measures and there was limited evidence that these measures reduce transmission in schools. Many studies were observational, providing low quality evidence.

CONCLUSION: Despite a paucity in robust data, existing evidence indicates a probable benefit of combining multiple mitigation measures to reduce SARS-CoV-2 transmission in ECEC and schools. Higher quality research is needed to support decision-making, especially considering the global spread of Omicron and the increase in transmissibility.

Epidemiology of SARS-CoV-2 cases and COVID-19 hospitalisations and deaths in children and adolescents

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INTRODUCTION: Children and adolescents have been largely spared from the direct effects of SARS-CoV-2. However, the potential impact on children was unclear with the emergence of the highly transmissible Omicron variant. We describe the epidemiology of COVID-19 using surveillance data in children and adolescents during the Omicron period, with a focus on Australia and specific countries relevant to the Australian context because of their testing and reporting capacity and public data availability.

METHODS: COVID-19 surveillance data were sourced from government websites. Country-specific data on COVID-19 public health and social measures (PHSM), school mitigation measures, vaccination rates, case trends, hospitalisations, deaths and genomic surveillance were included for Australia, Canada, Denmark, Finland, Netherlands, Singapore, South Africa, United Kingdom and United States. Excess mortality data were sourced from EuroMOMO and Our World in Data. We included additional data about COVID-19 trends in children and adolescents from new publications.

RESULTS: Schools were open for in-person learning in all countries but mitigation measures, testing criteria and vaccination rates varied considerably. Globally, the Delta variant was quickly replaced by Omicron subvariant BA.1 in late 2021, then BA.2 in early 2022, and then BA.4/BA.5 in June 2022. The age distribution of cases changed with restrictions easing and predominance of BA.1. BA.1 cases mainly occurred in young adults initially, with cases in children and adolescents increasing temporarily as schools reopened. Seroprevalence surveys in the United Kingdom and South Africa found a rapid rise of prior infection in children to high levels following the BA.1 wave. Hospitalisations briefly increased in children, primarily in 0-4 years, but remained the lowest amongst all age groups. This included admissions for COVID-19 treatment and incidental cases. Rates of multisystem inflammatory syndrome in children (MIS-C) were lower with BA.1/BA.2 than previous variants. COVID-19-related deaths and excess child deaths did not increase, where reported.

CONCLUSION: While cases and hospitalisations in children and adolescents temporarily increased with Omicron, MIS-C and deaths did not. It is difficult to compare trends between and within countries due to differences in testing criteria. Random sampling of the population can be used as a reliable indicator of age-specific SARS-CoV-2 case rates.

Component-resolved diagnosis of peanut allergy through flowcytometric staining of basophils (CytoBas)

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Background: Peanuts are the most common trigger of anaphylaxis, a life-threatening allergic reaction, in children. The gold-standard diagnostic test is a food-provocation which could risk in a reaction. Skin prick testing or serum-IgE measurements are safer, but not always specific and often require additional visits to define the risk for anaphylaxis. Basophil activation tests (BAT) can detect functional specific-IgE, but requires multiple tubes, is labour-intensive and time-consuming. Our research group recently developed recombinant allergens for flow cytometric staining of basophils (CytoBas). CytoBas was nearly 100% sensitive and specific for ryegrass pollen allergy, and has the capacity for rapid multiplex component-resolved differential diagnosis of allergies. In this study, we evaluated CytoBas for application of component-resolved diagnosis of peanut allergy.

Methods: Recombinant peanut allergens were produced with the baculovirus expression system in Sf21 cells. Following targeted biotinylation, recombinant Ara h 1, Ara h 2 and Ara h 6 were tetramerized with fluorochrome-conjugated streptavidin. Fresh leukocytes of patients and healthy controls were stained with these tetramers and antibodies to evaluate the allergen binding and activation of basophils. Multicolour flow cytometry was performed on the BD LSRII flow cytometer.

Results: Recombinant allergen tetramers were used to simultaneously stain and activate basophils of 7 patients and 3 controls. CytoBas diagnosed all patients as sensitized to Ara h 1, 2 and 6 compared to BAT where two patients were considered non-sensitised to Ara h 2 and 6 despite known clinical histories. Specificity and sensitivity were calculated based on a cut-off value obtained from observations. Preliminary data suggested CytoBas displays greater sensitivity compared to BAT for Ara h 2 and 6 with equivalent sensitivity for Ara h 1 and a lower specificity compared to BAT for Ara h 2 and 6 with equivalent specificity for Ara h 1. It is expected CytoBas will display greater sensitivity and specificity with greater sample sizes.

Conclusion: CytoBas can potentially overcome limitations in current diagnostic approaches through implementation as a single, multiplex flowcytometric assay for sensitive and specific detection of peanut allergy. Ideally, this test will include allergens specific for anaphylaxis as well as those associated with mild symptoms or tolerance.

Dynamic metabolic footprint analysis in a hollow-fibre infection model: ceftolozane-tazobactam *versus* a challenging *Pseudomonas aeruginosa* clinical isolate

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Introduction Extracellular metabolites from samples in an *in vitro* study like a hollow-fibre infection model (HFIM) could expedite the quantification of bacterial response to antibiotic exposure, compared to traditional microbiological culturing protocols, and potentially alleviate high time and material costs associated with these extensive pre-clinical experiments. Ceftolozane-tazobactam (C/T), the β-lactam antibiotic used in this study, is not yet approved for patients with cystic fibrosis (CF) and relies on off-label dosing regimens.

Methods A C/T-susceptible and multidrug-resistant mutator *Pseudomonas aeruginosa* CF clinical isolate, CW41, was used. CW41 was challenged with C/T as a continuous infusion at standard (4.5g/day) and high (9g/day) daily doses in the HFIM for 7-9days in biological replicates (n=4). Ceftolozane concentrations simulated clinically achievable concentrations in the lung lining fluid of a typical patient with CF, and were confirmed by LC-MS/MS. Total bacterial populations and resistant subpopulations were quantified and mathematically modelled.

Culture supernatant from HFIM bacterial samples collected at 0, 7, and 24h intervals from 23 to 215h were quenched and extracellular metabolites extracted. Metabolites were detected in untargeted LCMS-based metabolomics analysis, then subjected to correlation analysis with bacterial data, and manually filtered. Selected metabolites were co-modelled with their respective correlating bacterial population.

Results Both daily doses of C/T provided some initial killing, but resulted in treatment failure with amplified resistance from 48-72h onwards. Arginine metabolism and central carbon metabolism were two pathways enriched with ≥5 metabolites of the 91 metabolites identified. Secreted L-ornithine and assimilated L-arginine were highly correlated with the total bacterial population (0.82 and -0.79 respectively, $p<0.0001$). Ribose-5-phosphate, sedoheptulose-7-phosphate and trehalose-6-phosphate correlated with the resistant subpopulation (0.64, 0.64 and 0.67, respectively, $p<0.0001$), and were likely secreted as a result of resistant growth overcoming oxidative and osmotic stress induced by C/T exposure.

Conclusion Five extracellular metabolites were well described with mathematical modelling based on bacterial killing and resistant regrowth. This proof-of-concept study suggests further exploration with other antibiotics and *P. aeruginosa* strains is warranted to determine the generalizability of these findings. Ceftolozane-tazobactam should be administered in combination with other antibiotics for CF patients, aligning with current CF treatment guidelines.

The lipidome of lipid droplets changes significantly during viral infection in the brain

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We have recently demonstrated for the first time, that lipid droplets (LDs) play vital roles in facilitating the magnitude of the early antiviral immune response, in particular the production of interferon following viral infection, and control of viral replication in an astrocyte model. The CNS is made of numerous cell types, including neurons, astrocytes and microglia and there is evidence that these CNS cell may act differentially in response to viral infection. This study sought to examine the differential roles of CNS cell types in viral infection via examination of LD dynamics and the changing lipidome of cells following viral infection.

To determine if LD induction following viral infection is a common phenomenon in all CNS cell types, brain sections harvested from LCMV (lymphocytic choriomeningitis virus) infected mice were stained using antibodies for the main CNS cell types (neurons, astrocytes and microglia) and LDs. The upregulation of LDs *in vivo* was significantly localised to astrocytes, therefore, to look at this in more detail, we investigated LD induction in various astrocytic and neuronal models, *in vitro*. Viral mimic stimulation induced significant upregulation of LDs in both human and rodent astrocytic cell lines; however, no significant change was observed in neuronal cell lines complementing our *in vivo* work. We have optimised a method to isolate LDs from astrocyte cells to determine LDs lipidome prior to and following viral infection. Although there were minimal changes in lipid species at the whole cell level, there was a significant shift in the lipidome of LDs during infection. Most notably, there was a significant upregulation in glycolipids, particularly in triglycerides, with most of the upregulated fatty acids being polyunsaturated (PUFAs). There was also a trend of long, and very long fatty acids being upregulated in the LDs following infection, including the species docosahexaenoic acid and arachidonic acid, which have been previously shown to influence the immune environment of cells.

By understanding the intricacies of cell type specific responses within the CNS we will acquire better insights into the relationship of lipids during the antiviral response, allowing for the development of novel antiviral therapeutics.

Effect of different resistance mechanisms on bacterial killing and regrowth of *Pseudomonas aeruginosa* in response to meropenem

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Traditional pharmacokinetic/pharmacodynamic (PK/PD) indices are based on minimum inhibitory concentrations (MICs) and link the response of bacterial pathogens with their exposure to antibiotics. The PK/PD index most relevant for beta-lactam antibiotics (*e.g.* meropenem) is the time during which the unbound antibiotic concentration exceeds the MIC of the pathogen ($fT_{>MIC}$). These indices must be further explored for influencing factors to better understand the PK/PD relationship and ultimately provide optimum dosing of antibiotics. We evaluated whether the effect of meropenem on isogenic bacterial strains of *Pseudomonas aeruginosa* with different resistance mechanisms is predicted solely by MIC or depends on the mechanism of resistance.

Six isogenic *P. aeruginosa* strains with upregulated efflux pumps (PAΔmexR), a loss of entry porins (PAOD1), increased beta-lactamases (PAΔAD), or a combination of two of these resistance mechanisms (PAΔDMxR, PAOD1MxR and PAOD1ΔD) were used, plus the wild type strain (PAO1). The meropenem MIC of each strain was determined. Then they were exposed to meropenem (1-64 mg/L) in static concentration time-kill studies (SCTK) over 72 hours.

Among the studied strains 1xMIC was sufficient to suppress regrowth of PAOD1ΔD (16 mg/L) over 72 hours. In contrast, 2xMIC was needed to suppress regrowth of PAOD1 (8 mg/L), PAΔmexR (8 mg/L) and PAOD1MxR (32 mg/L), while 4xMIC was required against PAO1 (4 mg/L), PAΔAD (8 mg/L), and PAΔDMxR (16 mg/L). Differences across strains were most marked in the 1-2x MIC range of the SCTK concentrations.

These studies indicated that MIC alone was not predictive of the meropenem concentration required to suppress bacterial regrowth over 72h, and the concentration-effect relationship was more complex than $fT_{>MIC}$. Even at the same MIC the resistance mechanism might influence the meropenem concentration required to suppress regrowth.

These findings suggest that traditional PK/PD indices do not tell the full story when explaining the relationship between antibiotic exposure and bacterial response. It is possible that the mechanisms of resistance present may need to be taken into account when optimizing antibiotic dosing.

Isolating transmission-blocking nobodies against the *Plasmodium falciparum* PfPSOP12 protein

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Plasmodium 6-cysteine proteins are expressed throughout the life cycle of malaria parasites and are involved in processes necessary for parasite development. Several 6-cysteine proteins localise to the surface of the sexual stages of the parasite where they mediate molecular processes involved in parasite transmission from human to *Anopheles* mosquito, making them attractive transmission-blocking vaccine candidates[1]. PSOP12 is a 6-cysteine protein expressed in the sexual stage, but its function remains unclear. Reverse genetics revealed that PSOP12 is not essential for the sexual development of *Plasmodium berghei*, a mouse malaria species. However, antibodies against PbPSOP12 exhibited transmission-blocking activity[2]. In *P. falciparum*, the most lethal human malaria parasite species, PfPSOP12 is refractory to gene deletion highlighting its potential importance in parasite development. We have generated nanobodies against PfPSOP12 to understand its function and importance in malaria parasite transmission. We will use our collection of anti-PfPSOP12 nanobodies to determine its cellular localisation and expression in *P. falciparum*. Using standard membrane feeding assays, we will examine if PfPSOP12 nanobodies inhibit parasite transmission in the mosquito. To characterise the mechanisms of action for inhibitory PfPSOP12 nanobodies, we will use structural and biophysical approaches to understand theier binding characteristics and the identification of inhibitory epitopes. Our work will expand the understanding of PfPSOP12 in malaria transmission and assess its potential as a vaccine candidate.

References

1. Lyons, F. M. T., Gabriela, M., Tham, W.-H. and Dietrich, M. H. *Plasmodium* 6-Cysteine Proteins: Functional Diversity, Transmission-Blocking Antibodies and Structural Scaffolds. *Frontiers in Cellular and Infection Microbiology* 12, 1-20 (2022).
2. Arredondo, S. A. and Kappe, S. H. I. The s48/45 six-cysteine proteins: mediators of interaction throughout the *Plasmodium* life cycle. *International Journal for Parasitology*, 47, 409–423 (2017).

Determining new permeation pathway functionality of *Plasmodium berghei in vivo* using guanidinium hydrochloride

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Background

Malaria is one of the leading infectious diseases in the world and is caused by protozoan parasites of the species *Plasmodium*. *Plasmodium* parasites infecting humans have developed resistance to all antimalarial drugs, and it is therefore critical for new therapeutics to be developed.
New permeation pathways (NPPs) have been validated as a crucial modification of the host erythrocyte during development, which facilitate parasite acquisition of nutrients and are therefore an attractive therapeutic target. Understanding the channel's molecular structure is critical for targeted drug design. Rodent models of malaria are an effective tool in investigating protein structures *in vivo* and could be used to validate the structure of NPPs.

Aims

Activity of *P. falciparum* NPPs can effectively be determined hypotonically lysing cells. However, there is currently no method available for assessing the functionality of NPPs in rodent species *P. berghei*. This study therefore aimed to develop an erythrocyte osmotic lysis assay which would enable the determination of NPP functionality in infected rodent erythrocytes. Osmotic lysis assay will then be used in genetic modification studies of *P. berghei* to further investigate the structure of NPPs.

Methods & Results

Compounds were screened on infected rodent erythrocytes for their ability to selectively cause lysis. Guanidinium hydrochloride was found to effectively lyse infected erythrocytes with uninfected cells unaffected. The use of NPP inhibitors validated entry into the infected erythrocyte via the NPPs. Lysis assays on immature vs. mature parasites will be used to further confirm the occurrence of lysis on cells exhibiting NPPs at the erythrocyte surface.

Conclusion

Guanidinium hydrochloride is an effective compound in selectively causing lysis of infected rodent erythrocytes via the NPPs. Genetic modification of genes linked to NPPs in human malaria *P. falciparum* can now be investigated in an *in vivo* rodent model using guanidinium hydrochloride osmotic lysis assay.

Investigating immune evasion by the Hendra virus-g2 variant

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Hendra virus (HeV) is a highly pathogenic paramyxovirus that, together with Nipah virus and several related viruses forms the genus *Henipavirus*. The natural reservoir of HeV is fruit bats and spillover events can result in severe disease with high mortality in horses and humans (1). The ability of HeV to evade the interferon (IFN)-mediated innate immune response is considered to be a critical pathogenesis factor, and is mediated principally by accessory proteins encoded by the P gene; P, V, W and C through a variety of mechanisms (2). Recently, a second HeV variant, denoted genotype 2 (HeV-g2), was described. While most of the viral proteins have high amino acid homology, P, V and W show the greatest divergence (3). Here, we aimed to examine potential differences in the ability of the virus to inhibit the IFN pathway.

To examine and compare the capacity of P, V and W of HeV g1 and g2 to inhibit IFN induction and signaling pathways, we are using reporter gene assays and immunoprecipitation analysis of interactions with host immune proteins. Current data indicates a conserved ability to inhibit IFN signaling but with some potential significant differences. Assays to determine the molecular basis of these changes will be ongoing. This data provides the first indications that by acquiring mutations in the P gene, the Hendra variant may have acquired changes in IFN-antagonist function. Furthermore, additional work is planned with infected cells as well as analysis to assess changes in replication.

1. Halpin K, Rota P. A Review of Hendra Virus and Nipah Virus Infections in Man and Other Animals. Zoonoses - Infections Affecting Humans and Animals. 2014:997–1012.
2. Audsley MD, Moseley GW. Paramyxovirus evasion of innate immunity: Diverse strategies for common targets. World J Virol. 2013;2(2):57-70.
3. Wang J, Anderson DE, Halpin K, Hong X, Chen H, Walker S, et al. A new Hendra virus genotype found in Australian flying foxes. Virol J. 2021;18(1):197.

Rifaximin prophylaxis causes resistance to the last-resort antibiotic daptomycin

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Bacterial pathogens such as vancomycin-resistant *Enterococcus faecium* (VREfm) that are resistant to almost all antibiotics are among the top global threats to human health. Daptomycin is a new last-resort antibiotic for VREfm infections with a novel mode-of-action, but for which resistance has surprisingly and alarmingly been widely reported. The causes of such a rapid emergence of resistance to this novel antibiotic have been unclear. Here we show that the use of rifaximin, an unrelated antibiotic used prophylactically to prevent hepatic encephalopathy in liver disease patients, is unintentionally causing resistance to this last-resort antibiotic in VREfm. We show that mutations within the bacterial RNA polymerase complex confer cross-resistance to both rifaximin and daptomycin. Furthermore, VREfm with these mutations are spread globally across at least 5 continents and 20 countries, making this a major yet previously unrecognised mechanism of resistance. Until now, rifaximin has been considered 'low-risk' for development of antibiotic resistance. Our study shows this is not the case and that widespread rifaximin use may be compromising the clinical efficacy of daptomycin, one of the major last-resort interventions for multidrug resistant pathogens. These findings demonstrate that unanticipated antibiotic cross-resistance may potentially undermine global strategies designed to preserve the clinical use of last-resort antibiotics.

Characterising the role of IKKε in STING signalling

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Cyclic GMP-AMP (cGAMP) synthase (cGAS) detects cytosolic double-stranded DNA (dsDNA), a common hallmark of infection, and produces the secondary messenger cGAMP, which binds and activates stimulator of interferon genes (STING). STING recruits TANK-binding kinase 1 (TBK1) to induce type I interferon (IFN) production. This recruitment is mediated by the TBK1-binding motif (TBM; residues 369-377). cGAS-STING activation also activates nuclear factor kappa B (NF-κB) and the mitogen-activated protein kinases to produce proinflammatory cytokines; however, how STING mediates these pathways remains poorly understood. Recent studies have established the importance of non-IFN STING responses in the pathogenesis of STING-mediated autoinflammatory disease, protection against HSV-1 and anti-tumour immunity. This illustrates the importance of studying the mechanisms that mediate STING non-IFN responses, which can provide the groundwork for new therapeutics development. TBK1 and its homologue IκB kinase epsilon (IKKε) have recently been shown to induce canonical NF-κB activation in response to cGAS-STING activation. However, the mechanism of how IKKε is involved in STING activation has not yet been clarified. Here, we conducted co-immunoprecipitation (Co-IP) assay using *Sting*$^{-/-}$ immortalized bone marrow-derived macrophages (iBMDMs) reconstituted with doxycycline-inducible tagged version of STING to demonstrate that STING recruits IKKε following activation. Furthermore, a mutation within STING TBM, previously reported to abrogate TBK1 recruitment, failed to recruit IKKε suggesting STING recruits IKKε similarly to TBK1. However, we identified a potential additional interaction between IKKε and STING using structural modelling. Mutagenesis studies confirmed that this secondary region in IKKε is critical for its ability to interact with STING to elicit immune responses.

Accelerated Bacille Calmette Guérin (BCG) reactions: more than meets the eye

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Background
An accelerated local injection site reaction following Bacille Calmette-Guérin (BCG) vaccination has been associated with underlying tuberculosis (TB), in high TB-prevalence settings. The clinical significance of an accelerated BCG reaction in individuals without TB symptoms, particularly in low TB-prevalence countries, is unclear. We aimed to determine the incidence, and investigate for clinical implications, of an accelerated BCG reaction in asymptomatic adults in low and high TB-prevalence settings. This is important in an era when BCG vaccination is increasingly being considered for novel applications.

Methods
In this cohort study nested within an international randomised trial of BCG vaccination (the BRACE trial), we used vaccine safety surveillance data and interferon-gamma release assays to test for latent TB infection. Multivariate logistic regression analysis was used to investigate determinants of accelerated BCG reaction formation.

Results
Amongst 1987 BCG-vaccinees, an accelerated BCG reaction occurred in 755 (38%) adults. Regional lymphadenopathy following BCG vaccination was twice as likely in participants with an accelerated reaction compared to those without an accelerated reaction (OR 2.18, 95% CI 1.25-3.77, p<0.01). Although accelerated reactions, compared with non-accelerated reactions, were more frequently painful, tender, erythematous and/or swollen within the first fourteen days of vaccination, they did not meet criteria for a serious adverse event following immunisation. Prior mycobacterial exposure, through prior BCG vaccination (OR 2.47, 95%CI 1.94-3.15, p<0.001) or latent TB infection (OR 4.18, 95%CI 1.17-15.0, p=0.03), and female sex (OR 1.26, 95%CI 1.03-1.56, p=0.03), were key determinants for the occurrence of an accelerated BCG reaction.

Conclusion
In both low and high-TB prevalence settings, an accelerated BCG reaction is associated with prior mycobacterial exposure. The development of an accelerated local reaction to BCG vaccination in an individual without prior history of BCG vaccination, should prompt consideration of further investigations for potential underlying TB infection.

Don’t take it for pomegranate: MR1 presents dietary antigens to modulate MAIT cell activation

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Mucosal Associated Invariant T-cells (MAIT cells) are a critical component of early antimicrobial defence, representing up to 4% of T-cells in peripheral blood but up to 40% in the liver and up to 10 % of intestinal T-cells (1). The principal method of activation for MAIT cells is through recognition of riboflavin derivative 5-OP-RU. This antigen is presented by MHC I-related (MR1), a ubiquitously expressed monomorphic antigen-presenting molecule that presents vitamin B metabolites to MAIT cells, although other activating ligands such as drug molecule diclofenac have also been described (2), suggesting that the chemical space for MR1 ligands is much more diverse than what is currently known. MAIT cells are abundant in barrier tissues such as the liver, lungs, and gut. As such, there is reason to believe that metabolites in the gut may be presented by MR1 and modulate MAIT cell activity.

Here, we use a fluorescence polarisation competitive binding assay we previously established to screen for dietary and naturally derived ligands from a commercial screening kit. One ligand, ellagic acid (EA), a polyphenol abundant in pomegranate and berries, showed strong binding to MR1. Functional analysis of EA shows that whilst EA itself does not activate MAIT cells, gut microbe metabolites of EA, urolithins, show inhibitory properties through a downregulation of activation marker CD69 on MAIT cells when presented by MR1. Urolithins also demonstrate appreciable binding to MR1 through fluorescence polarisation, indicating that they are able to be presented by MR1. Taken together, these data demonstrate that dietary molecules are able to exert a direct influence on MR1-MAIT axis and suggests that diet may directly influence MAIT cell activation status and thus direct modulation of at least one arm of the immune system is possible through diet.

References

1. Dusseaux M, Martin E, Serriari N, Péguillet I, Premel V, Louis D, et al. Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161^{hi} IL-17–secreting T cells. Blood. 2011;117(4):1250.
2. Keller AN, Eckle SBG, Xu W, Liu L, Hughes VA, Mak JYW, et al. Drugs and drug-like molecules can modulate the function of mucosal-associated invariant T cells. Nat Immunol. 2017;18:402.

Examining The Proteomic Shift in Molluscs Following Viral Challenge and Immune Priming

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Molluscs are major contributors to Australian aquaculture industries, with an estimated value of $3.11B AUD by 2025. Molluscs such as abalone lack adaptive immune systems and rely solely on innate immunity for antimicrobial defence. We have recently demonstrated that “immune priming” abalone with poly (I:C) before infection with Haliotid Herpesvirus-1 (HaHV-1) significantly reduces the otherwise approximate 90% mortality rate in these animals. However, there is little understanding of the abalone immune response and the proteins that might be involved in a successful antiviral response. This study aimed to investigate the proteomic shifts within the hemolmph and hemocytes of the economically important hybrid tiger abalone (hybrid of *Haliotis laevingata* and *Haliotis rubra*) during infection, to determine potential key antiviral proteins and immune pathways.

Mass spectrometry was performed on isolated proteins from hemolymph plasma and hemocytes from mock, HaHV-1 infected, and poly (I:C) primed abalone at 24 and 48hrs post-stimulation. Gene ontology analysis was carried out on proteins uniquely detected in poly (I:C) primed and HaHV-1 infected abalone to determine their potential antiviral roles. The HaHV-1 infected proteomic profile included 26 and 156 uniquely detected hemocyte and plasma proteins, respectively, which displayed roles mostly pertaining to cell structure maintenance and oxidative stress responses. Of the 97 plasma proteins uniquely detected in primed abalone, 32 possessed roles mostly related to autophagy and cell structure maintenance, whereas 11 of the 15 unique hemocyte proteins have exhibited pattern recognition receptor signaling and protein regulation activity. Proteins detected in the poly (I:C) proteomic profile that were absent in HaHV-1 infected animals have previously exhibited antiviral signaling functions, including within the Paracellular, Hedgehog, Notch, and Hippo immune pathways; indicating that these signaling pathways may be important in virus resistance in Australian abalone.

Here, we examine the proteomic profiles of poly (I:C) primed and HaHV-1 infected abalone for the first time. This study has shed light on the potentially important proteins that may contribute to abalone survival following poly (I:C) treatment. This work will provide knowledge on producing novel antimicrobial therapeutics in molluscs, which may reduce the economic burden of pathogens such as HaHV-1 on Australian aquaculture industries.

The role of the inflammasome adaptor ASC in gastric tumourigenesis

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Inflammasomes are key regulators of innate immunity in chronic inflammatory and autoimmune diseases, however, their role in inflammation-associated tumourigenesis remains unclear. In gastric cancer (GC), we discovered a pro-tumourigenic role for the key inflammasome adaptor apoptosis-associated speck-like protein containing a CARD (ASC) in the *gp130*$^{F/F}$ spontaneous mouse model of intestinal-type GC [1]. Specifically, we identified interleukin 18 (IL-18) as the major effector cytokine; and that the pro-tumourigenic ASC inflammasome activity resides within the tumour epithelium. However, the identity of the specific pattern recognition receptor(s) (PRRs) that activate tumour-promoting inflammasomes during GC is unknown.

We investigated the role of the best-characterised inflammasome-associated PRR, nucleotide-binding domain, and leucine-rich repeat containing receptor, pyrin domain-containing (NLRP) 3, in GC. In gastric tumours of *gp130*$^{F/F}$ mice, genetic ablation of NLRP3 did not lessen tumour burden [2]. Similarly, tumourigenesis-associated cellular processes namely, proliferation, apoptosis, and inflammation, in addition to inflammasome activation, were not altered between *gp130*$^{F/F}$ and *gp130*$^{F/F}$:*Nlrp3*$^{-/-}$ mice. Leading us to believe that another PRR was activating the ASC inflammasome in GC.

To this end, we employed a proteomics approach in which ASC was isolated in *gp130*$^{F/F}$ tumours using an immunoprecipitation method. Proteins associating to the isolated ASC complex were identified using mass spectrometry. Consequent computational analysis led to the identification of several candidate PRRs which have been validated in patient cohorts. We are also investigating the functional requirement of these PRRs for inflammasomes, and cellular processes associated with tumourigenesis in GC using CRISPR knockdown of these PRRs in human GC cell lines.

Overall, identifying PRRs that activate the ASC inflammasome in gastric tumourigenesis provides the potential for the development of PRR-directed targets for inhibitors for use as anticancer agents in GC.

[1] Deswaerte V, et al. *Cancer Res*, 78:1293-1307, 2018
[2] West AJ, et al. *Front Oncol*, 12: 830350, 2022

CREB-regulated immunosuppression in the GBM tumour microenvironment

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Glioblastoma (GBM) is the most common and aggressive type of primary brain cancer in adults. GBM is characterised by an immunosuppressive tumour microenvironment, which hinders the effectiveness of the immune system or immunotherapy modalities. The immunosuppression is largely driven by infiltrating tumour-associated macrophages and microglia (TAMs). Previous studies show that the expression of anti-inflammatory/immunosuppressive factors in macrophages and microglia is regulated by the transcription factor cyclic AMP response element binding protein (CREB). However, this has not been confirmed in TAMs in GBM.

Therefore, this research aims to investigate whether CREB activation in TAMs modulates immunosuppression in the GBM microenvironment.

To investigate CREB activation in TAMs in GBM patient samples, multiplex immunohistochemistry was performed using macrophage and microglial subtype markers, including CD68, TMEM119 and the immunosuppressive macrophage marker, CD163. My data shows that around 80% of CD163+ macrophages and microglia exhibit high pCREB expression, suggesting a positive correlation between CREB activation and immunosuppressive characteristics in TAMs.

To further investigate if CREB activation is induced by secreted factors from GBM cells, monocytic THP-1 cells and microglial HMC-3 cells were cultured in GBM-conditioned media from different GBM cell lines. CREB activation was observed in THP-1 and HMC-3 cells cultured in GBM-conditioned media. This result suggests the presence of CREB-activating factors in the conditioned media, which may drive macrophage and microglial polarisation toward a pro-tumour state.

Future experiments will investigate whether inhibiting CREB in macrophages and microglia blocks the pro-tumour polarisation by looking at pro- and anti-inflammatory cytokine profiles, antigen presentation, and phagocytic capacity.

Assessing the improvement in accuracy of *Plasmodium vivax* serological exposure markers through use of different genetic sequences

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In most malaria-endemic countries, malaria elimination is largely hindered due to the absence of tools to identify individuals with hidden *Plasmodium vivax* liver-stage parasites, known as hypnozoites. Our lab has recently developed a panel of serological exposure markers that can identify *P. vivax* hypnozoite carriers for the first time. The accuracy of our serological exposure markers could be impacted if the circulating strains of *P. vivax* have high levels of diversity in protein sequence compared to the proteins used in our serological tool. We aimed to replace the previously screened serological exposure markers with the regionally prevalent genetic haplotypes, then determining whether this could improve the accuracy of the surveillance tool. To address this aim, we used a Multiplexed-Luminex assay to measure total IgG antibody responses against the panel of serological exposure markers using the reference strain (n=6) and their selected genetic haplotypes (n=9). Responses were measured at the last timepoint of a yearlong observational Brazilian cohort (n=935), where *P. vivax* infection history was known from monthly PCR. A Receiver-Operating-Characteristic (ROC) curve was generated for each individual *P. vivax* protein assessed and enabled visualisation of the sensitivity and specificity trade-off when using different binary cut-offs to classify individuals as exposed to *P. vivax* in the prior 9-months or not. The Area-Under-Curve (AUC) reflects the overall accuracy of each individual *P. vivax* protein marker. Our preliminary data shows only 1/6 of the serological exposure markers tested were impacted by the parasite genetic diversity, as its genetic haplotype showed a higher accuracy. Our results demonstrate our serological exposure markers are mostly not impacted by parasite genetic diversity, reassuring the reliability of our serology surveillance tool across multiple geographic areas. This is an important step in the development of the markers from a laboratory-based assay to a point-of-contact test.

The ARTC2-P2X7 axis regulates the diverse function of MAIT, γδT, and NKT cells

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Unconventional T cells, such as MAIT, γδT, and NKT cells, recognise non-peptide antigens and rapidly secrete a wide range of cytokines, including IFN-γ, IL-4, and IL-17, upon activation. Despite these cytokines often mediating opposing immune responses, the mechanisms that regulate the diverse function of unconventional T cells are poorly understood.

As ARTC2-mediated P2X7 activation on mouse T-bet$^+$ NKT cells results in reduced IFN-γ production and cell death, we investigated how this axis collectively affects T-bet$^+$, IFN-γ-producing MAIT1, γδT1, and NKT1 cells. We show that MAIT1, γδT1, and NKT1 cells highly co-express ARTC2 and P2X7, and are susceptible to the effects of ARTC2-mediated P2X7 activation, which include loss of surface CD27 expression, functional impairment, and cell death. Notably, ARTC2/P2X7 blockade greatly increased the number of peripheral IFN-γ-producing MAIT1, γδT1, and NKT1 cells recovered after stimulation *ex vivo* without affecting IL-17-producing cells. Furthermore, we demonstrate that subsets of IFN-γ-producing lymphocytes can also co-produce IL-4 in a manner dependent on ARTC2/P2X7 blockade, including a novel IL-4$^+$IFN-γ$^+$ MAIT cell subset that has previously eluded detection. As ARTC2 blockade reduced cell death *ex vivo*, we next developed a novel strategy for the enrichment and adoptive transfer of rare MAIT cells at the steady state. Importantly, ARTC2 blockade underpinned the successful adoptive transfer of MAIT cells into an NKT/γδT cell-deficient mouse model, where we demonstrate higher levels of homeostatic proliferation by MAIT cells *in vivo* in the absence of competing NKT and γδT cells.

Consequently, this study highlights a common mechanism that regulates the diverse function of the distinct unconventional T cell subsets. As ARTC2/P2X7 blockade increased the viability and functional capacity of MAIT, γδT, and NKT cells *ex vivo*, this strategy will likely facilitate future studies into these cells. As IL-4/IFN-γ co-production was partially dependent on ARTC2/P2X7 blockade, ARTC2-mediated P2X7 activation may regulate the secretion of functionally opposing cytokines by unconventional T cells and other lymphocytes.

Characterisation of Streptococcus pyogenes reference strains

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Streptococcus pyogenes vaccine development requires from broadly applicable immunoassays to measure vaccine immunogenicity. The reliability of current functional. Current assays including whole bacteria is limited by diversity across S. pyogenes isolates available to labs. The Australian Strep A Vaccine Initiative (ASAVI) has undertaken to establish a collection of reference isolates to advance the development of internationally standardised immunoassays, enabling comparison and interpretation of data from different research sites.

Selection of isolates representing 4 different S. pyogenes emm-types was guided by molecular epidemiology, clinical relevance, and previous laboratory characterisation: M1, M12, M53 and M75. Bacterial growth kinetics were studied in standard broth and animal-free media, and gene expression of key virulence factors measured by RT-qPCR. Reference genomes were generated and analysed for phylogeny. Genomic stability with serial passage were also assessed. Functional characteristics were assessed using adhesion and internalisation assays, flow cytometry, and Western blot.

Each isolate was phylogenetically representative of their emm-type. Growth was fastest for all isolates in animal-free media. Relative expression of spyCEP, spyAD and slo varied between isolates, growth medium, and over time. These data were used to generate high density master cell banks according to Good Laboratory Practice principles. All processes and batch manufacturing records were reviewed and approved by an independent bio-regulatory expert to assure high quality

We have manufactured and characterised a set of 4 well-characterised S. pyogenes reference isolates banks to streamline global vaccine development efforts. These master cell banks are ready to use and will be freely available for research purposes worldwide.

Effects of Inflammation and Infection on MR1 Expression

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Antigen presentation is critical in initiating adaptive immunity to defend against infection. This involves the display of antigens by Major Histocompatibility Complex (MHC) molecules to T cells for their stimulation and immune effector functions. The non-classical monomorphic MHC class I-related protein 1 (MR1) is one of the most highly evolutionarily conserved, but the least understood such molecules. MR1 presents vitamin B metabolite antigens from various bacteria and yeasts to highly abundant mucosal-associated invariant T (MAIT) cells. This facilitates their activation and subsequent microbial killing activities.

Without ligand binding, MR1 mainly resides in the endoplasmic reticulum, resulting in low surface expression and limited interaction with MAIT cells. Some studies have shown that stimulation with toll-like receptor (TLR) ligands or bacterial infection increases MR1 surface expression and antigen presentation in some cell lines, however, none of these were demonstrated *in vivo* or in primary cells. Hence, there is no clear understanding how infection or inflammation modulates the expression of MR1. To address this knowledge gap, we are investigating how different TLR ligands and bacterial infection affect the expression of MR1 *in vivo* in mouse models. To overcome the difficulty in detecting MR1 expression in cells, a novel MR1-reporter model has been employed and allows the detection of MR1 expression within cells by the level of a fluorescent reporter protein, tdTomato. Verification of any changes in MR1-tdTomato fluorescence has been performed in wild-type mice via intracellular staining or MR1 cell-surface detection. This study reveals downregulated MR1 expression in mouse peritoneal cavity cells from both the TLR ligand lipopolysaccharide and during infection with *Staphylococcus aureus*. In contrast, stimulation with CpG failed to change MR1 expression in mice. Overall, rather than being enhanced after infection, we find MR1 expression is turned off upon inflammation. This may serve to prevent the overactivation of MAIT cells.