Victorian Infection & Immunity Network

Young Investigator Symposium Series

15 – 17 November 2021

www.viin.org.au
#viinyis, #YoungVIIN2021

Welcome to the 2021 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 2021 Young Investigator Symposium.

Special welcome to our keynote speakers: Dr Michelle Boyle of QIMR-Berghofer and Professor Colin Pouton of the Monash Institute of Pharmaceutical Sciences, Monash University.

This is the 14th year that the VIIN has convened a symposium for young investigators and the second time in virtual format – a necessary decision in the face of the COVID-19 pandemic.

We are indebted to many who have been vital to bringing the 2021 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
Laura Cook, University of Melbourne
Andrew Fleetwood, Baker Institute
Carlo Giannangelo, Monash Institute of Pharmaceutical Sciences
San Lim, Hudson Institute of Medical Research
Rhea Longley, Walter and Eliza Hall Institute
Joyanta Modak, Deakin University
Nicole Messina, Murdoch Children's Research Institute
Ursula Norman, Monash University
Jason Paxman, La Trobe University
Linda Reiling, Burnet Institute
Patrick Schriek, University of Melbourne
Rebecca Smith, VIIN
Yanie Tayipto, Walter and Eliza Hall Institute
Ryan Tseng, University of Melbourne
Jinxin Zhao, Monash University

- Communications Workshop speakers: Dr Shane Huntington, CEO of Little Big Steps and Catherine Somerville, Senior Media Advisor, University of Melbourne
- Careers Panel speakers: Dr Hayley Joseph, Australian Red Cross LifeBlood; Dr Jesse Toe, IP Group plc; Dr Anubhav Srivastava, Dimerix Bioscience; Dr Yu-Wei Lin, Certara; Dr Emma Petrie, Translational Research program at the VCCC.
- The 50+ Session chairs and judges for oral presentations and posters, which is a reflection of the increasing 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.

Prof Paul Hertzog and Prof Heidi Drummer, Co-Convenors of VIIN

Thank you to our workshop hosts

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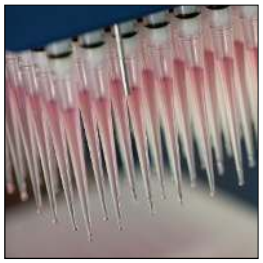
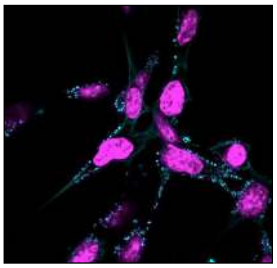
Thank you to the sponsors of prizes for Best Poster, Science Bites and People's Choice Awards (continued)

For links to sponsor websites: https://www.viin.org.au/event/viin-young-investigator-symposium-2021

Victorian Infection & Immunity Network

Connecting Researchers, Students, Health Care and Industry to solve problems in infectious diseases and immunology

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 postgraduate and postdoctoral 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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VIDRL Victorian Infectious Diseases Reference Laboratory

CSIRO

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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 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 2021 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:

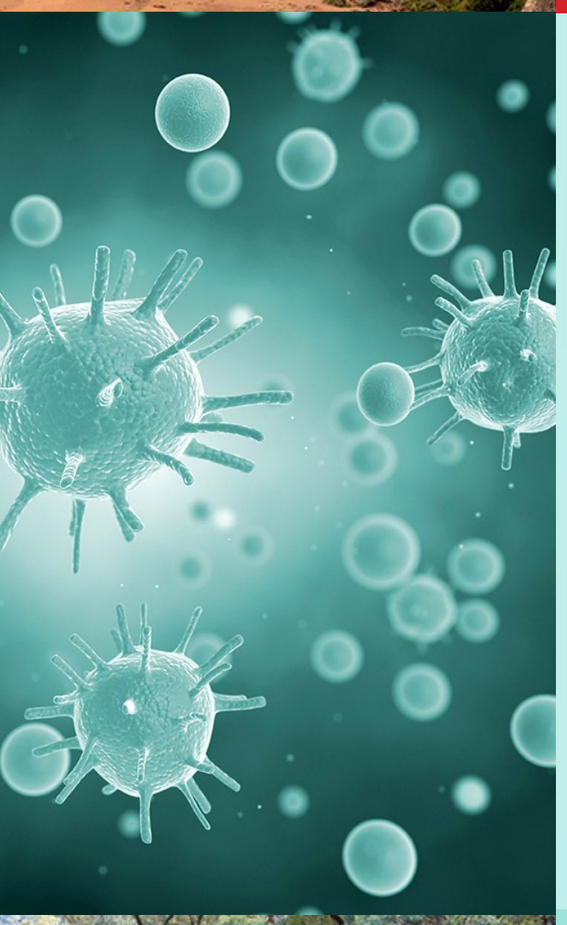
- ePoster prizes for best student and best post-doc
- Science Bites prizes for best student and best post-doc (3 minute oral presentations)
- Best VIIN Young Investigator Symposium PhD talk (10 minute oral presentation)
- People's Choice Awards – stay tuned!

12TH LORNE INFECTION & IMMUNITY 2022

HYBRID CONFERENCE | 16 - 18 FEBRUARY

www.lorneinfectionimmunity.org

EARLY-BIRD REGISTRATION: WEDNESDAY 24 NOVEMBER 2021
POSTER ABSTRACTS CLOSE: TUESDAY 14 JANUARY 2022

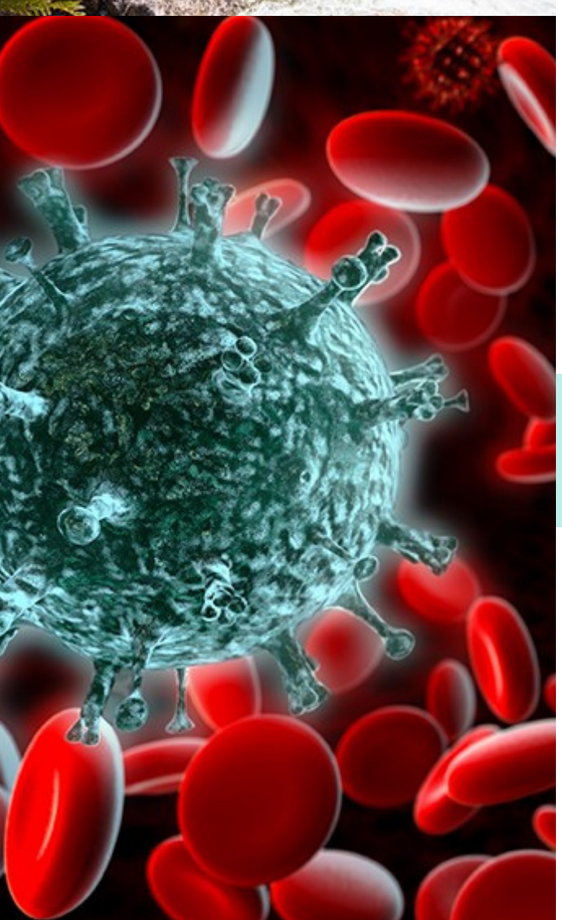


INTERNATIONAL SPEAKERS

Richard Alm, Boston University, USA
Sanjay Chotirmall, NTU, Singapore
Ashraf Ibrahim, Lundquist Institute, USA
Iliyan Iliev, Cornell University, USA
Carolyn King, University of Basel, Switzerland
Suzanne Noble, University of California San Francisco, USA
Maria-Grazia Pizza, GSK, Italy
Caetano Reis e Sousa, Francis Crick Institute, UK

NATIONAL SPEAKERS

Jeremy Barr, Monash University, VIC
Justin Beardsley, University of Sydney, NSW
Lea-Ann Kirkham, Telethon Kids Institute, WA
Kate Quinlan, University of New South Wales, NSW
Kylie Quinn, RMIT, VIC
Sudha Rao, QIMR Berghofer Medical Research Institute, QLD
Leann Tilley, University of Melbourne, VIC
Chris Tonkin, WEHI, VIC
David Tscharke, The Australian National University, ACT



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The Annual Meeting of the

Victorian Infection & Immunity Network

AID | Australian Infectious Diseases Research Centre

Program-at-a-Glance 2021

MONDAY 15 NOVEMBER
SESSION 1: 9am – 10.30am
Keynote speaker: Professor Colin Pouton, Monash Institute of Pharmaceutical Sciences
Talks selected from abstracts
Technical workshop
SESSION 2: 10.45am – 12:30pm
Talks selected from abstracts
Technical workshop
SESSION 3: 1.30pm – 3.30pm
Concurrent poster sessions I, II and III
SESSION 4: 3.45pm – 5.00pm
Talks selected from abstracts
Daily prize announcements

TUESDAY 16 NOVEMBER
SESSION 5: 9am – 11am
Careers Panel and Q&A • Dr Anubhav Srivastava, Dimerix Bioscience • Dr Jesse Toe, IP Group plc • Dr Hayley Joseph, Australian Red Cross LifeBlood • Dr Yu-Wei Lin, Certara • Dr Emma Petrie, Translational Research program at the VCCC
SESSION 6: 11.15am – 1pm
Keynote speaker: Dr Michelle Boyle, QIMR-Berghofer
Talks selected from abstracts
Technical workshop
SESSION 7: 1.10pm – 2.10pm
"Lunch with the speakers" **(Registration open until 4pm Monday)**
SESSION 8: 2.20pm – 4.40pm
Talks selected from abstracts
Technical workshop
Daily prize announcements

WEDNESDAY 17 NOVEMBER
SESSION 9: 9am – 11am
Communications Workshop and Q&A • Catherine Somerville, University of Melbourne • Dr Shane Huntington, Little Big Steps
SESSION 10: 11.15am – 2pm
Talks selected from abstracts
Technical workshops
Daily prize announcement
Grand prize announcement
SESSION 11: 6.30pm – 8.30pm
Trivia night **(Registration open until 2.30pm Wednesday)**

Monday 15 November

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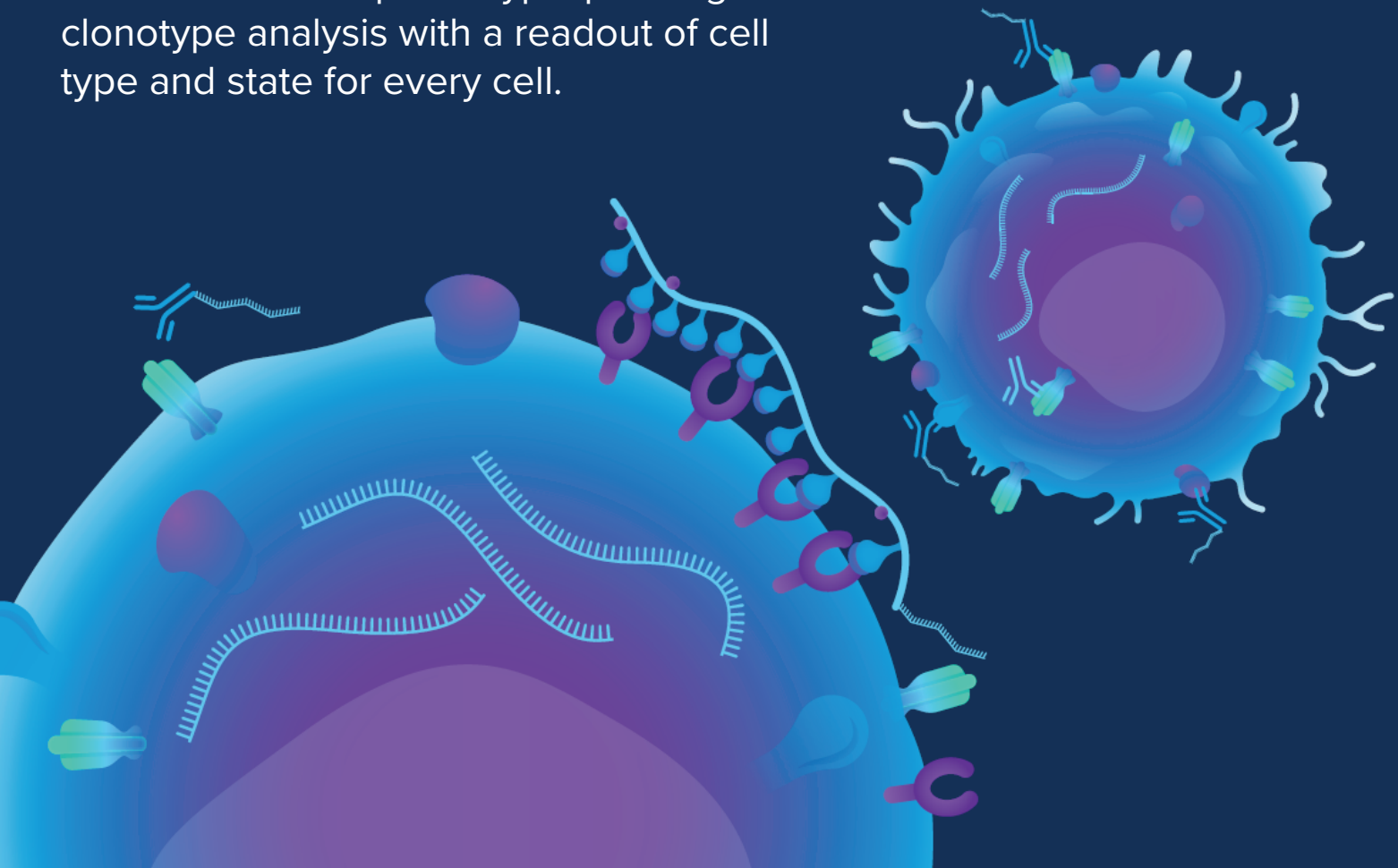

SESSION 1

Monday 15 November: 9am – 10.30am

09:00 – 09:10	**Welcome and Acknowledgement of Country** **Chairs: Linda Reiling, Burnet Institute and Carlo Giannangelo, Monash Institute of Pharmaceutical Sciences**
09:10 – 09:40	**Keynote Speaker I**
09:10	**Keynote speaker:** Professor Colin Pouton, Monash Institute of Pharmaceutical Sciences, Monash University **mRNA vaccines and therapeutics: COVID-19 and beyond**
09:40 – 10:10	**Science Bites I**
09:40	**Evidence for an epididymal-specific, phospholipid-targeting human defensin in immunomodulation** Guneet Bindra, Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science (PhD)
09:43	**Characterising T cell responses following seasonal influenza vaccination** Olivia Campisi, Department of Microbiology and Immunology, University of Melbourne (Honours)
09:46	**Application of serological markers for the assessment of the spatial transmission of *Plasmodium vivax* infections in Papua New Guinea** Natalie Cerovac, Population Health and Immunity Division, Walter and Eliza Hall Institute, Department of Medicine, Dentistry and Health Sciences, University of Melbourne (Honours)
09:49	**The role of an innate-like T cell subset during *Plasmodium* sporozoite infection** Phoebe Dewar, Department of Microbiology and Immunology, University of Melbourne (PhD)
09:52	**Variants strike back: Understanding vaccine-induced antibody responses to emerging SARS-CoV-2 RBD variants** Ebene Haycroft, Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne (Masters)
09:55	**Knockdown of PTEX impairs the haemoglobin digestion pathway in *Plasmodium falciparum*** Thorey Jonsdottir, Life Science, Burnet Institute, Department of Microbiology and Immunology, University of Melbourne (PhD)
09:58	**Using *Anopheles* salivary antibody biomarkers to assess the effectiveness of personal insect repellent in Southeast Myanmar** Ellen Kearney, Life Sciences, Burnet Institute (PhD)
10:01	**Alternate Synthesis and the Evaluation of 2 Aminobenzimidazole Antimalarials** Jomo Kigotho, Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences (PhD)
10:10 – 10:25	**Technical workshop – Millennium Science** **Immune Cell and Repertoire Profiling at Single Cell Resolution** **Geoffrey McDermott, Science & Technology Advisor**
10:25 – 10:30	**Concluding Remarks and People's Choice Prize Announcement**
10:30 – 10:45	**Morning tea break**

New Reagents and Technologies for Flow Cytometry and Imaging

Focus

- Spectral and Low Spillover dyes
- Brighter Secondary Antibodies
- Luminex
  - RNA Multiplexing
  - Hallmarks of Cancer
  - Apoptosis
  - Coronavirus

Date

November 15, 2021

Time

11:55am-12:25pm

Location

VIIN Young Investigator Symposium

Speaker

Anthony Park
Flow Cytometry, Imaging and Luminex
Reagent Specialist

Contact:

kevin.lau@thermofisher.com **OR** Anthony.park@thermofisher.com

ThermoFisher SCIENTIFIC

SESSION 2

Monday 15 November: 10:45am – 12:30pm

10:45 – 10:50	**Welcome and Acknowledgement of Country** **Chairs: Andrew Fleetwood, Baker Institute and Ryan Tseng, University of Melbourne**
10:50 – 10:55	**Sponsor message - KNF**
	Sponsor message – American Society for Microbiology
10:55 – 11:55	**ORAL PRESENTATIONS I**
10:55	**Immune signature of acute pharyngitis in a *Streptococcus pyogenes* human challenge trial** Jeremy Anderson, Infection and Immunity, Murdoch Children's Research Institute (PhD student)
11:05	**Molecular surveillance of asymptomatic *Plasmodium falciparum* in high-transmission regions in the context of interventions** Dionne Argyropoulos, Department of Microbiology and Immunology, University of Melbourne, Bio21 Institute (PhD student)
11:15	**PD-1 Inhibits T Cell Activation by Decreasing Division Destiny** Melissa Butler, Immunology Division, Walter and Eliza Hall Institute (PhD student)
11:25	**An untargeted target identification approach for novel aminobenzimidazole antimalarials identifies Exportin-1 as a potential target** Matthew Challis, Drug delivery, disposition and dynamics, Monash Institute of Pharmaceutical Sciences (PhD student)
11:35	**Pathogen-tailored transcriptional networks of T follicular helper cells** Lennard Dalit, Immunology, Walter and Eliza Hall Research Institute (PhD student)
11:45	**Withdrawn**
11:55 – 12:25	**Technical workshop – ThermoFisher Scientific** **Anthony Park, Technical Sales Specialist** **New Reagents and Technologies for Flow Cytometry and Imaging**
12:25 – 12:30	**Concluding Remarks and People's Choice Prize Announcement**
12:30 – 13:30	**Lunch break**

SESSION 3 (CONCURRENT STREAMS)

Monday 15 November: 1:30pm – 3:30pm (POSTER I)

13:30 – 13:40	**Welcome and Acknowledgement of Country** **Chairs: Laura Cook, University of Melbourne and Joyanta Modak, Deakin University**
13:40 – 15:30	**E - POSTER SESSION I (concurrent)**
13:40	**Functional evaluation of common NOD2 gene variants in patients with antibody deficiency and gastrointestinal complications** Ebony Blight, Department of Immunology and Pathology, Monash University (Honours)
13:45	**Behavioural and Psychological Outcome of Co-Designing COVID-19 Health Communication Messages with Culturally and Linguistically Diverse Communities** Jasper Liang, Optimise Group, Burnet Institute (Honours)
13:50	**The information, communication and support needs of families undergoing paediatric COVID-19 testing** Lily Richard, Vaccine Uptake Group, Murdoch Children's Research Institute (Honours)
13:55	**The Role of Antiviral Soluble Mediators in Mollusc Haemolymph** Angus Watson, Department of Physiology, Anatomy, and Microbiology, La Trobe University (Honours)
14:00	**Why some SARS-CoV-2 variants infect wild type mice and rats** Shruthi Mangalaganesh, Australian Centre for Disease Preparedness, CSIRO (Undergraduate)
14:05	**Understanding Bacteria's secret weapon: Phylogeny, function, and structure of Autotransporter proteins** Kaitlin Clarke, La Trobe Institute of Molecular Biology, La Trobe University (Masters)
14:10	**A novel structure of SARS-CoV-2 Nsp1/5′-UTR and interplay: Incites viral translational regulation and implications for potential therapeutics, vaccines** Wilma Lopes, Monash Biomedicine Discovery Institute, Monash University (Masters)
14:15	**Characterisation of Bacterial Dsb Proteins for Therapeutic Applications** Lachlan Mitchell, La Trobe Institute for Molecular Science, La Trobe University (Masters)
14:20	**Atypical evolutionary signatures driving the global evolution of emm4 Group A *Streptococcus*** Chaw Thwe, Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, University of Melbourne (Masters)
14:25	**Aged, MLKL-deficient mice develop multifocal inflammatory lesions and exhibit altered peripheral circulating white blood cell counts** Emma Tovey Crutchfield, Inflammation Department, Walter Eliza Hall Institute of Medical Research (Masters)
14:30	**Drug screening to uncover novel aspects of *Salmonella* infection**

	Jiyao Gan, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research (ECR)
14:35	**Sex differences in renal and systemic inflammation in a new mouse model of diet-induced obesity** Maria Jelinic, Centre for Cardiovascular Biology and Disease Research, La Trobe University (ECR)
14:40	**Interferon-Induced Transmembrane Protein 3 (IFITM3) SNPs and COVID-19 disease severity** Xiaoxiao Jia, Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, University of Melbourne (ECR)
14:45	**Early life infection is associated with proinflammatory, atherogenic, and diabetogenic metabolomic and lipidomic profiles at 12 months of age** Toby Mansell, Inflammatory Origins, Murdoch Children's Research Institute (ECR)
14:50	**T-bet dependent CD4+ effector differentiation is intertwined with memory formation in viral settings** Amania Sheikh, Immunology, Walter Eliza Hall Institute of Medical Research (ECR)
14:55	**Genetic mutations in host TMPRSS2 reduced COVID-19 infection in patients and potential drugs showed viral clearance in cohort studies** Naveen Vankadari, Biomedicine Discovery Institute, Monash University (ECR)
15:00	**Arbidol impairs SARS-CoV-2 spike glycoprotein trimerization and reduced mortality in adult COVID-19 patients in a cohort study** Naveen Vankadari, Biomedical Discovery Institute and Department of Biochemistry, Monash University (ECR)
15:05	**The balance of IL-12 and IL-23 determines the bias of MAIT1 versus MAIT17 responses during bacterial infection** Huimeng Wang, Peter Doherty Institute of Infection and Immunity, University of Melbourne (ECR)
15:10	**Management of Tuberculosis Infection (TBI) in Victorian children: a retrospective clinical audit of TBI management, and factors affecting treatment completion** Rebecca Holmes, Melbourne Medical School, University of Melbourne
15:15 – 15:20	**People's Choice voting**
15:20 – 15:30	**Concluding Remarks and People's Choice award announcement**
15:30 – 15:45	**Afternoon tea break**

SESSION 3 (CONCURRENT STREAMS)

Monday 15 November: 1:30pm – 3:30pm (POSTER II)

13:30 – 13:40	**Welcome and Acknowledgement of Country** **Chairs: Nicole Messina, Murdoch Children's Research Institute and Ryan Tseng, University of Melbourne**
13:40 – 15:30	**E - POSTER SESSION II (concurrent)**
13:40	**Rational design of antisense oligonucleotides modulating the activity of TLR7/8 agonists** Arwaf Alharbi, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research & Monash University (PhD)
13:45	**Investigating the role of the unfolded protein response in Legionella pneumophila infection** Manal Alshareef, Hudson Institute of Medical Research, Monash University / Saudi Arabian Cultural Mission (PhD)
13:50	**The Development of a Novel Antimalarial Class with Slow to Moderate Erythrocytic Stage Activity** Brodie Bailey, Chemical Biology & Infection and Immunity, Walter and Eliza Hall Institute (PhD)
13:55	**Whole Body Analysis of Tissue-Resident Immune Cells** Allison Clatch, Department of Microbiology and Immunology, Peter Doherty Institute, University of Melbourne (PhD)
14:00	**Understanding the mechanisms regulating GILZ, a key determinant of immune responses** Megan Cristofaro, Rheumatology Research Group, Centre for Inflammatory Diseases, School of Clinical Sciences, Monash University (PhD)
14:05	**The role of RIP kinases in bacterial gut infection** Vik Ven Eng, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research (PhD)
14:10	**Molecular mechanisms of lipid presentation by CD1b and TCR recognition** Rachel Farquhar, Department of Biochemistry and Molecular Biology, Monash University (PhD)
14:15	**Differential activation of innate immune responses by Bacteroides fragilis and their outer membrane vesicles (OMVs)** William Gilmore, Physiology, Anatomy and Microbiology, La Trobe University (PhD)
14:20	**Integrated immune networks in SARS-CoV-2 infected pregnant women reveal differential NK cell and unconventional T cell activation** Jennifer Habel, Department of Microbiology and Immunology, University of Melbourne (PhD)
14:25	**Dendritic cell apoptotic bodies as antigen presenting vesicles within an Influenza A virus infection model** Amy Hodge, Department of Biochemistry & Genetics, La Trobe Institute for Molecular Science (PhD)
14:30	**Investigating the in vitro degradation behaviour of bistriazines, a potent novel antimalarial class**

	Peiyuan Luo, Drug Delivery, Disposition and Dynamics, Global Health Therapeutic Program Area, Monash Institute of Pharmaceutical Sciences, Monash University (PhD)
14:35	**Molecular regulation of CCR4 ligands by GM-CSF and ILl4** Tanya Lupancu, Department of Medicine, University of Melbourne (PhD)
14:40	**The Design, Synthesis and Evaluation of Novel Metalloaminopeptidase Inhibitors as Antimalarial Agents** Mahta Mansouri, Medicinal chemistry/Global Health Therapeutics Area, Monash Institute of Pharmaceutical Sciences (PhD)
14:45 – 14:50	**People's Choice voting**
14:50 – 14:55	**Concluding Remarks and People's Choice award announcement**
14:55 – 15:45	**Afternoon tea break**

SESSION 3 (CONCURRENT STREAMS)

Monday 15 November: 1:30pm – 3:30pm (POSTER III)

13:30 – 13:40	**Welcome and Acknowledgement of Country** **Chairs: Yanie Tayipto, Walter and Eliza Hall Institute and Carlo Giannangelo, Monash Institute of Pharmaceutical Sciences**
13:40 – 15:30	**E - POSTER SESSION III (concurrent)**
13:40	**TREML4 receptor regulates inflammation and innate immune cell death during polymicrobial sepsis** Joseph Menassa, La Trobe Institute for Molecular Science, La Trobe University (PhD)
13:45	**Develop a Nanobody Platform to Enable High-Resolution Structural Determination of Candida auris ABC Transporter Protein CDR1 by Cryogenic Electron Microscopy (cryo-EM)** Shukla Promite, Division of Biomedical Science and Biochemistry, Research School of Biology, ANU College of Science, The Australian National University (PhD)
13:50	**The role of anti-malarial immunity in the spontaneous clearance of molecular-detectable *Plasmodium* spp. infection** Merryn Roe, School of Population Health and Preventative Medicine, Malaria and Infectious Diseases Epidemiology Group, Burnet Institute & Monash University (PhD)
13:55	**Advances in drug target characterization: A new UV-Vis spectroscopy approach for protein investigation** Carlos Fernando Santos Martin, Department of Biochemistry and Genetics, La Trobe University (PhD)
14:00	**Role of SOCS3b in zebrafish innate immunity** Mohamed Luban Sobah, School of Medicine, Deakin University (PhD)
14:05	**The hijacking of lipid synthesis during flavivirus infection** Alice Trenerry, Department of Microbiology and Immunology, University of Melbourne (PhD)
14:10	***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)
14:15	**Identification and characterisation of the pH-dependent membrane-targeting saltwater crocodile defensin CpoBD13** Scott Williams, Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science (PhD)
14:20	**The identification of addiction to a human kinase inhibitor in *Plasmodium falciparum*** Tayla Williamson, School of Health and Biomedical Sciences, RMIT University (PhD)
14:25	**The mechanisms of *P. aeruginosa* OMV biogenesis alters their cargo composition and biological functions** Lauren Zavan, Department of Physiology, Anatomy and Microbiology, La Trobe University (PhD)

14:30	**Understanding natural immunity to pre-erythrocytic *P. vivax* proteins in a longitudinal cohort** Kael Schoffer, Population Health and Immunity, Walter and Eliza Hall Institute of Medical Research, Research assistant
14:35	**To vaccinate or not to vaccinate? COVID-19 vaccine intentions amongst priority groups in Victoria** Darren Suryawijaya Ong, Vaccine Uptake Group, Murdoch Children's Research Institute, Research assistant
14:40	**Association of prenatal antibiotics and mode of birth with otolaryngology surgery in offspring: A national data linkage study** Isobel Todd, Infection and Immunity, Murdoch Children's Research Institute Research assistant
14:45	**Changes in infection-related hospitalizations in children following pandemic restrictions: an interrupted time-series analysis of total population data** Isobel Todd, Infection and Immunity, Murdoch Children's Research Institute, Research assistant
14:48	**Dynamics of gut-resident lymphocytes** Andreas Obers, Peter Doherty Institute, University of Melbourne, PhD student
14:48 – 14:55	**People's Choice voting**
14:55 – 15:00	**Concluding Remarks and People's Choice award announcement**
15:30 – 15:45	**Afternoon tea break**

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SESSION 4

Monday 15 November: 3:45pm – 5pm

15:45 – 15:55	**Welcome and Acknowledgement of Country** **Chairs: Jinxin Zhao, Monash University and Aaron Brice, Australian Centre for Disease Preparedness, CSIRO**
15:55 – 16:00	**Sponsor message – Crux Biolabs**
16:00 – 16:35	**ORAL PRESENTATIONS II**
15:55	**The good, the bad and the ugly: The functional IgA response in convalescent COVID-19 patients** Samantha Davis, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, University of Melbourne(PhD student)
16:05	**High frequency human MLKL mutation causes innate immune response defects and hematopoietic dysfunction in CRISPR-cas9 generated mouse model.** Sarah Garnish, Inflammation Division, Walter and Eliza Hall Institute (PhD student)
16:15	**Durability of B-cell memory to SARS-CoV-2 infection and vaccination** Gemma Hartley, Department of Immunology and Pathology, Monash University (PhD student)
16:25	**The value of antibody avidity in malaria vaccine responses** Jessica Horton, Disease Elimination, Burnet Institute, (PhD student)
16:35 – 16:55	**SCIENCE BITES II**
16:35	**Molecular and functional mechanisms underlying age-related changes in influenza virus-specific CD8+ T-cells across human lifespan** Carolien van de Sandt, Department of Microbiology and Immunology University of Melbourne at the Peter Doherty Institute (ECR)
16:38	**Design of an Australian Facilities Survey on Management of Polioviruses and potentially infectious materials (PIMs)** Simran Chahal, School of Medicine, Deakin University & Health and Biosecurity, CSIRO, Research assistant
16:41	**P3-Mumbubvax intervention adaptation for general practitioners: a qualitative interview study** Carol Jos, Vaccine Uptake Team, Murdoch Children's Research Institute, Research assistant
16:44	**Antibody responses to P. falciparum transmission-stage antigens in participants following a human experimental malaria infection study** Shirley Lu, Life Sciences, Burnet Institute, Research assistant
16:48	**Functional and transcriptional differences in monocytes from children with obesity compared to children of healthy weight** Siroon Bekkering, Inflammatory Origins AND Internal Medicine (resp.), Murdoch Children's Research Institute AND Radboud University Medical Center (The Netherlands) (ECR)
16:55 – 17:00	**Sponsor message – BMG Labtech**
17:00 – 17:05	**Concluding Remarks and People's Choice Prize Announcement**

Tuesday 16 November

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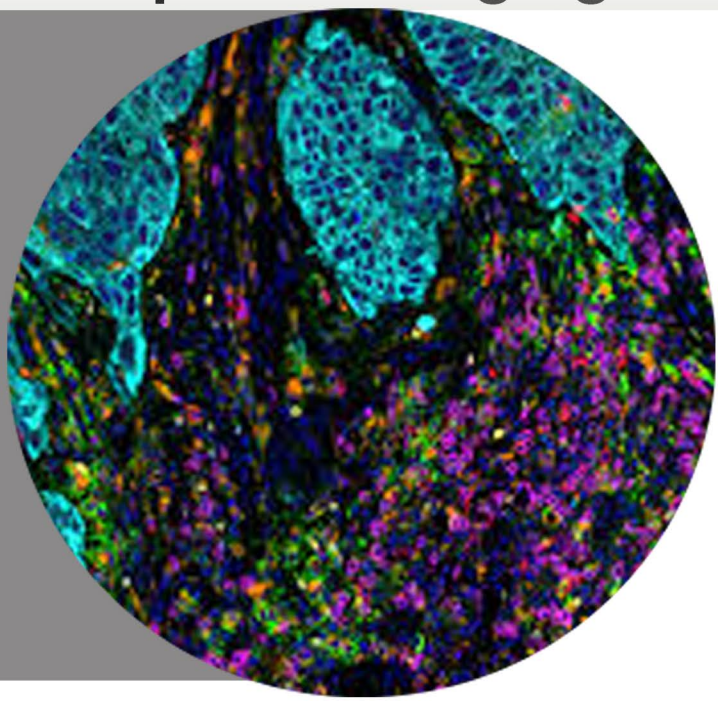
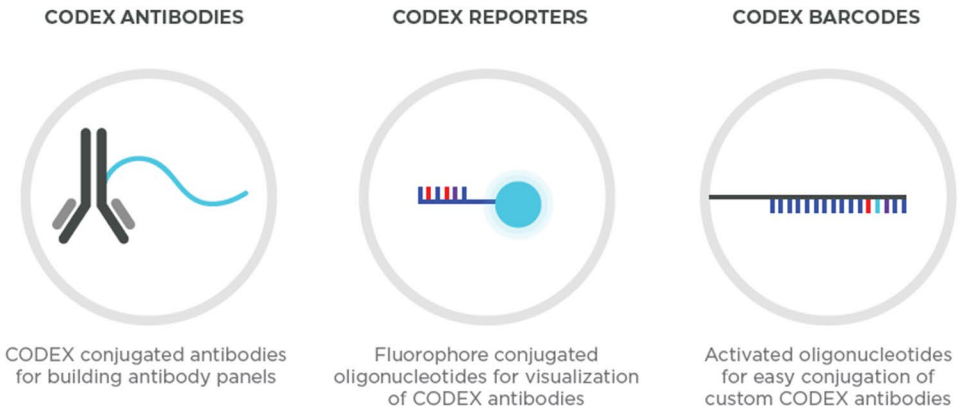
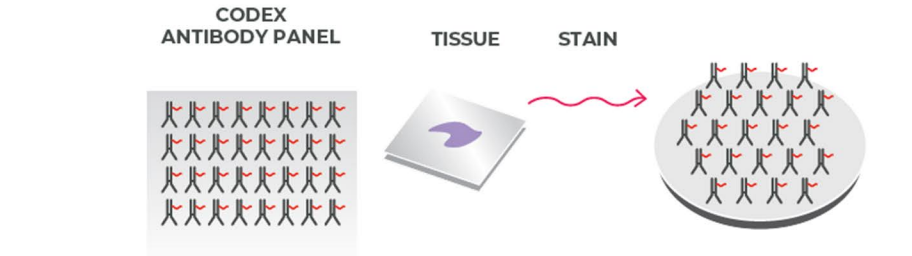
- Full spatial context, not limited to regions of interest
- Single cell resolution down to 600nm/250nm (20x/40x objective)
- Single step staining protocols preserves samples
- Simple benchtop fluidics system

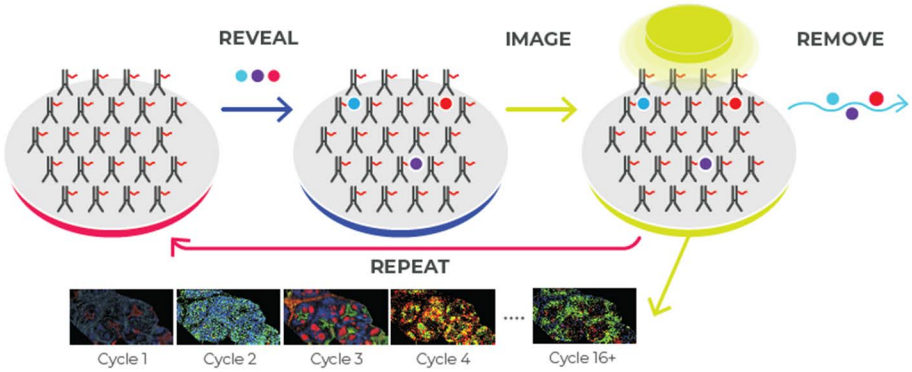




3 MAIN COMPONENTS OF CODEX TECHNOLOGY



SINGLE-STEP STAINING TO PRESERVE SAMPLE INTEGRITY



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1

COVID-19 Cytokine Panels:

- ***Early warning system*** *for cytokine storm*[1]
- *Risk stratification & targeted therapeutics*[1]
- *Multiplex profiling for complete cytokine picture*[2]

2

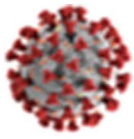
COVID-19 Antigen assay:[3]

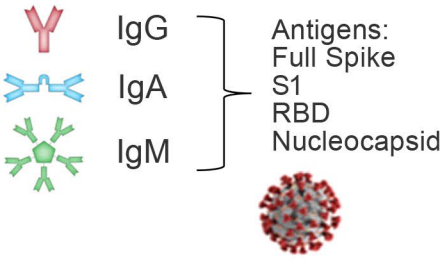
- ***Potential for PCR-sensitivity*** *for pre-symptomatic detection of virus*
- *Simple, fast immunoassay*
- *Quantitative measurement of viral load*[4]

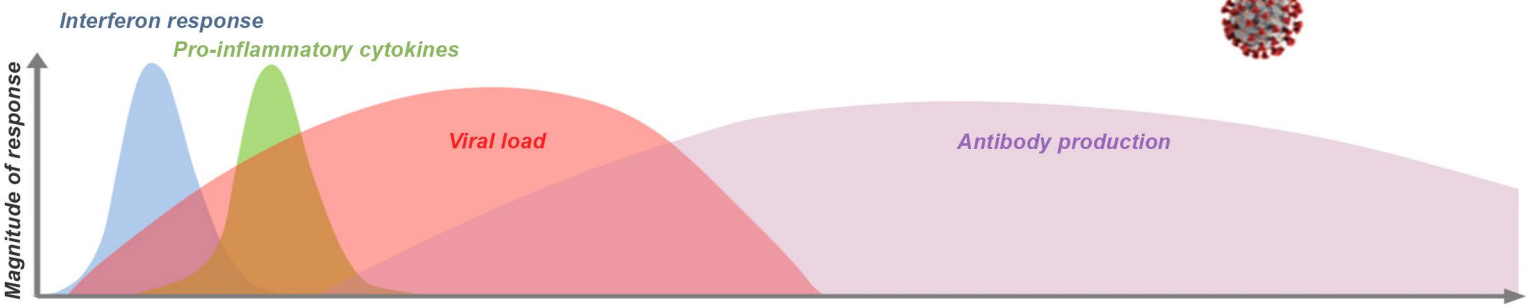
3

COVID-19 High Definition Serology:[3]

- ***Comprehensive profiling*** *of Ab response*
- *Earliest detection of immune response*
- *Quantitative titer assessments*
- *Establish thresholds for protective immunity*







1. Recent data and preliminary clinical studies
2. Complete Simoa cytokine menu
3. Products In development
4. Pre-immunity

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SESSION 5

Tuesday 16 November: 9am – 11am

09:00 – 09:05	**Welcome and Acknowledgement of Country** **Chairs: Catarina Almeida, University of Melbourne and Jason Paxman, La Trobe University**
09:05 – 09:10	**Sponsor message - Abcam**
09:10 – 11:00	**Careers Panel**
09:10	**Dr Anubhav Srivastava, Project Manager Dimerix Bioscience**
09:25	**Dr Jesse Toe, Investment Analyst, Life Sciences IP Group plc**
09:40	**Dr Hayley Joseph, Project Manager Australian Red Cross LifeBlood**
09:55	**Dr Yu-Wei Lin, Associate Director and Pharmacometrician, Certara**
10:10	**Dr Emma Petrie, Program Manager, Translational Research VCCC Alliance**
10:25	**Panel Q&A with audience**
10:55 – 11:00	**Concluding Remarks**
11:00 – 11:15	**Morning tea break**

SESSION 6

Tuesday 16 November: 11.15am – 1pm

11:15 – 11:25	**Welcome and Acknowledgement of Country** **Chairs: Linda Reiling, Burnet Institute and Joyanta Modak, Deakin University**
11:25 – 11:55	**Keynote Speaker II**
11:25	**Keynote speaker:** Dr Michelle Boyle, QIMR-Berghofer **Mechanisms of antibody development in human malaria infection**
11:55 – 12:10	**Technical Workshop – Geneworks** **Brenton Short** **Magic markers – Next Gen technologies for biomarker discovery**
12:10 – 12:50	**SCIENCE BITES III**
12:10	**The emerging role of lipid droplets acting as modulators for innate immune signalling** Jay Laws, Department of Physiology Anatomy and Microbiology, La Trobe University (PhD)
12:13	**Variations in microRNA length: a new source of disease biomarkers?** Alexandra McAllan, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research (PhD)
12:16	**Cyclopropyl amide antimalarials act by disrupting Plasmodium falciparum pyrimidine metabolism**

	Abbey McCorquodale, Monash Institute of Pharmaceutical Sciences, Monash University (Honors)
12:19	**Influenza infection of human macrophages** Tina Meischel, Department of Microbiology and Immunology, University of Melbourne (PhD)
12:22	**Single-cell analysis of αβ versus γδ T cell development** Seungyoul Oh, St Vincent's Institute of Medical Research, University of Melbourne (PhD student)
12:25	**Evading the host immune response in neuronal grafts using immune-cloaked human stem cell-derived midbrain dopamine progenitors** Chiara Pavan, Florey Institute, University of Melbourne (PhD)
12:28	**Defining the mechanisms of action of antibodies against Plasmodium vivax malaria** Kaitlin Pekin, Burnet Institute, Monash University (Honors)
12:31	**Profiling the antibody response 6 years following reduced-dose HPV vaccination in adolescent Fijian girls** Chau Quang, Infection and Immunity, Murdoch Children's Research Institute (Masters)
12:34	**Investigating the antimalarial activity of novel heterospirocyclic compounds in drug-resistant parasites** Liana Theodoridis, Department of Physiology, Anatomy and Microbiology,La Trobe University (Masters)
12:37	**Multi-Pronged CAR-T Cells To Eliminate Cutaneous T Cell Lymphoma In Vitro And In Vivo** Van To, School of Clinical Sciences, Monash University/ Hudson Institute of Medical Research (PhD)
12:40	**Inflammation dependent differentiation of two distinct VAT Treg populations shape systemic metabolism** Santiago Valle Torres, Department of Microbiology and Immunology, The Peter Doherty Institute (PhD)
12:43	**MAIT cells expand in the absence of NKT and γδT cells** Calvin Xu, Department of Microbiology and Immunology, University of Melbourne, (PhD student)
12:50 – 12:55	**Sponsor message - Perkin Elmer**
12:55 – 13:00	**Concluding Remarks and People's Choice Prize Announcement**

SESSION 7

Tuesday 16 November: 1.10pm – 2:10pm

13:10 – 14:10	**Lunch with the speakers – separate sessions**

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SESSION 8

Tuesday 16 November: 2:20pm – 4.40pm

14:20 – 14:25	**Welcome and Acknowledgement of Country** **Chairs: Laura Cook, University of Melbourne and San Lim, Hudson Institute of Medical Research**
14:25 – 14:30	**Sponsor message – Walter and Eliza Hall Institute of Medical Research**
14:30 – 15:10	**ORAL PRESENTATIONS III**
14:30	**Increased immunopathology and perturbed immune dynamics during influenza virus and arbovirus co-infection** Isabelle Foo, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, University of Melbourne (PhD student)
14:40	**Structural Basis of Coronavirus E protein interactions with human PALS1 PDZ domain** Airah Javorsky, Department of Biochemistry and Genetics, La Trobe University (PhD)
14:50	**Influenza A virus causes maternal and fetal pathology via innate and adaptive vascular inflammation** Osezua Oseghale, School of Health and Biomedical Science, RMIT University (PhD student)
15:00	**Pomalidomide as an immunomodulatory agent to enhance NK cell anti-HIV immunity** Rachel Pascoe, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, University of Melbourne (PhD student)
15:00 – 15:15	**Technical Workshop – Scientifix** **Nikki Tsoudis, Director and Co-founder of Scientifix Pty Ltd.** **Elegance in Simplicity. In-Fusion Cloning Fosters Boundless Imagination**
15:15 – 15:50	**ORAL PRESENTATIONS IV**
15:15	**Snotwatch: When data goes Viral** Rana Sawires, Faculty of Medicine, Nursing and Health Sciences, Department of Paediatrics, Monash University (PhD student)
15:25	**Mouse Mx1 inhibits HSV-1 at a Late stage in the Virus Replication Cycle** Melkamu Tessema, Department of Microbiology and Immunology, University of Melbourne (PhD student)
15:35	**Illuminating the chemical space for MR1-restricted ligands: a binding assay using fluorescence polarisation** Carl Wang, Department of Biochemistry and Molecular Biology, Monash University (PhD student)
15:45	**Lipid composition is altered in immune cells and impacts susceptibility to ferroptosis** Pooranee Morgan, Baker Heart and Diabetes Institute, La Trobe University (PhD)
15:55	**Immune responses in the respiratory tract and blood of COVID-19 patients reveal mechanisms of disease severity** Wuji Zhang, Department of Microbiology and Immunology, University of Melbourne (PhD student)
15:55 – 16:00	**Sponsor message - AGRF**
16:00 – 16:05	**Concluding Remarks and People's Choice Prize Announcement**

Wednesday 17 November

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SESSION 9

Wednesday 17 November: 9am – 11am

09:00 – 09:05	**Welcome and Acknowledgement of Country** **Chairs: Rhea Longley, Walter and Eliza Hall Institute and Nicole Messina, Murdoch Children's Research Institute**
09:05 – 09:10	**Sponsor message – BMG Labtech**
09:10 – 12:00	**Communications workshop**
09:10	**Communicating science to the media** Catherine Somerville, Senior Media Advisor, University of Melbourne
09:50	**Effective communication of research for successful careers** Dr Shane Huntington, CEO Little Big Steps
10:30	Q&A with speaker panel
10:55 – 11:00	**Concluding Remarks**
11:00 – 11:15	**Morning tea break**

SESSION 10

Wednesday 17 November: 11:15am – 2pm

11:15 – 11:20	**Welcome and Acknowledgement of Country** **Chairs: Ursula Norman, School of Clinical Sciences, Monash University and Yanie Tayipto, Walter and Eliza Hall Institute**
11:20 – 11:25	**Sponsor message – John Morris Group**
11:25 – 13:05	**ORAL PRESENTATIONS SESSION V**
11:25	**A structural basis underpinning biased T cell receptor recognition of an immuno-dominant HLA-A2 restricted epitope from the SARS- CoV-2 spike protein** Priyanka Chaurasia, Biomedicine Discovery Institute, Monash University (ECR)
11:35	**Discrete tissue microenvironments instruct diversity in resident memory T cell function and plasticity** Susan Christo, The Peter Doherty Institute for Infection and Immunity, University of Melbourne (ECR)
11:45	**Increased basal and induced phosphoinsitol-3-kinase signalling in B- and T-cells of healthy adults carrying the *PTPN22* R620W mutations** Emily Edwards, Department of Immunology and Pathology, Monash University (ECR)
11:55	**Circulating microRNA biomarkers enable accurate identification of COVID-19 patients** Ryan Farr, Australian Centre for Disease Preparedness, CSIRO (ECR)
12:05	**Single-cell landscape of tissue-resident memory T cell development**

	Raissa Fonseca, Department of Microbiology and Immunology, University of Melbourne (ECR)
12:15	**IL-23 in arthritic and inflammatory pain development** Kevin Lee, Department of Medicine (RMH), University of Melbourne (ECR)
12:25	**Chromate stress dysregulates *Pseudomonas aeruginosa* molybdenum homeostasis** Eve Maunders, Department of Microbiology and Immunology, University of Melbourne (ECR)
12:35	**Analysis of the Dynamics and Composition of Lipid Droplets During Viral Infection** Ebony Monson, Department of Physiology, Anatomy and Microbiology, La Trobe University (ECR)
12:45	**SARS-CoV-2 infection in children does not necessitate establishment of adaptive SARS-CoV-2-specific immunological memory** Louise Rowntree, Department of Microbiology and Immunology,The University of Melbourne (ECR)
12:55	**Repeated *Plasmodium falciparum* infection in humans drives the clonal expansion of an adaptive γδ T cell repertoire** Anouk von Borstel, Department of Biochemistry and Molecular Biology, Monash University (ECR)
13:05	**People's Choice voting**
13:10	**Technical workshop – Genscript Biotech** **How Wing Leung, Field Application Scientist** **Optimizing target recognition for immune-based therapy and vaccine development**
13:25 – 14:00	**Concluding remarks and grand prize announcements** **VIIN Co-Convenors:** • **Professor Heidi Drummer, Burnet Institute and** • **Professor Paul Hertzog, Hudson Institute of Medical Research**

SESSION 11

Wednesday 17 November: 6:30pm – 8.30pm

18:30 – 18:40	**Welcome, House-keeping and Acknowledgement of Country**
18:40 – 20:30	**TRIVIA NIGHT**

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 #YoungVIIN2021 and #viinyis. Please follow us @The_VIIN where we will be live tweeting symposium highlights.

SLACK: To chat to VIIN YIS organisers presenters and attendees join us on slack @ https://join.slack.com/t/viinyounginve-3mn2142/shared_invite/zt-yb827806-dJNmJCDMsxQc5a5s5rf4rQ

Please remember: no data! Thank you.

Registrations for the "Lunch with the speakers" and "Trivia night" remain open:

Lunch with the speakers: Register here (open until 4pm Monday)

Trivia night: Register here (open until 2.30pm Wednesday)

ABSTRACTS
SESSION 1

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Recombinant antibodies:
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Evidence for an epididymal-specific, phospholipid-targeting human defensin in immunomodulation

Guneet Bindra*[1], Cassandra Humble[1], Scott Williams[1], Fung Lay[1], Kha Phan[1] and Mark Hulett[1]

[1]*Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, VIC 3086, Australia*

* = presenting author

Defensins belong to the superfamily of cationic host defense peptides, comprising of small cysteine-rich innate immune peptides with antimicrobial and anticancer activity. Plant defensins, NaD1 and TPP3, and human β-defensins, HBD2 and HBD3, have been shown to exert their activity by interacting with specific anionic membrane phospholipids, known as phosphoinositides. Although of low abundance in the membrane, phosphoinositides play pivotal role in cell growth, proliferation and survival.

To further validate the importance of defensin-phospholipid interaction, a computational search was performed, which identified HBD14 as a potential phospholipid binding defensin. HBD14, a human β-defensin, represents a poorly defined member of β-defensin found in the male epididymis. In this study, HBD14 has been characterised in terms of its phosphoinositide-binding specificity, its direct cytotoxic and membranolytic activity against fungal and tumour cells, as well as its immunomodulatory activity.

HBD14 was successfully expressed and purified, with quality control confirmed by SDS-PAGE, immunoblot, mass spectrometry and circular dichroism spectroscopy analyses. Protein-lipid overlay assays revealed that HBD14 binds to an array of different phospholipids, including phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$), which was further validated by liposome binding assay. In contrast to previously characterised defensins, HBD14 does not induce antimicrobial or anticancer activity against the human fungal pathogen *Candida albicans* or human tumour cell lines. Notably, however, HBD14 induced a potent inflammatory cytokine release from peripheral blood mononuclear cells, suggesting a role in modulating the immune system. In addition, western blot analysis confirmed an upregulation of downstream effector of $PI(4,5)P_2$, phosphorylated Akt (p-Akt), eluding to the involvement of PI3K/Akt pathway in cytokine expression and release.

This study, therefore, presents the first evidence of a non-lytic defensin that solely acts as an immunomodulatory peptide, speculatively via a phosphoinositide-mediated pathway, in order to maintain immune regulation and homeostasis in the male epididymis.

Characterising T cell responses following seasonal influenza vaccination

Olivia Campisi[1*], Sheena Sullivan[2], Jose A. Villadangos[1,3], Annette Fox[2] and Laura Cook[1]

[1]Department of Microbiology and Immunology, University of Melbourne at The Peter Doherty Institute for infection and Immunity, Melbourne, VIC, [2] WHO Collaborating Centre for Reference and Research on Influenza at The Peter Doherty Institute for infection and Immunity, Melbourne, VIC, [3]Department of Biochemistry and Pharmacology, Bio21 Institute, University of Melbourne, Melbourne, VIC,

* = presenting author

Background: Vaccination against influenza mitigates the risk of developing severe disease, however, recent studies suggest that protection elicited by vaccination may be decreased in those who have been previously vaccinated (1). The mechanism behind this is undetermined but evidence suggests a contributing role for CD4+ T cells. We sought to characterise the antibodies and memory T cells generated following influenza vaccination to identify T cell correlates of protective immunity.

Method: We recruited a cohort of individuals receiving a 2020 influenza vaccination with a history of none, or multiple, prior flu vaccinations, and obtained blood pre-vaccination and at day 7 post-vaccination. To interrogate the T cell response, we optimised an activation induced marker assay using PBMCs, analysed by spectral cytometry. This assay quantifies antigen specific CD4+ T cells by co-expression of CD25 and OX40, and CD8+ T cells by co-expression of CD69 and 4-1BB after 44-48 hours antigen stimulation. Flu-specific antibody titres were determined by hemagglutinin inhibition assay.

Results: Our optimised assay enables quantification of antigen specific CD4+ and CD8+ T cell responses, even at very low frequencies. Our analysis panel allows us to measure the contribution of important subsets, including T follicular helper, T regulatory and T helper 1 cells and their IL-10 and IFNγ cytokine production, to these responses. Cohort analysis is ongoing, with our preliminary data indicating that both CD4+ and CD8+ T cell responses are biased against the same antigenic targets recognised by antibodies following vaccination, this was associated with increased haemagglutinin-driven IL-10 production.

Conclusion: We report here an optimised 24-colour spectral cytometry assay to quantify CD4+ and CD8+ T cell responses following flu vaccination that enables a deep phenotypic characterisation of these cells. This assay can be applied to study T cell responses to various vaccines and will allow us to better understand why protection is decreased with multiple vaccinations, enabling design of more efficacious vaccines.

References

1. Richards, K.A., et al., *Evidence That Blunted CD4 T-Cell Responses Underlie Deficient Protective Antibody Responses to Influenza Vaccines in Repeatedly Vaccinated Human Subjects.* J Infect Dis, 2020. **222**(2): p. 273-277.

Application of serological markers for the assessment of the spatial transmission of *Plasmodium vivax* infections in Papua New Guinea

Natalie Cerovac[1*], Shazia Ruybal-Pesántez[1,2,3], Rhea Longley[1,3], Leanne Robinson[1-4], Ivo Mueller[1,3] and Maria Ome-Kaius[1,3,4]

[1]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; [2]Burnet Institute, Melbourne, Australia; [3]Department of Medical Biology, University of Melbourne, Melbourne, Australia; [4]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea

* = presenting author

Despite upscaling control efforts, *Plasmodium vivax*, a major human malaria parasite, continues to be a barrier to malaria elimination. This is largely due to the ability of *P. vivax* to sequester within liver cells, resulting in asymptomatic infections that cannot be detected by current diagnostic tools[1]. Dormant liver parasites, known as hypnozoites, can activate and cause a relapse of disease, thus sustaining onwards community transmission. Detection of these silent infections is crucial to identifying pockets of residual transmission and progressing malaria elimination. Serological exposure markers can identify individuals with prior *P. vivax* exposure who may be harbouring hypnozoites[1]. This study aimed to classify children based on prior *P. vivax* exposure and determine risk factors for recurrent infections caused by hypnozoites. Samples from a 2013 longitudinal child cohort study in Papua New Guinea (PNG)[2] (n=395) were used to identify children exposed to *P. vivax* within the preceding 9 months. 3 of the 12 villages assessed were found to have significantly higher proportions of prior exposure, which suggests that spatial location may be a risk factor for recurrent *P. vivax* infections. These findings may contribute to a new way of identifying and managing residual transmission in PNG.

1. Longley RJ, White MT, Takashima E, Brewster J, Morita M, Harbers M, Obadia T, Robinson LJ, Matsuura F, Liu ZSJ *et al*. (2020) Development and validation of serological markers for detecting recent Plasmodium vivax infection. *Nature Medicine*. 26(5):741-749.
2. Ome-Kaius M, Kattenberg JH, Zaloumis S, Siba M, Kiniboro B, Jally S, Razook Z, Mantila D, Sui D, Ginny J *et al*. (2019) Differential impact of malaria control interventions on P. falciparum and P. vivax infections in young Papua New Guinean children. *BMC Medicine*. 17(1).

The role of an innate-like T cell subset during *Plasmodium* sporozoite infection

Phoebe M. Dewar[1*], Christopher D. Goodman[2], Anton Cozijnsen[2], Troi Pediongco[1], Adam G. Nelson[1], Sheilajen Alacantara[1], Lisa H. Verzier[3], Justin A. Boddey[3], Zhenjun Chen[1], Huimeng Wang[1], Geoffrey I. McFadden[2], William R. Heath[1], Moriya Tsuji[4], James McCluskey[1], Marcela de Lima Moreira[1], Daniel Fernandez-Ruiz, Jordana Grazziela Coelho-dos-Reis[5,6], and Sidonia BG. Eckle[1]

[1] Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Parkville, VIC, Australia [2] School of BioSciences, The University of Melbourne, Parkville, VIC, Australia [3]The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia [4]Aaron Diamond AIDS Research Center, Department of Medicine, Columbia University Irving Medical Center, New York, New York, United States [5] Instituto René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brazil [6]Department of Microbiology, Institute for Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

* = presenting author

Variants strike back: Understanding vaccine-induced antibody responses to emerging SARS-CoV-2 RBD variants

Ebene R Haycroft[1*], Ester Lopez[1], Kevin J Selva[1], Samantha K Davis[1], Adam K Wheatley[1], Samuel J Redmond[1], Jennifer A Juno[1], Nicholas A Gherardin[1], Dale I Godfrey[1,2], Wai-Hong Tham[3,4], Stephen J Kent[1,5,6], and Amy W Chung[1].

[1]Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Victoria., [2]Australian Research Council Centre of Excellence for Advanced Molecular Imaging, University of Melbourne, Melbourne, Victoria., [3]The Walter and Eliza Hall Institute of Medical Research, Parkville, Melbourne, Victoria., [4]Department of Medical Biology, University of Melbourne, Melbourne, Victoria., [5]Melbourne Sexual Health Centre, Department of Infectious Diseases, Central Clinical School, Monash University, Melbourne, Victoria. [6]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, University of Melbourne, Melbourne, Victoria.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19 disease, remains a challenge to worldwide public health. The emergence of viral variants with acquired mutations in the surface spike protein has raised concerns for the impact of such mutations on allowing escape from vaccine-induced immunity. The receptor binding domain (RBD) of the spike is a prominent target for neutralizing antibodies, and mutations at this site can generate loss of recognition by antibodies.

Given the importance of understanding the impact of various mutations on vaccine effectiveness, we evaluated antibody binding features and neutralization activity to 44 naturally occurring RBD mutations from plasma of two dose Pfizer (BNT-162b2) vaccine recipients (n = 18; two-weeks post second dose) as well as convalescent SARS-CoV-2-infected individuals with mild/moderate disease (n = 15; median: 38 days). RBD-specific antibody responses and neutralization activity were characterized via a high throughput multiplex assay.

BNT-162b2-vaccine recipients induced significantly higher levels of overall IgG binding responses compared to convalescent individuals across all 44 RBD variants assessed ($p < 0.001$). In comparison to the ancestor wild type (WT) strain (Median $1/IC_{50}$: 716), a reduced neutralization capacity of vaccine-plasma to several mutations was also exhibited, including to the RBD of beta (B.1.351), gamma (P1), eta (B.1.525), kappa (B.1.617.1) and iota (B.1.526) (Median $1/IC_{50}$: 257, 238, 554, 674, 338 respectively). To a slightly lesser extent, we observed a decrease in the neutralization capacity of vaccine-plasma against delta (B.1.617.2) (Median $1/IC_{50}$: 572) compared to wild type.

Our study shows that BNT-162b2-vaccine recipients induce high levels of IgG responses to the RBD of SARS-CoV-2 with potent neutralization capacity. However, some various naturally induced RBD mutations confer reduced neutralization sensitivity by vaccine-plasma. Understanding immune escape by SARS-CoV-2 is critical for global public health to provide insight into potential mechanisms for emerging viral variants to subvert vaccine-induced antibody immunity.

Knockdown of PTEX impairs the haemoglobin digestion pathway in *Plasmodium falciparum*

Thorey K. Jonsdottir[1,2*], Brendan Elsworth[1], Simon Cobbold[3], Mikha Gabriela[1,4], Paul R. Sanders[1], Catherine Nie[1], Malcolm McConville[3], Hayley E. Bullen[1], Brendan S. Crabb[1,2] and Paul R. Gilson[1,2]

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* = presenting author

During its blood-stage the human malaria parasite *Plasmodium falciparum* resides within the red blood cell (RBC). The parasite deploys its own translocation machinery called PTEX to export hundreds of proteins across the parasite's encasing vacuole membrane and into the RBC compartment to establish host cell modifications. Conditional knockdown of PTEX core components, HSP101 and PTEX150 and EXP2, results in rapid growth arrest. Interestingly, parasite cells with depleted PTEX150 or HSP101 have accumulation of undigested haemoglobin (Hb) inside the parasite, suggesting that proteolytic processing of this metabolite is impaired. This implies that PTEX might also be involved in the trafficking of Hb proteases. Early-acting Hb proteases are trafficked to the parasite surface where they enter Hb containing vesicles *en route* to the food vacuole where Hb digestion occurs. We looked specifically into one of these proteases, FP2a, with regards to PTEX association and trafficking. By using biochemical- and immunoprecipitation assays we found that FP2a food vacuole targeting relies on two superficially distinct steps: (i) FP2a requires unfolding before crossing the parasite plasma membrane, and (ii) extraction into the parasitophorous vacuole space appears to be HSP101-dependent. This study indicates that HSP101 might help chaperone Hb proteases within the PV space and thereby play an intermediate role in the trafficking of these proteases to the food vacuole. Overall this data provides new insights into the role of PTEX in protein translocation.

Using *Anopheles* salivary antibody biomarkers to assess the effectiveness of personal insect repellent in Southeast Myanmar

Ellen Kearney*[1,2], Paul Agius[1,2,3], Win Han Oo[1], Julia Cutts[1,4], Katherine O'Flaherty[1], Aung Thi[5], 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], Punam Amratia[6], Kefyalew Addis Alene[6,7], Peter Gething[6,7], Brendan Crabb[1], James Beeson[1], Naanki Pasricha[1], Victor Chaumeau[8,9], Julie A Simpson[2], Freya JI Fowkes[1,2,3]

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* presenting author

Innovative approaches that enhance vector surveillance capacity are urgently needed to advance the malaria elimination agenda, as current tools are inefficient and insensitive. Human antibodies to *Anopheles* salivary proteins could serve as proxy biomarkers of vector exposure and malaria transmission, providing a surrogate outcome measure in vector-control intervention effectiveness trials but evidence for the appropriateness of this approach is limited.

This study uses data from a stepped-wedge cluster randomised control trial that demonstrated repellent distribution was protective of *Plasmodium* spp. infection. As the association is likely moderated by reduced *Anopheles* biting, we sought to quantify the association between repellent distribution and antibodies to *Anopheles* salivary proteins by ELISA in 14,128 samples, measured monthly over 15-months. Furthermore, as personal repellent may be more effective for populations most at risk (*i.e.* migrants and forest-goers), we estimated the extent to which the effect of repellent was moderated by risk group.

We observed no instantaneous effect of repellent on antibody levels to *Anopheles* salivary proteins (b=0.01; 95%CI=-0.03, 0.05), however estimation of a series of lagged effects of repellent distribution (*i.e.* modelling a gradual antibody decay from prolonged use) showed reduced antibody levels after transition to repellent (*i.e.* repellent distribution 6-months prior saw a 0.03-unit (95%CI=-0.08, 0.03) decrease in antibody levels). More specifically, we observed reductions in antibody levels for migrants (6-month lag: b=-0.10; 95%CI=-0.21, -0.01) and forest dwellers (b=-0.05; 95%CI=-0.10, 0.00), but not village residents (b=0.02; 95%CI=-0.04, 0.08).

These findings suggest antibodies to *Anopheles* salivary proteins could be an informative trial outcome measure and provide important parameters on antibody decay dynamics to inform the design of future studies assessing the effectiveness of vector-control interventions.

Alternate Synthesis and the Evaluation of 2-Aminobenzimidazole Antimalarials

Jomo K. Kigotho[1], Shane M. Devine[1], Darren J. Creek[2], Raymond S. Norton[1], and Peter J. Scammells[1]

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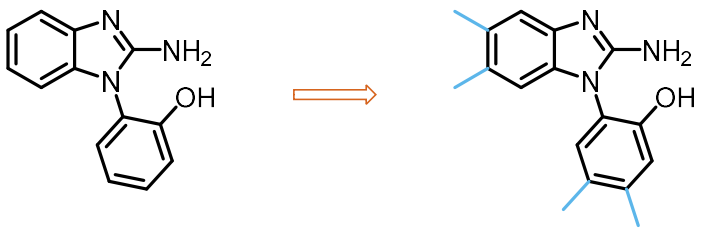
Malaria is a parasitic disease caused by species of *Plasmodium* and infects over 200 million people each year, resulting in over 400,000 deaths. While these numbers have been decreasing annually, the COVID-19 pandemic has disrupted malaria control and prevention efforts so this trend may be reversed temporarily. Resistance to frontline therapies is currently emerging, requiring the development of new antimalarials operating via novel mechanisms of action. A series of 2-aminobenzimidazoles (ABIs), containing a crucial N^1-phenol, were found to be potent inhibitors of both drug-sensitive and drug-resistant strains of *Plasmodium falciparum*, suggesting a possibly novel mechanism of action.[1] Substitution around the benzimidazole had not yet been explored due to regioselectivity issues in the synthesis, so an alternate route was developed to explore the positional impact of various substituents. All compounds were assessed for antiparasitic activity against *P. falciparum* and, while several had improved activity, a tetramethylated ABI was 10-fold more potent than the unsubstituted parent compound, with an IC_{50} of 5 nM. This ABI series contains phenol and amine moieties that are susceptible to glucuronidation, so the metabolism of several promising ABIs was evaluated and certain substituents reduced the rate of glucuronidation and some stopped this metabolism entirely. Future work will focus on combining both changes that enhance antimalarial activity, and substituents which reduce the rate of glucuronidation, to develop even more promising candidates which are both highly potent and metabolically stable.



Pf 3D7 IC_{50} = 47 ± 2 nM *Pf* 3D7 IC_{50} = 4.9 ± 0.5 nM

(1) Devine, S. M.; Challis, M. P.; Kigotho, J. K.; Siddiqui, G.; De Paoli, A.; MacRaild, C. A.; Avery, V. M.; Creek, D. J.; Norton, R. S.; Scammells, P. J., Discovery and Development of 2-Aminobenzimidazoles as Potent Antimalarials. *Eur. J. Med. Chem.* **2021**, *221,* 113518.

ABSTRACTS
SESSION 2

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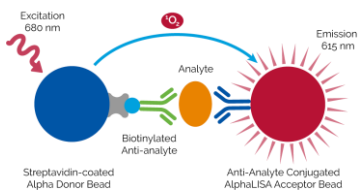

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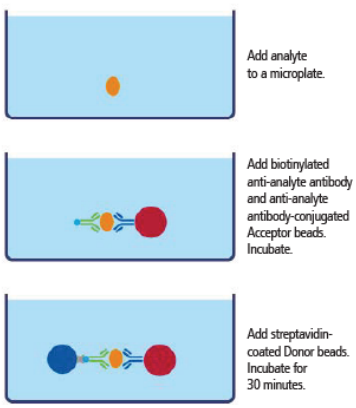

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For the Better

Immune signature of acute pharyngitis in a *Streptococcus pyogenes* human challenge trial

Jeremy Anderson[1,2]*, Samira Imran[1,2], Hannah R Frost[1], Kristy I. Azzopardi[1], Sedi Jalali[1,2], Boris Novakovic[1,2], Joshua Osowicki[1,2,3#], Andrew C Steer[1,2,3#], Paul V Licciardi[1,2#] Daniel G Pellicci[1,2,4#]

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Streptococcus pyogenes causes at least 750 million infections and more than 500,000 deaths each year. No vaccine is currently available for *S. pyogenes* and our understanding of the immunological response associated with infection is limited. Human challenge models offer unique and exciting opportunities to interrogate the immune response to infectious diseases. Here, we used high-dimensional flow cytometric analysis and multiplex cytokine and chemokine assays to study serial blood and saliva samples collected during the early immune response in human participants challenged with *S. pyogenes.* An immune signature of experimental human pharyngitis was characterised by: 1) elevation of IL-1Ra, IL-6, IFN-γ, IP-10 and IL-18; 2) increases in innate dendritic cell and monocyte populations within the blood; 3) migration of B-cells and CD4+ T-cell subsets (Th1, Th17, Treg, T_{FH}); and 4) activation of unconventional T-cell subsets, γδTCR+Vδ2+ T-cells and MAIT cells. These findings demonstrate that *S. pyogenes* infection generates a robust early immune response that is critical for the engagement of key adaptive immune cells involved in host protection. These data provide important insights that will assist the evaluation of future *S. pyogenes* vaccines and therapeutics, which are urgently needed to address the unmet public health burden of uncontrolled disease caused by this pathogen.

Molecular surveillance of asymptomatic *Plasmodium falciparum* in high-transmission regions in the context of interventions

Dionne C. Argyropoulos[1*], Mun Hua Tan[1], Kathryn E. Tiedje[1], Christiana O. Onwona[2], Anita Ghansah[2], Abraham R. Oduro[3], Kwadwo A. Koram[2], and Karen P. Day[1]

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* = presenting author

Molecular surveillance is pivotal to fully grasp the impact of malaria control interventions in high-transmission settings. However, the majority of *Plasmodium falciparum* infections in these regions are asymptomatic with low gDNA and are multiclonal. Molecular tools have been created to monitor *P. falciparum* populations yet are seldom validated on the reservoir of asymptomatic infection and in high-transmission settings. We present a population genetic study that compares the performance of a biallelic 20 single nucleotide polymorphisms (SNPs) barcode and 10 polymorphic microsatellite markers on asymptomatic *P. falciparum* isolates in a high-seasonal transmission setting in northern Ghana, West Africa. *P. falciparum* multilocus infection haplotypes were constructed from SNPs and from microsatellites for the same isolates across two age-stratified cross-sectional surveys before and after an indoor residual spraying (IRS) intervention which led to a >90% reduction in transmission intensity and 35.7% reduction in the *P. falciparum* prevalence. The multiplicity of infection (MOI) and genetic diversity parameters were compared between the two markers. Strikingly, 10 SNP loci (50%) had minor allele frequencies ≤10% in the population at both time points (i.e., pre- and post-IRS). Using *THE REAL McCOIL* method to estimate MOI from the SNP-genotyped isolates, we found that it could not reliably estimate isolate MOI when compared to other methods, including *msp2* typing. Population genetic analyses of the SNP infection haplotypes showed low expected heterozygosity, high genetic relatedness, and the presence of clones in the population. However, microsatellite analysis revealed that infection haplotypes were highly diverse with low genetic relatedness, as all multilocus haplotypes were unique. This SNP barcode originated from surveillance in Senegal and yet has proven to be unsuitable for this location in northern Ghana. These data posit that this proposed SNP barcode is not suitable to assess MOI or genetic diversity in this high-transmission setting. This leads us to question the utility of SNP barcode in high-transmission if it cannot deal with multiclonal infections and must be customised for parasite populations at local geographic scales. This study further highlights the utility of microsatellites with multiple alleles per locus as a neutral marker in high-transmission settings.

PD-1 Inhibits T Cell Activation by Decreasing Division Destiny

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* = presenting author

Co-inhibitory receptors such as programmed death receptor 1 (PD-1) are expressed on T cells after activation and are known to inhibit T cell responses. However, despite the establishment of PD-1-blockade as a potent cancer immunotherapy, the precise mechanisms by which PD-1 modulates T cell proliferative responses are not yet fully understood. Upon activation, T cells undergo a controlled division burst to form a pool of antigen-specific effector cells. Previous work using quantitative T cell assays has demonstrated that parameters including division entry, subsequent division rate, cell survival, and the number of times the cells divide before returning to quiescence (termed division destiny) determine the size and duration of the division burst (1, 2). These key variables are independently controlled by the type and strength of the signals received upon activation via the T cell receptor (TCR), co-stimulatory and cytokine inputs (1, 2). We applied this in-depth understanding to investigate the precise role of PD-1 signalling in naïve T cell proliferative responses.

We developed a quantitative dendritic cell−T cell co-culture assay for controlled delivery of co-stimulatory and co-inhibitory signals, including PD-1, to T cells in vitro. Using this system, we discovered that PD-1 signalling reduced proliferation of naïve T cells by specifically decreasing division destiny, with no effect on cell survival. Not only did we confirm a reduction in IL-2 production in response to PD-1 signalling, but we also uncovered a novel IL-2-independent pathway for PD-1-mediated inhibition. Furthermore, we show that interference with CD28 signalling is a major mechanism for the inhibitory function of PD-1, suggesting the reduction in division destiny and IL-2 production is a consequence of attenuated CD28 activity. These findings have uncovered a key mechanism for how PD-1 exerts its inhibitory function to control the proliferative potential of activated T cells.

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2. Heinzel, S. *et al*. 2017. A Myc-dependent division timer complements a cell-death timer to regulate T cell and B cell responses. *Nat. Immunol.* 18: 96.

An untargeted target identification approach for novel aminobenzimidazole antimalarials identifies Exportin-1 as a potential target

Matthew P. Challis[1*], Ghizal Siddiqui[1], Amanda De Paoli[1], Lee Yeoh[3,4], Stuart A. Ralph[5], Raymond S. Norton[2], Peter J. Scammells[2], Shane M. Devine[2] and Darren J. Creek[1]

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[2]Monash Institute of Pharmaceutical Sciences, Medicinal Chemistry, Monash University, Parkville, VIC 3052
[3]Burnet Institute of Medical Research and Public Health, Melbourne
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* = presenting author

Current antimalarial treatments are failing due to the emergence of resistance to the only frontline antimalarials available, the artemisinins. Therefore, the identification of new antimalarial compounds with novel mechanisms of action is urgently needed. The aminobenzimidazoles (ABIs) are a novel class of antimalarial that have excellent potency against the blood stage of *P. falciparum*, however, their mechanism of action is currently unknown, limiting their scope for further development.

To investigate the mechanism of action of the ABIs, an initial 'multi-omics' approach encompassing metabolomics, proteomics and peptidomics experiments was employed. Blood stage *P. falciparum* parasites were treated with 1 μM of our lead ABI, identifying over 600 metabolites, with a depletion of haemoglobin derived peptides observed as the major metabolic profile. The proteomics analysis also revealed a dysregulation of proteins associated with transcription and translation regulation following ABI treatment.

Subsequently, i*n vitro* generation of ABI-resistant *P. falciparum* was performed in a step-wise manner over a period of 4-8 months. We succeeded in producing three independent parasite lines, which demonstrated a 2-3 fold increase in IC_{50} when compared to the parent Dd2 line. Whole genome sequencing identified a shortlist of proteins of interest, with 9 genes possessing single nucleotide polymorphisms and two regions of copy number amplification identified in at least one of the three lines.

An untargeted chemoproteomic pulldown approach utilizing alkyne functionalized ABI probes was performed, taking advantage of a copper catalyzed click chemistry reaction and untargeted proteomics to enrich for, and subsequently identify, binding targets of the ABIs. From a lysate of 2292 detected proteins, we identified four proteins significantly enriched by the ABI probe when compared to multiple negative controls. One of the enriched proteins, exportin-1 (PF3D7_0302900) also had a single point mutation in one of our ABI resistant lines.

The identification of exportin-1 as a protein of interest from two independent and unbiased target identification approaches has led us to consider it a putative ABI target, and currently further work to confirm direct ABI-exportin-1 binding is underway. Exportin-1 represents an exciting, novel ABI target and will hopefully assist in the development of the ABIs into antimalarial drug candidates.

Pathogen-tailored transcriptional networks of T follicular helper cells

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T follicular helper (Tfh) cells are necessary for B cells to form high-affinity class switched antibodies, long-lived plasma cells, and memory B cells. Tfh cells promote the B cell response by providing co-stimulatory signals through cognate interactions as well as secreted cytokines. Tfh cell differentiation is instructed by the transcription factor Bcl6, which acts through repression of target genes to inhibit alternative CD4+ effector fates. However, we and others have shown that Tfh cells can also co-express the lineage defining transcription factors of T effector subsets, such as T-bet in type 1 inflammatory responses. Instructed by these transcriptional regulators, Tfh cells produce diverse combinations of cytokines and chemokine receptors and can be grouped into separate subpopulations (Tfh1/Tfh2/Tfh17). It has been suggested that this enables functional heterogeneity in Tfh to tailor pathogen-specific germinal centre responses. To test this hypothesis, we have explored diverse Tfh responses in distinct viral, bacterial, and helminth infections, to identify transcriptional regulation of Tfh subtypes, as well as their functional differences. Using ZsGreen-Tbet reporter mice, we show that Tbet is expressed in Tfh cells in a context dependent manner. We demonstrate that T-bet expression correlates to the distinct expression of Tfh-produced cytokines in viral, bacterial and helminth infections. To build a transcriptional map of Tfh cell heterogeneity, we have performed RNAseq of Tfh cells during diverse infectious challenge, and single cell RNAseq analysis of human tonsil Tfh. Initial RNAseq analysis shows that Tfh cells from different infections form separate and distinct clusters on dimensionality reduction plots. Gene expression analysis highlights a core Tfh signature but also identifies an infection-dependent profile of known and previously unknown factors. Our results define a blueprint of Tfh diversity and may identify ways to direct this process for immunotherapies for antibody-mediated diseases, such as Lupus and asthma, where skewed Tfh diversity impacts disease.

ABSTRACTS
SESSION 3
POSTER I

Functional Evaluation of Common *NOD2* Gene Variants in Patients With Antibody Deficiency And Gastrointestinal Complications

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Background: Predominantly antibody deficiency (PAD) is the most common inherited immunodeficiency and presumed to be caused by rare genetic mutations. Despite genomic advances, >70% remain genetically undiagnosed. Approximately 20% of PAD patients suffer from gastrointestinal disease. Common genetic variants (minor allele frequency >1%) in the NOD2 gene (R702W, G908R, and L1007fsX1008) are the three major risk alleles for gastrointestinal disease. NOD2 is a pattern recognition receptor that recognises peptidoglycan fragment muramyl dipeptide (MDP) and is critical for defence against bacteria. In this study, we examined whether NOD2 variants are associated with PAD and whether these impact on NOD2 function.

Methods: Carriership for three NOD2 variants (R702W, G908R, and L1007fsX1008) was determined in 75 PAD patients from whole exome sequencing data, and using Sanger sequencing in 75 healthy adult controls. NOD2 function was determined through in vitro stimulation of peripheral blood mononuclear cells with L18-MDP and detection of intracellular TNFα by flow cytometry. Stimulation with LPS and media-only were used as positive and negative controls, respectively.

Results: The R702W variants was detected in 8 controls and 8 patients, G908R in 2 controls and 1 patient, and L1007fsX1008 in 2 patients. No homozygotes were detected. Monocytes from healthy adults and patients with and without the R702W showed similar median TNFα production (P=0.39) after L18-MDP stimulation.

Conclusion: Our PAD cohort did not display increased presence of NOD2 variants. The R702W does not impact on NOD2 function to induce TNFα in healthy controls or PAD patients. In ongoing studies, phosphorylation of p38 in L18-MDP stimulated monocytes will be measured in individuals with and without R702W. This will give new insights into the functional consequences of NOD2 variants on the NOD2 pathway, and their association with gastrointestinal disease in PAD. If an effect is confirmed, this would provide a rationale for the use of therapies in these patients.

Behavioural and Psychological Outcome of Co-Designing COVID-19 Health Communication Messages with Culturally and Linguistically Diverse Communities

Jasper Liang[1*]

[1]*Burnet Institute*

* = presenting author

In the COVID-19 pandemic, community cooperation is crucial for public health measures to be effective and for vaccination goals to be reached. In particular, Cultural and Linguistically Diverse (CALD) communities deserve more attention given that they are disproportionately affected and tend to have inadequate health literacy. Such inadequacy may be attributed to a wide range of barriers. These barriers could be categorized by the Capacity, Opportunity and Motivation Model of Behavior (COM-B), and they demonstrate the need for behavioral intervention. However, research evidences have shown that pre-designed / unidirectional interventions in health communication may have inconsistent and fragile efficacy, potentially due to participants' mistrust and self-perceptions not being considered. Co-designing the behavioral intervention with community members could solve these issues. Though the contents vary between different projects due to its nature, principles and guidelines of co-design have been developed and the methodology have been shown to have both directly (communication barriers are reduced) and indirectly (positive emotions) beneficial outcomes. Given the paucity of co-design work in the context of COVID-19 health communication, the current project aims at doing so with CALD communities in Victoria, followed by evaluating community members' direct (behavioral change) and indirect (self-efficacy and general well-being) outcomes. In alignment with the co-design methodology, evaluation measures would also be co-created with community members. Although the project is still at its early stages, the team hopes CALD communities in Victoria would be motivated to adhere to public health measures, get vaccinated and to have increased sense of empowerment.

The information, communication and support needs of families undergoing paediatric COVID-19 testing

Lily Richard[*1], Jess Kaufman[1,2] and Margie Danchin[1,2]

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* = presenting author

Introduction

COVID-19 testing for children can be a stressful and confusing process for families. COVID-19 testing is a crucial public health measure against the current pandemic that impacts parents' needs for information, communication and support. While recent data suggests that many Australian parents are unsure about when a child might need a COVID-19 test, there is limited research exploring parents' broader information needs or how they navigate the COVID-19 testing journey. This study aims to explore the information, communication and support needs of parents and children prior to, during and after COVID-19 testing and provide recommendations for improvement.

Methods

As part of a larger mixed methods study, we administered an online survey to parents of children tested for COVID-19 at the Royal Children's Hospital Respiratory Infection Clinic between July and December 2020. The survey explored when families sought or received information and their experience of care across five timepoints of the COVID-19 testing patient journey. Participant demographics were used to describe differences in information-seeking behaviour and experience of care across variables such as the child's age, the family's cultural and linguistic diversity and the child's COVID-19 test result.

Results

Of the 250 participants of the larger study, 51% completed this survey (128/250). Before their child's test, parents did not look information (98/128; 77%) and were not worried about their child's COVID-19 test (123/128; 96%). Parents were most likely to look for information before their child's COVID-19 test (30/128; 23%) which related to their child's COVID-19 symptoms and where to get their child tested. Most families were satisfied with the information received during their test at the Royal Children's Hospital (119/128; 93%). Families were "frustrated" by "conflicting" post-test isolation from the Royal Children's Hospital and the Department of Health. This particularly affected families of COVID-19 positive children.

Conclusion

The information and supports made available by the Royal Children's Hospital and the Department of Health in relation to paediatric COVID-19 testing should be tailored to when parents most often interact with health information.

The Role of Antiviral Soluble Mediators in Mollusc Haemolymph

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Molluscs are major contributors to aquaculture industries in Australia, estimated to reach $3.11B AUD by 2025. Molluscs such as abalone lack adaptive immune systems, and solely rely on innate immunity and soluble mediators for antimicrobial defence. We have recently demonstrated that “priming” abalone via poly(I:C) injections before Haliotid Herpesvirus-1 (HaHV-1) infection significantly improves the survival rate of these animals. However, we still don’t understand the mechanisms by which this works. There is a lack of understanding of how soluble mediators contribute to antiviral responses. Therefore, this study aimed to examine the proteomic shifts between mock and infected Jade Tiger abalone (hybrid of *Haliotis laevingata* and *Haliotis rubra*) in order to determine potential key players in this response.

Proteins were extracted from haemolymph of abalone that were either infected with HaHV-1 or primed with poly(I:C). Proteomic analysis revealed 50 post HaHV-1 infection, and 73 proteins following poly(I:C) stimulation. Only 11 proteins were upregulated across both groups, 8 of which were not detected in mock infected abalone; suggesting to us that these upregulated proteins are likely involved in antiviral defence or possess immune-related roles. These upregulated proteins included a RAB-15 homolog and c-type lectin (CTL), both with described immune roles in other species, supporting our hypothesis that soluble mediators contribute to this innate antiviral response. There are currently no methods to fluorescently image or quantify HaHV-1 infection in abalone, and therefore we next aimed to optimise methods to analyse this. Abalone nerves extracted from HaHV-1 infected abalone were sectioned and stained with anti-3G1.1 and 2G4 dsRNA antibodies. We found that these antibodies were able to bind to HaHV-1 virus in the nerves, allowing us to fluorescently visualise viral localisation to the nerves for the first time, but also to quantify this infection, and use this as a screening tool for future infection trials of abalone.

Here, we identify proteins that may play vital roles in the innate immune response of these animals. HaHV-1 is a devastating infection in Australian abalone, and therefore this work will add knowledge around how we can create novel therapeutics.

Why some SARS-CoV-2 variants infect wild type mice and rats

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COVID-19 pandemic has resulted in significant global mortality and morbidity; the virus can expand its host range, evolve and reinfect humans, thus complicating response and recovery. SARS-CoV-2 virus isolates in circulation until mid-2020 did not infect wildtype mice (*Mus musculus*) as the virus spike protein was unable to interact effectively with the mouse-angiotensin-converting-enzyme-2 (mACE2) receptor to promote viral entry, triggering global efforts to develop transgenic mice models (Callaway., 2020). Subsequent variants containing N501Y mutation in the spike protein, including Alpha, Beta and Gamma variants of concern, started infecting wildtype mice, leading to questions about which mutations are essential for mouse adaptation, whether the globally significant Delta variant could infect wildtype mice, and the extent of risk posed by the mouse plague in Australia and rat plagues in different parts of the world. We developed an advanced biomolecular dynamics model and predicted that that aromatic substitutions at either spike position 501 or 498, but not both, must occur for mouse adaptation. Our *in silico* results also identified that mouse adaptation could be enhanced by mutations in positions 417, 484, 486, 493 and 499 (especially K417N/T, E484K, Q493K/R), but that these enhancing mutations cannot sustain mouse infectivity by themselves. Our theoretical predications were validated with results from all twenty *in vitro* or *in vivo* studies reported to date on SARS-CoV-2 infecting wild-type mice (Kuiper et al., 2021). These mutations also appear to result in more favourable binding interactions with ACE2 residues of rats (*Rattus rattus, Rattus norvegicus*). Reassuringly, our study shows that the Delta variant lacks the essential aromatic mutation for infecting mice. By analysing 2.4 million SARS-CoV-2 sequences on 'GISAID', we have identified 41 countries where the variants capable of infecting mice are in circulation, and therefore recommend enhanced and targeted field surveillance in areas where humans come into contact with mice and rats, for instance sewers that contain excreted virus. Currently, we are extending this approach to 53 rodents whose ACE2 sequences are available.

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Understanding Bacteria's secret weapon: Phylogeny, function, and structure of Autotransporter proteins

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In Gram-negative bacteria, the Autotransporters (Ats) are largest group of outer membrane and secreted proteins, that are responsible for a wide array of pathogenic phenotypes from many medically relevant bacterial pathogens. Each AT protein contains both the primary secretion machinery (translocator domain) for transport to the bacterial surface and the functional cargo (passenger domain) that directly contributes to disease [1]. The passenger domains display astounding functional diversity including host adhesion, bacterial aggregation/biofilm formation, invasion, intracellular motility, and immune evasion, along with enzymatic activities such as serine proteases, lipases, and sialidases that act as cytotoxins and in nutrient acquisition [2, 3]. Collectively, ATs contribute to a wide range of bacterial diseases, including whooping cough, urinary tract infections, nosocomial infections, diabetic ulcers, sepsis, and meningitidis.

Despite their abundance and important role in bacterial diseases, ATs are poorly understood and there is no adequate classification system to describe the functional classes of the protein family. To address this, I have utilised insights from our own research and other published literature, to develop a phylogenetics-based classification system that, for the first time, classifies ATs into groups according to their molecular structure and function.

This new classification system has provided new insights and information to further characterise and understand the relationships between ATs. Using this system, I have directed my attention towards characterising ATs from a relatively unknown group, that includes the ATs TcfA and Vag8 from the pathogen Bordetella pertussis (whooping cough). With protein crystals for both targets, I am currently using a combination of structural biology (Australian Synchrotron) along with other experimental techniques to understand the molecular mechanisms of these ATs and their role in promoting whooping cough. Again, we have used our new AT classification system to identify AT functions with use in medical applications. Currently, I am re-purposing the AT toxins to create the first AT platform for the intracellular delivery of therapeutics to human tissue. Such a medical innovation would be highly beneficial to medicine, as 30% of all human therapeutics are peptide/protein based, which cannot cross human cell membranes.

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Structure of SARS-CoV-2 Nsp1 and 5′-UTR RNA complex: Incites viral translational regulation and implications for potential therapeutics, vaccines

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The SARS-CoV-2 virus is the cause of the ongoing Coronavirus disease 19 (COVID-19) pandemic, which causes pneumonia and lower respiratory tract infections (ARDS). To understand the pathogenicity and mode of action of SARS-CoV-2, it is important to portray the whole repertoire of expressed viral proteins. Recent studies showed that the SARS-CoV-2 leader protein Nsp1 has a role in shutting down host protein production. However, how Nsp1 modulates host translation is still unknown. Here, we present a structure of Nsp1 from SARS-CoV-2 in complex with the SL1 (RNA) region of the SARS-CoV-2 5′UTR supported with experimental studies. Our findings demonstrate how SARS-CoV-2 Nsp1 regulates self and host translation via a bipartite mechanism, binding to self RNA and hijacking host ribosomes. We also employed molecular dynamics and simulations to model the real-time stability and functional dynamics of the Nsp1/SL1 complex. The studies also identify potential inhibitors and their modes of action for inhibiting viral protein/RNA complex formation. This advanced our understanding of the mechanism of the first viral protein synthesised in a human cell to regulate self and host translation. Understanding SARS-CoV-2 Nsp1 structure and function, as well as its interactions with viral RNA and the ribosome, will pave the way for the development of live attenuated vaccines and possible therapeutic targets for this disease.

Keywords: Coronavirus, 40S Ribosome, COVID-19, Vaccine strategy, Nsp1, viral infection

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Characterisation of Bacterial Dsb Proteins for Therapeutic Applications

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The disulfide bond (Dsb) forming machinery in bacteria are central mediators of bacterial pathogenesis as they catalyse the folding of a wide array of virulence proteins in a variety of different pathogens[1]. As a result, these proteins have been extensively researched as potential therapeutic targets for the development of antimicrobial agents to combat a range of infections.
This project aims to investigate unexplored Dsb systems in pathogenic bacteria. Specifically, we have characterised the Dsb system from *Bordetella pertussis*, the causative agent for whooping cough and a global concern due to a high levels of antimicrobial resistance[2].
Using a combination of structural biology and biochemical analysis, we have comprehensively characterised *B. pertussis* DsbA a key virulence protein essential for pertussis toxin formation and secretion during pathogenesis. Outcomes of this research will provide insight into *Bordetella pertussis* pathogenesis and inform the future development of DsbA inhibitors.

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Atypical evolutionary signatures driving the global evolution of *emm4* Group A *Streptococcus*

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Group A *Streptococcus* (GAS) is a major human pathogen, causing over 500,000 deaths annually. GAS are epidemiologically classified into over 250 *emm* types based on the nucleotide variation in 150 base pairs of the *emm* gene. Among them, *emm4* is one of the top ten clinically relevant *emm* types in high-income countries, causing invasive and non-invasive diseases. Recent genome surveillance studies have identified the clonal expansion of an *emm4* sub-lineage in North America, which exhibits a range of atypical evolutionary features such as gene loss within prophage regions. Our study aims to investigate the spread of this new sub-lineage globally, with a particular focus on clinical isolates from Australia. We compiled a genome database of 536 global *emm4* isolates revealing that different *emm4* genotypes with unique evolutionary features are dispersed globally. Interrogation of the accessory genome revealed multiple stepwise prophage degradation pathways that may have occurred independently in different *emm4* sub-lineages, indicating a common yet evolutionary independent phenomenon that may have attributed to prophage degradation. Bayesian phylogenomic analyses revealed that prophage degradation occurred during the 1960s, yet no significant differences in mutation rates were observed between sub-lineages associated with degraded and complete phage profiles. Further investigations are required to determine the biological and evolutionary consequences of these different *emm4* sub-lineages. Yet, these findings highlight the need for enhanced surveillance to detect and track the spread of clinically relevant GAS strains globally.

Aged, MLKL-deficient mice develop multifocal inflammatory lesions and exhibit altered peripheral circulating white blood cell counts.

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Necroptosis is an inflammatory form of lytic programmed cell death. Unlike immunologically quiet apoptosis, necroptosis culminates in bursting open of cellular membranes, Damage Associated Molecular Pattern (DAMP) release and an innate immune response. This programmed necrosis has recently been implicated in human pathology borne of inflammation, such as psoriasis and rheumatoid arthritis. With an ever-expanding list of potential indications for necroptosis-blocking drugs, the two most downstream effectors of necroptosis, MLKL and its obligate activating kinase RIPK3, are being closely scrutinized as druggable targets. Small molecule inhibitors of necroptosis have shown promise in several murine models of inflammatory disease and in phase II human clinical trials. Whilst proven safe for use up to 3 months in humans, the long-term safety of these drugs remains in question. This question is particularly poignant after two recent reports of MLKL loss of function mutations segregating with a novel progressive neurodegenerative disorder or MODY diabetes in two families. We sought to predict long-term side effects by aging a cohort of wild-type and necroptosis deficient, MLKL or RIPK3 knock-out, mice. We showed that aged necroptosis deficient mice lack any overt neurological or diabetic phenotype. Incidentally, we also found that aging MLKL deficient mice exhibit altered peripheral circulating white blood cell counts and multifocal inflammatory lesions. Overall, our work provides important preliminary pre-clinical safety data and prompts future research that examines a possible link between genetic MLKL deficiency and inflammation.

Drug screening to uncover novel aspects of *Salmonella* infection

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Salmonella species are among the most common foodborne pathogens. As an intracellular pathogen, *Salmonella* employs multiple virulence factors to interfere with critical host cell pathways to achieve successful colonization of the host. Past studies have provided many molecular insights into *Salmonella*-host cell interactions. However, many aspects of *Salmonella* pathogenesis are still not well understood. Furthermore, emerging antibiotic-resistant *Salmonella* strains represent a significant clinical threat, and it is crucial to develop novel anti-*Salmonella* therapeutics.

Previous studies have established that IFNγ plays a pivotal role driving the clearance of *Salmonella* from infected hosts. Although several elegant examples of IFNγ-triggered host defense mechanisms have been elucidated, the activities of many IFNγ-regulated genes remain elusive. In this project, we aimed to uncover novel aspects of IFNγ-regulated, *Salmonella*-host cell interactions via conducting a macrophage-based drug screen. A drug library of 3,088 compounds, mainly comprised of FDA approved drugs, were used to treat RAW264.7 cells before cells were stimulated with IFNγ and subsequently infected with *Salmonella*. The infection was examined by confocal microscopy and analyzed through the CellProfiler data analysis pipeline. From the primary screen, we identified 86 drugs that inhibited *Salmonella* intracellular replication with IFNγ treatment when compared to untreated samples, while 121 drugs appeared to enhance *Salmonella* intracellular replication. Our preliminary data suggests that IFNγ stimulation alters *Salmonella* host cell infection outcomes from various drug treatments.

Sex differences in renal and systemic inflammation in a new mouse model of diet-induced obesity

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Obesity is a leading cause of chronic kidney disease (CKD) and there are major sexual dimorphisms in the development of obesity and its associated complications (i.e. obese pre-menopausal women are more prone to developing type-2 diabetes, but obese men are more likely to develop CKD). The mechanisms behind these dimorphisms are poorly understood, which is at least partly due to a lack of reliable animal models of obesity in females. Indeed, female rodents are highly resistant to weight gain and metabolic disturbances in response to traditional dietary and genetic models of obesity, leading to an overwhelming majority of studies in males only. Thus, we aimed to address this limitation, and developed a robust diet-induced rodent model of obesity that accurately reflects the clinical presentation of obesity in both sexes and characterized renal and circulating immune cell profiles in obese and healthy settings. Six-week-old male and female C57BL/6 mice were fed either a high-fat diet (43% kcal in food) with high sugar and salt in their drinking water (10% high fructose corn syrup and 0.9% NaCl; HFSS), or normal chow diet (NCD) for 10 weeks. Physiological parameters were measured weekly and fortnightly. At end point, blood and renal immune cell populations were characterized using flow cytometry. Mice fed a HFSS diet displayed accelerated weight gain, hyperglycemia and pre-hypertension, regardless of sex ($P < 0.05$; compared to NCD of same sex). In males, HFSS significantly increased B cells (B220+) and proinflammatory monocytes (Ly6C^{Hi}/CD11b+ cells) in both the blood and kidney ($P < 0.05$; compared to NCD males). Strikingly, females were completely protected from these obesity-induced increases in B cells and proinflammatory monocytes. These findings suggest that possibly B cells or proinflammatory monocytes are crucial drivers of renal disease in obese males, and females are protected against obesity-induced CKD due to suppression of this mechanism. Future studies should test the therapeutic potential of targeting these aspects of the immune response in obesity to reduce renal damage and dysfunction.

Interferon-Induced Transmembrane Protein 3 (IFITM3) SNPs and COVID-19 disease severity

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SARS-CoV-2 has greatly threatened the world with COVID-19 disease from early 2020, leading to more than 234 million infected cases and 3.8 million deaths to date. Rapidly evolving strains are causing major concerns at the moment due to upsurged infection and ICU admission rate. There are several expected risk factors for increased disease severity including age, pregnancy, underlying co-morbidities and immunosuppression in patients with compromised immune system such as cancer and auto-immunity diseases. However, previously healthy young and middle-aged individuals can also succumb to severe COVID-19 and the reasons are unknown. Therefore, the mechanisms underlying differential COVID-19 outcomes still remains unclear. Interferon-Induced Transmembrane Protein 3 (IFITM3) plays a crucial role in the host defense by controlling viral replication such as influenza virus and SARS coronavirus. Single nucleotide polymorphisms (SNPs) of IFITM3 have been shown to strongly correlated with disease severity and morbidity in influenza and other viral diseases. IFITM3 rs12252-C/C allele is associated with a rapid disease progress and lower survival rate compared to rs12252-T/C or rs12252-T/T influenza patients. Additionally, rs34481144-A and rs6598045-G can affect influenza disease outcome. In this study, we sought to investigate the role of IFITM3 SNPs in COVID-19 severity. Blood samples were collected from total of 185 SARS-CoV-2 positive patients in Australia and China, including 64 patients recovered at home, 97 patients admitted to hospital and 24 patients to ICU. Seventy-nine SARS-CoV-2 negative patient blood samples were collected as healthy control. IFITM3 SNPs rs12252, rs34481144 and rs6598045 were sequenced form collected patients DNA samples. Inflammatory cytokines and chemokines were correlated with IFITM3 SNPs. Our study sheds light on the mechanism underlying the role of IFITM3 SNPs in disease outcomes of COVID-19 patients, and provides insights into understanding and preventing severe COVID-19, beneficial for future immunotherapies.

Early life infection is associated with proinflammatory, atherogenic, and diabetogenic metabolomic and lipidomic profiles at 12 months of age

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Introduction: Infection is linked to later cardiovascular and metabolic disease, but the mechanisms are poorly understood, particularly in early life, when most infection occurs. We investigated the relationship of infectious burden (from birth to 12 months) with NMR metabolomic and LC/MS lipidomic profiles at 12 months of age, and whether inflammation mediated these effects.

Methods: Plasma metabolomics and lipidomics were quantified in 12-month plasma from 555 infants in the Barwon Infant Study who had complete data on parent-reported infections from repeated questionnaires in the first year of life. In linear regression models adjusted for confounders, the exposure was total number of infections as a continuous variable, and the outcomes were 12-month metabolomic and lipidomic measures. We investigated whether inflammation (with the biomarkers glycoprotein acetyls (GlycA) and high-sensitivity C-reactive protein (hsCRP)) mediated these effects using structural-equation modeling.

Results: A higher number of infections was associated with higher inflammation markers and phenylalanine; and with lower high-density lipoprotein cholesterol, apolipoprotein A1, and docosahexaenoic acid. In lipidomic analysis, higher number of infections was associated with higher phosphatidylethanolamines and lower plasmalogens; and lower ceramide and hexosylceramides species. Higher 12-month GlycA was associated with similar, more pronounced profiles. GlycA mediated a substantial proportion of the leading associations between infections and metabolomic and lipidomic measures (9.2-39.9%). hsCRP showed little evidence for mediating the relationship between infections and metabolomic/lipidomic differences.

Conclusion: Higher infectious burden in infancy is associated with pro-inflammatory, pro-atherogenic, and diabetogenic metabolomic and lipidomic profiles. Inflammation may play a key role in mediating the metabolic effects of infection. These findings suggest potentially modifiable pathways linking early life infection, inflammation, and cardiometabolic risk.

T-bet dependent CD4$^+$ effector differentiation is intertwined with memory formation in viral settings

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Following viral infection, naive CD4$^+$ T cell differentiate into heterogenous effector cells. These effector cells coordinate the cellular and antibody-mediated responses to clear infection and promote protection. Differentiation toward T-helper 1 (TH1) cells mediates inflammation and pathogen clearance, while T follicular helper (TFH) cells facilitate germinal center reactions for the generation of high-affinity antibodies. In contrast, a diverse range of memory cells provide protection against repeated infection with the same pathogen. The ontogeny of distinct CD4$^+$ memory populations is unclear. We have previously demonstrated that the TH1-asscociated transcription factor T-bet is important for TH1 and TFH differentiation following LCMV infection. To investigate the relatedness of CD4$^+$ effector cells to distinct memory populations, we investigated T-bet's role in memory cell differentiation and maintenance. Following infection, during CD4$^+$ T cell expansion, there was no change in memory precursor cells in T-bet-deficient LCMV-specific cells compared to their controls. However, during the memory phase, there was a complete loss of effector memory and tissue resident memory cells in non-lymphoid tissues. These observations suggest that either effector TH1 and TFH cells may seed distinct memory populations and/or that T-bet plays a role in the maintenance of CD4$^+$ memory. We are using a novel cellular barcoding strategy to investigate these hypothese and elucidate the relationship between distinct effector cells and the CD4$^+$ T cell memory pool. Addressing these questions will be crucial to improve strategies that promote CD4$^+$ T cell memory formation during vaccination.

Arbidol impairs SARS-CoV-2 spike glycoprotein trimerization and reduced mortality in adult COVID-19 patients in a cohort study

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The ongoing COVID-19 pandemic and the emergence of variations have increased the complexity of infection, demanding effective and promising therapies to treat infected individuals. We describe the mechanism of action of Arbidol (umifenovir) in the treatment of coronavirus disease 2019 (COVID-19) by preventing SARS-CoV-2 spike glycoprotein trimerization. We also discovered a distinct sequence similarity between influenza H3N2 and SARS-CoV-2. In addition, a cohort of 504 hospitalised COVID-19 patients was assembled to test the efficacy of Arbidol alone or in combination with other approved COVID19 drugs Oseltamivir and Ritonavir. We first notice that factors such as older age, lower SpO2 level, larger lesion, early admission date, and pre-existing conditions have been linked to higher mortality. After segregating patient characteristics or conditions and concurrent antiviral drug use, Arbidol was found to be promising and associated with lower mortality in the cohort and reduced lesion absorption (P=02023) based on a chest CT scan, and its efficacy was higher when combined with Oseltamivir. The overall mortality rate in the cohort was 15.67% and the OR for Arbidol is 0·183 (95% CI, 0·075 to 0·446; P<0·001). Through structural studies, we also demonstrate the mechanism of Arbidol action on spike protein. Arbidol, a broad-spectrum antiviral drug, accelerated lesion absorption and reduced mortality in COVID19 patients. We anticipate that understanding the mechanism of action of Arbidol will aid in the development of new SARS-CoV-2 therapeutics.

Keywords: Antiviral; COVID-19; cohort, Spike glycoprotein, lesion absorption

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Genetic mutations in host TMPRSS2 reduced COVID-19 infection in patients and potential drugs showed viral clearance in cohort studies

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The SARS-CoV-2 is responsible for the COVID-19 pandemic, causing severe acute respiratory distress syndrome (ARDS) that has resulted in over 4.7 million deaths worldwide. Understanding the unique cell entry mechanism of SARS-CoV-2 via processing its spike-glycoprotein has vast implications in the development of potential therapeutics. The spike glycoprotein of SARS-CoV-2 binds to the host receptor ACE2 and is activated by the host serine protease Furin and TMPRSS2 via proteolytic activation for subsequent entrance. Here, we present how TMPRSS2 recognizes and activates the SARS-CoV-2 spike using structural, molecular, clinical, and computational studies. Second, we discovered TMPRSS2 cleavage sites in the S2 domain of the SARS-CoV-2 spike and demonstrated the structure as a complex including the catalytic triad of enzyme processing. We next performed whole-exome sequencing for healthy and COVID-19 patients (n523) and identified a key mutation rs12329760 (V160M) in the TMPRSS2 gene that results in a decreased infection rate in clinically diagnosed COVID19 patients and provides the possible reason behind the differential infection rate among the individuals. We also structurally demonstrate how mutations in the host genome reduce infection. We also present potential drugs to block TMPRSS2 and a cohort of studies showing that Chemostat and Nafamostat are associated with faster lesion absorption in the lungs of infected patients. These findings contribute to our understanding of the mechanism of TMPRSS2 processing causing increased virulence, as well as insight into the highest quality intervention options and widen the knowledge of host factors in viral infection.

The balance of IL-12 and IL-23 determines the bias of MAIT1 versus MAIT17 responses during bacterial infection

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Mucosal-associated invariant T (MAIT) cells are a major subset of innate-like T cells mediating protection against bacterial infection through recognition of microbial metabolites derived from riboflavin biosynthesis. MAIT cells differentiate into two main subpopulations with diverse functions, namely T-bet-expressing MAIT1 and RORγt-expressing MAIT17 cells. Previously, we reported that ICOS and IL-23 provide essential signals for optimal MR1-dependent activation and expansion of MAIT17 subsets during bacterial infection. However, optimal activation requirements for MAIT1 cells, *in vivo*, remain unclear. Here, in a model of tularemia, in which MAIT1-responses predominate, we demonstrate that IL-12 and IL-23 promote MAIT1 cell expansion during acute infection and that IL-12 is indispensable for ensuring the differentiated MAIT1 phenotype and cytokine production. A combination of IL-12 and synthetic antigen, 5-OP-RU, was able to expand MAIT1 cells systemically in mice. Furthermore, our findings demonstrate that bias toward MAIT1 or MAIT17 responses, as seen during different bacterial infections, is determined and modulated by the balance between IL-12 and IL-23. The findings explain the mechanisms of MAIT cell polarisation and open opportunities for manipulation of MAIT cells immunity.

Management of Tuberculosis Infection (TBI) in Victorian children: a retrospective clinical audit of TBI management, and factors affecting treatment completion

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Publish Consent Withheld

ABSTRACTS
SESSION 3
POSTER II

RATIONAL DESIGN OF ANTISENSE OLIGONUCLEOTIDES MODULATING THE ACTIVITY OF TLR7/8 AGONISTS

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Oligonucleotide-based therapeutics have the capacity to engage with nucleic acid immune sensors to activate or block their response. However, a detailed understanding of these immunomodulatory effects is currently lacking. Here, we show that gene targeting 2′-O-methyl (2′OMe) gapmer antisense oligonucleotides (ASOs) can have opposing activities on Toll-Like Receptors 7 and 8 (TLR7/8), leading to divergent suppression of TLR7 and activation of TLR8, in a sequence-dependent manner. Through a screen of 192 2′OMe ASOs and sequence mutants, we characterized the structural and sequence determinants of these activities. Importantly, we identified core motifs preventing the immunosuppressive activities of 2′OMe ASOs on TLR7. Based on these observations, we designed oligonucleotides strongly potentiating TLR8 sensing of Resiquimod, which preserve TLR7 function, and promote strong activation of phagocytes and immune cells. We also provide proof-of-principle data that gene-targeting ASOs can be selected to synergize with TLR8 agonists currently under investigation as immunotherapies, and show that rational ASO selection can be used to prevent unintended immune suppression of TLR7. Accordingly, we propose that rational selection of TLR8-potentiating ASOs could present new opportunities in the therapeutic development of bifunctional ASOs with gene-targeting and immunostimulatory activities. Taken together, our work characterizes the immumodulatory effects of ASOs to advance their therapeutic development.
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Investigating the role of the unfolded protein response in *Legionella pneumophila* infection

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L. pneumophila, the environmental gram-negative pathogen, is an intercellular bacterium that survives in the environment by multiplying in the free-living amoebae. When transmitted to humans, it targets phagocytic immune cells within the lung, such as macrophages and monocytes. Interestingly, *Legionella* secrets > 300 virulence factors or "effector" proteins into the cell via its type-IVB secretion system. These effectors manipulate various host processes to evade elimination by phago-lysosomal degradation and other innate immune responses to infection. In fact, using its effector proteins, *Legionella* hijacks vital processes within the cell to use the available sources and to replicate. For example, when infecting human macrophages, *Legionella* recruit's proteins from the endoplasmic reticulum (ER) membranes to form its intracellular replication vacuole, the *Legionella*-containing vacuole (LCV). It also causes a loss of ER homeostasis and ER stress within infected host cells.

My project focuses on infecting human macrophage cell line (THP1) with *L. pneumophila* to study the effect of ER stress and unfolded protein responses to *Legionella*, we found that treating THP1 cells with Tunicamycin and Thapsigargin, the ER stress and unfolded protein response activators, for two hours can inhibit *Legionella* replication significantly up to 48 hours post-infection. On the other hand, these drugs do not affect the translocation of *Legionella* effector proteins. Also, pre-treating THP1 cells with Thapsigargin helps them survive *Legionella* infection longer and protects the host cell from *Legionella*-induced apoptosis up to 48h post-infection. We have also found that *Legionella* infection limits and inhibits activation of ER stress and the unfolded protein response via multiple mechanisms such as blocking of XBP-1 mRNA splicing and inhibition of number of UPR stress genes.

The Development of a Novel Antimalarial Class with Slow to Moderate Erythrocytic Stage Activity

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Malaria has long been heralded a “preventable and curable disease.” However, parasite resistance against all currently available antimalarials, including the first-line treatment Artemisinin combination therapy (ACT), threatens our efforts to control the disease. An urgent need has arisen towards the development of antimalarials with novel mechanisms of action.

In collaboration with Janssen Pharmaceuticals and Medicines for Malaria Venture, a high-throughput screen was undertaken against the asexual blood stage of *Plasmodium falciparum*, identifying several novel antimalarial classes. One of these series is the focus of the present studies and is mediated by an unknown mechanism of action. Medicinal chemistry optimisation has generated potent nanomolar inhibitors which have been used to characterise the series’ activity in parasites phenotypically.[1] The series was identified to act with a slow to moderate rate of kill and are equipotent in *P. falciparum* multidrug resistant strains. Mechanistic studies are currently underway in the hope of identifying a novel *P. falciparum* therapeutic target.

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Whole Body Analysis of Tissue-Resident Immune Cells

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Tissue-resident memory T (T_{RM}) cells are a non-circulating lymphocyte population that are principally located in peripheral tissues. T_{RM} cells provide rapid protection against a wide range of infections and cancer; hence, enhancing T_{RM} cell formation and persistence is an attractive means for establishing durable immunity. While many studies have dissected the properties of T_{RM} cells within peripheral tissues in mice, our knowledge of human T cells has been largely derived from blood sampling. In collaboration with Austin Health, we have established the first Australian Donation and Transplantation Biobank that provides access to a wide range of healthy human organs. Using this resource, we performed a whole-body analysis of T_{RM} cells across barrier and non-barrier tissue sites. We employed multiparameter flow cytometry and scRNAseq to resolve distinct T_{RM} cell populations across the gut, skin, liver and spleen. We observed intra- and inter-organ T_{RM} cell heterogeneity based on the expression of tissue residency markers CD69 and CD103, and inhibitory molecules such as PD-1 and CD244. Furthermore, we have demonstrated how the tissue microenvironment influences various T_{RM} cell functional capabilities. Together, this holistic characterisation of T_{RM} cells across solid organs underscores the importance of investigating local tissue immunity which cannot be discovered by conventional blood sampling. The results of this study will direct novel tissue-specific immunotherapies aimed to promote and establish durable tissue immunity.

Understanding the mechanisms regulating GILZ, a key determinant of immune responses

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A key outcome of glucocorticoid (GC) treatment in patients with inflammatory and autoimmune diseases is upregulation of the glucocorticoid-induced leucine zipper (GILZ). GILZ acts as a natural brake against activation, expansion and effector responses of B cells, CD4 T cells, macrophages and dendritic cells, and collectively these effects confer protection from damage in autoimmune diseases. GILZ is downregulated in inflammatory contexts and restoration of GILZ is an attractive therapeutic avenue. Developing a strategy to achieve this requires an understanding of the mechanisms governing GILZ abundance. Here, we show K48 and K63 linked polyubiquitination and proteasomal degradation of GILZ, which we found to have a half-life approximately 45-60 minutes. Importantly, the stability of the GILZ protein was not altered by GC treatment or by stimulation of cells with inflammatory signals, including agonists of toll-like receptors (TLRs) 4, 7 and 9, although these ligands rapidly stifled GILZ transcription. This demonstrates that GILZ abundance is regulated through gene transcription rather than protein turnover, which remains stable. We identified a non-redundant E3 ligase of GILZ, deletion of which more than doubled GILZ half-life. This discovery provides guidance towards a potential mechanism for manipulating GILZ abundance and its associated consequences across the immune system.

The role of RIP kinases in bacterial gut infection

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Enteropathogenic *Escherichia coli* (EPEC) is a diarrhoeagenic gut bacteria that utilises a type III secretion system to translocate effector proteins into host cells and modify cell signaling. One such effector is EspL, which has been characterised to inhibit host cell necroptosis and associated inflammatory pathways by directly cleaving RIPK1 and RIPK3 at their conserved RHIM domains. Preliminary experiments using *Ripk1*$^{-/-}$*Ripk3*$^{-/-}$*Casp8*$^{-/-}$ mice inoculated with *Citrobacter rodentium* – the model organism for EPEC, demonstrated heightened susceptibility to infection, suggesting a role for RIP kinases in the clearance of enteropathogens.

Examination of disease in various single and compound knockout mice revealed that both RIPK1 and RIPK3 provides protection against *C. rodentium* infection. Notably, RIPK3 plays a significant role in moderating local gut pathology. More interestingly, flow cytometry analysis of the colonic lamina propria from infected *Ripk1*$^{-/-}$*Ripk3*$^{-/-}$*Casp8*$^{-/-}$ mice showed a marked reduction in the T-helper 17 and T-regulatory cell populations, which are important for mediating bacteria-induced colitis. This is consistent with the improved disease phenotype exhibited by wildtype mice infected with an *espL* deleted *C. rodentium* mutant. Here, qPCR evaluation of inflammatory cytokine levels found an increased expression of *Il22*, but not *Il17a* in the colons of mutant infected mice compared to wildtype controls. Thus far, these results show for the first time, a link between RIPK1/3 (innate immunity) and T cell responses (adaptive immunity), which will be further investigated to better inform the significance of RIP kinases in bacterial pathogenesis and maintenance of gut homeostasis.

Molecular mechanisms of lipid presentation by CD1b and TCR recognition

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Recognition of antigens by T-cell receptors (TCRs) on the surface of T-cells is central to the immune response. Lipid antigens are presented by CD1 molecules and have recently entered the spotlight for antigenic lipid presentation and TCR recognition. CD1b has already been extensively studied for the role it plays in in *Mycobacterium tuberculosis* infection, however, we know very little about immune responses to $CD1b^+$ cell interactions and related diseases including lupus, and psoriasis. It is not surprising that CD1b is capable of presenting self-lipids, with lipids playing a critical role in binding cleft stabilisation throughout protein production of CD1b (1). However, the concept of self-lipids acting as antigenic targets is novel with crystal structures and functional data showing TCR interaction and T-cell activation by CD1b presenting self-lipids (2).

To understand the mechanisms behind this auto-reactivity, presentation of self-lipids by CD1b has been established with numerous lipid species. The structures of CD1b in complex with phosphatidylinositol (CD1b-PI) and CD1b-PI in complex with an autoreactive TCR (CD1b-PI-BC8B) were determined via x-ray crystallography. These structures bring us closer to elucidating the mechanism behind the CD1b-autoreactive axis, opening up an untapped area of research into the potential role of CD1b in self lipid presentation and consequent autoimmunity.

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Differential activation of innate immune responses by *Bacteroides fragilis* and their outer membrane vesicles (OMVs)

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

To do this, the size and composition of purified *B. fragilis* OMVs was characterized, revealing that immunostimulatory products including peptidoglycan, LPS, nucleic acids and proteins were associated with *B. fragilis* OMVs. Additionally, we observed the enrichment of specific protein cargo into OMVs compared to their parent bacteria. The ability of OMVs to enter and deliver their cargo to intestinal epithelial cells was determined using confocal microscopy, and their potential to activate innate immune receptors compared to their parent bacteria was determined using HEK-Blue reporter cell lines. Whilst *B. fragilis* bacteria could only activate Toll-like receptor (TLR)-2, *B. fragilis* OMVs induced the activation of TLR2 and TLR4, in addition to intracellular TLR7 and NOD1 that detect bacterial RNA and peptidoglycan, respectively. Currently, we are elucidating the mechanisms underpinning the differences in receptor activation between *B. fragilis* OMVs and their bacteria, and the subsequent immunological outcomes.

Collectively, our results demonstrate that *B. fragilis* OMVs activate different immune signalling pathways compared to their parent bacteria, revealing novel roles for OMVs secreted by the intestinal microbiota in activating host immune responses.

Integrated immune networks in SARS-CoV-2 infected pregnant women reveal differential NK cell and unconventional T cell activation

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Pregnancy poses a greater risk for severe COVID-19, however the underlying immunological changes associated with SARS-CoV-2 infection during pregnancy are poorly understood. We defined immune responses to SARS-CoV-2 in pregnant and non-pregnant women during acute and convalescent COVID-19 up to 258 days post symptom onset, quantifying 217 immunological parameters. Additionally, matched maternal and cord blood were collected from COVID-19 convalescent pregnancies. Although serological responses to SARS-CoV-2 were similar in pregnant and non-pregnant women, cellular immune analyses revealed marked differences in key NK cell and unconventional T cell responses during COVID-19 in pregnant women. While NK cells, γδ T cells and MAIT cells displayed pre-activated phenotypes in healthy pregnant women when compared to non-pregnant age-matched women, activation profiles of these pre-activated NK and unconventional T cells remained unchanged at acute and convalescent COVID-19 in pregnancy. Conversely, activation dynamics of NK and unconventional T cells were prototypical in non-pregnant women in COVID-19. In contrast, activation of αβ $CD4^+$ and $CD8^+$ T cells, T follicular helper cells and antibody-secreting cells was similar in pregnant and non-pregnant women with COVID-19. Collectively, our study provides the first comprehensive map of longitudinal immunological responses to SARS-CoV-2 infection in pregnant women, providing insights into patient management and education during COVID-19 pregnancy.

Dendritic cell apoptotic bodies as antigen presenting vesicles within an Influenza A virus infection model

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The apoptotic cell disassembly process occurs post apoptosis and is characterised by the generation of membrane-bound extracellular vesicles, termed apoptotic bodies. Apoptotic bodies are reported to carry biomolecules, such as nucleic acids and proteins, and have been suggested to have a pivotal role in mediating intercellular communication. Extracellular vesicles released by immune cells have been shown to provide a novel mode of immune regulation suggesting a vital role within our immune system. However, the importance of the generation of apoptotic bodies and the apoptotic cell disassembly process by dendritic cells (DCs); professional antigen presenting cells essential for directing a functional adaptive immune system through T cell activation, has not been fully elucidated.

Therefore, to identify the functional role of DC-derived apoptotic bodies, a series of cell biological analyses using the DC cell line, DC2.4, was conducted via time lapse microscopy, fluorescent microscopy, and flow cytometry. Time lapse microscopy and flow cytometry analyses reveal that apoptotic DCs undergo the apoptotic cell disassembly process as regulated by Rho-associated Kinase 1 (ROCK1) to form dynamic membrane blebs, followed by the generation of membrane protrusions and release of apoptotic bodies. Furthermore, DC-derived apoptotic bodies retain important immune signalling molecules necessary for efficient antigen presentation to cognate T cells, including Major Histocompatibility Complex (MHC) Class I and II, and co-stimulatory molecules CD80 and CD86. Our findings indicate that Influenza A virus infected DCs also undergo the apoptotic cell disassembly process and release apoptotic bodies which carry peptide-MHC complexes that can directly activate antigen-specific CD8+ T cells *in vitro*. Furthermore, we concluded that the direct presentation of antigen to $CD8^+$ T cells via DC-derived apoptotic bodies was transporter associated with antigen processing (TAP) dependent as apoptotic TAP knockout DCs release apoptotic bodies which cannot activate $CD8^+$ T cells. Collectively, this research provides insights into DC-derived apoptotic bodies as efficient antigen-presenting vesicles with the potential to induce antigen-specific T cell responses thereby aiding adaptive immunity.

Investigating the *in vitro* degradation behaviour of bistriazines, a potent novel antimalarial class

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Malaria, a bloodborne protozoan infection, is responsible for half a million deaths worldwide on a yearly basis. Among six species which can infect humans, *Plasmodium falciparum* causes the most severe disease. Resistance to current frontline therapy, artemisinin, has emerged in sub-Saharan Africa, which bears the largest burden of *P. falciparum* infection. Hence, there is an urgent need for the discovery of antimalarials with novel mechanisms of action. Bistriazines compounds were identified in a high throughput screen and shown to exhibit single digit nanomolar potency against asexual blood stage *P. falciparum* parasites. Furthermore, they display no cross-resistance with artemisinin or chloroquine. However, the lead bistriazine compound demonstrated a short half-life of approximately 2 hours in human liver microsomes in *in vitro* studies. This raises concerns whether bistriazines will be potentially unstable under *in vitro* parasite culture systems. With the aid of liquid chromatography mass spectrometry, we showed that bistriazines were stable in water, with 98% of parent drug retained after 48 hours incubation. In comparison, in culturing media (complete RPMI) and red blood cells (2% haematocrit), bistriazines significantly degraded (40% and 50% of parent drug degraded respectively) over 48 hours. Furthermore, our results showed that the degradation of bistriazines was significantly increased by the presence of parasites. Approximately 7% and 60% of the parent drug degraded over 2 hours when exposed to *P. falciparum* at 1% and 10% parasitaemia respectively, demonstrating the positive correlation between the degradation rate and parasite load. It has not been shown whether the rapid bistriazine degradation is associated with its antimalarial activity. Hence, continuous effort is being dedicated to identify the degradation products of the bistriazines and their potential contribution to the *in vitro* potency of the compound.

MOLECULAR REGULATION OF CCR4 LIGANDS BY GM-CSF AND IL4

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The CCR4 receptor is expressed by Th17 and regulatory T cells, and an imbalance between these two T cell subpopulations is thought to drive autoimmunity and its associated chronic inflammation. CCL17 and CCL22 are the functional ligands of the CCR4 receptor. These chemokines share a nucleotide homology of 32%, are found in close proximity to each other on both the human and mouse chromosomes, yet they are differentially expressed in various autoimmune diseases. In rheumatoid arthritis (RA), CCL17 is highly upregulated in the synovial fluid of patients while CCL22 is detected at very low levels, despite it being constitutively expressed in healthy controls. These variable expression patterns suggest that despite their common function they might be differentially regulated.

GM-CSF and its receptor are currently being targeted in clinical trials for various inflammatory, autoimmune diseases. In RA, GM-CSF is highly upregulated in the synovial fluid of patients, and it has also been shown to upregulate CCL17 expression. IL4 is another cytokine that upregulates CCL17 expression, but it is detected at very low levels in RA patients. These cytokines have contrasting roles in inflammation but they both upregulate CCL17 production by promoting JMJD3 demethylase activity and IRF4 expression.

We report here that GM-CSF and IL4 can also upregulate CCL22 expression in human monocytes, human macrophages, and mouse macrophages. CCL22 upregulation is variably dependent on JMJD3 and IRF4 in these cell types, which suggests distinct signalling pathways in different cell types. Moreover, GM-CSF and IL4 independently activate the transcription factors STAT5 and STAT6, respectively, and their activation is crucial for CCL17 and CCL22 expression in all three cell types. This variable regulation of these seemingly similar chemokines sheds light on the nuances of cell types and their role in autoimmune diseases. As therapies begin targeting more specific, downstream mediators, delineating the signalling pathways activated by key inflammatory cytokines, and discerning differences between immune cell types, will increase the efficacy of future therapies.

The Design, Synthesis and Evaluation of Novel Metalloaminopeptidase Inhibitors as Antimalarial Agents

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Malaria remains a major burden on health resources worldwide. The recurrent emergence of resistance to the current antimalarials highlights the urgent need for novel therapeutics that target new pathways.[1] Metalloaminopeptidases play an important role in haemoglobin digestion which is an essential pathway in the intra-erythrocytic parasite. Previous work has demonstrated that targeting certain aminopeptidases, in particular the *Plasmodium falciparum* M1 and M17 aminopeptidases (*Pf*A-M1 and *Pf*A-M17 respectively), results in parasite death.[2,3] Current work within the group employs structure-based drug design to optimise and advance analogues capable of dual inhibition of both enzymes.[4] The new compounds in this study investigated the effect of altering the substituents occupying the enzymes' S1 pocket in order to improve the pharmacokinetic profile while maintaining desirable activity. This new aromatic series demonstrated encouraging antiparasitic activity and physicochemical profiles, presenting opportunities for further development into potent antimalarial compounds with a novel mode of action.

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ABSTRACTS
SESSION 3
POSTER III

TREML4 receptor regulates inflammation and innate immune cell death during polymicrobial sepsis

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Sepsis is a biphasic disease characterized by an acute inflammatory response, followed by a prolonged immunosuppressive phase. Therapies aimed at controlling inflammation help to reduce the time patients with sepsis spend in intensive care units, but they do not lead to a reduction in overall mortality. Recently, the focus has been on addressing the immunosuppressive phase, often caused by apoptosis of immune cells. However, molecular triggers of these events are not yet known. Using whole-genome CRISPR screening in mice, we identified a triggering receptor expressed on myeloid cells (TREM) family receptor, TREML4, as a key regulator of inflammation and immune cell death in sepsis. Genetic ablation of Treml4 in mice demonstrated that TREML4 regulates a host of cellular responses, particularly of innate immune cells, during polymicrobial sepsis, leading to an overall increase in survival rate, both during the acute and chronic phases of the disease.

Develop a Nanobody Platform to Enable High-Resolution Structural Determination of *Candida auris* ABC Transporter Protein CDR1 by Cryogenic Electron Microscopy (cryo-EM)

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A recent emerging multi-drug resistant (MDR) superbug, *Candida auris*, dramatically impacts the healthcare sector with increased mortality and associated economic problems worldwide[1]. According to the CDC, over 90% of US citizens are resistant to the most effective antifungal agent fluconazole[2]. However, the exact molecular resistance mechanism is still unclear but involves the ATP binding cassette (ABC) transporter CDR1. My project aims to develop a nanobody platform to enable high-resolution structural determination of *C. auris* ABC transporter protein CDR1 by cryo-EM. The mechanisms of CDR1 remain unexplored. As CDR1 is a membrane protein, most of its sequence resides within the membrane and undergoes dynamic conformational changes to enable transport across membranes. Hence, to visualize the CDR1 drug-bound structure by cryo-EM, we need to trap a fixed conformation for high-resolution structure determination. To resolve this, we will use a single domain antibody (nanobody) that binds with CDR1 in the presence of a drug to trap the confirmation specific for drug binding. Nanobodies can be expressed on yeast synthetically; thus, it not only reduces the use of animals in research but is cost- effective and confers rapid production. We have expressed pdd-mTurquoise2-Strep tagged CDR1 in *Saccharomyces cerevisiae* by multi fragment homologous gap repair protocol[3]. In the presence of a drug such as fluconazole, we will determine the drug binding affinity with CDR1 by GFP-based thermal-shift assay. Our next aim is to establish a nanobody library on yeast surface by using the modified protocol from McMahon et al[4]. To enrich and select the nanobodies specific for drug-bound CDR1, we will utilize fluorescent activated cell sorting and fluorescence-detection size-exclusion chromatography. Finally, we will determine the drug-bound CDR1 structure by cryo-EM. This project will show a new pathway to use nanobody as a prospective tool to discover conformationally selective nanobodies for CDR1 and aid in elucidating the structure of this protein that will give light for future drug discovery.

References

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The role of anti-malarial immunity in the spontaneous clearance of molecular-detectable Plasmodium spp. infection

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The Greater Mekong Sub-Region is approaching malaria elimination. However, in areas of very low transmission there are high proportions of sub-clinical Plasmodium spp. infections, that can act as reservoirs of transmission and are a barrier to elimination efforts. There is emerging evidence that molecular-detectable sub-clinical infections can spontaneously clear in the absence of antimalarial treatment. Naturally acquired immunity, which has been shown to protect against high density parasitemia, may also play a role in determining the natural course of (untreated) sub-clinical infections. We sought to quantify the association between antibody markers of immunity and spontaneous clearance of molecular-detectable sub-clinical infections.

A nested cohort study was undertaken in Cambodia recruiting 150 asymptomatic individuals with uPCR-detectable Plasmodium spp. infections at baseline. Individuals were sampled monthly for 12 months to evaluate the duration of infections. Antimalarial blood-stage antibodies specific for merozoite antigens PfAMA1 and PfMSP2 were quantified by ELISA. Accelerated time failure models were used to estimate the relative reduction of infection duration associated with immunity.

Spontaneous clearance was observed in 96% of baseline infections, with median infection duration of 63 days (range 17-301). Seroprevalence at baseline was 42.7% and 38% for anti-PfAMA1 and PfMSP2 IgG, respectively. Doubling of antibody levels at the preceding timepoint (time varying) were associated with a reduced median duration of all P. spp. infections by 18% (~11 days) (aTR0.82 95% CI:0.71-0.94) for PfAMA1 and by 13% (~8 days) (aTR0.87 95% CI:0.79-0.96) for PfMSP2.

Naturally acquired antibodies are associated with a reduction in the duration of molecular-detectable malaria infections. This has important implications for our understanding of the drivers of the epidemiology of the malaria infectious reservoir in the Greater Mekong Sub-region.

Advances in drug target characterization: A new UV-Vis spectroscopy approach for protein investigation

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Protein characterization plays a key role in the discovery and development of new drug targets and therapeutics. Among the different characteristics of proteins, their specific melting temperatures provide important information on their stability and greatly help in the process of drug design. This presentation gives a an overview on the importance of drug target characterization and a new approach in the use of UV-Vis spectroscopy to measure the thermal stability of proteins and interaction studies relevant for new drug design.

Role of socs3b in zebrafish innate immunity

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The suppressor of cytokine signalling 3 (SOCS3) protein is part of a negative feedback loop that controls signalling by several key cytokines such as IL-6, G-CSF and IL-1β. Through this mechanism, SOCS3 regulates important cellular processes such as haematopoiesis and inflammation, ensuring that they are maintained at homeostatic levels. To complement studies carried out in mammalian models, the function of the zebrafish *socs3b* orthologue was investigated. A global gene knockout was generated using CRISPR/Cas9, which unlike the mouse *Socs3* knockout was viable, providing a opportunity to study the impacts of global SOCS3 ablation throughout the life course. Zebrafish *socs3b* knockout embryos displayed elevated levels of myeloid progenitors during primitive hematopoiesis and an increase in neutrophils during definitive hematopoiesis. During adulthood, *socs3b* knockout zebrafish developed an inflammatory phenotype characterized by uveitis, with extensive infiltration of neutrophils and macrophages into the eye. Infiltration of neutrophils was also observed in several other tissues such as kidney and spleen. Wounding assays conducted on embryos further revealed that macrophages were more active in the mutants, with an elevation in chemokine markers. These findings identify a conserved role for *socs3b* in the regulation of neutrophil production and inflammation, including an additional role in the activation of embryonic macrophages.

The hijacking of lipid synthesis during flavivirus infection

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Flaviviruses are a genus of positive sense single strand RNA viruses (+ssRNA), which include several clinically important and widespread mosquito-borne viruses such as dengue, West Nile, Zika and yellow fever viruses. These viruses have a demonstrated ability to invade new environments with ease and mutate to cause increasingly severe disease symptoms, and the imminent threat of climate change and population increase has the potential to greatly alter the epidemiology of these viruses. Controlling their spread, however, depends mostly on mosquito control and vaccines and targeted therapeutics are severely lacking. Upon entry in a cell, flaviviruses cause a drastic rearrangement of the host cell lipid landscape, sequestering and upregulating lipid synthesis to provide substrates for increased metabolism and the formation of membranous replication complexes. Perturbing the synthesis of certain lipid classes has been demonstrated to attenuate the replication of some viruses and could therefore be a potentially effective antiviral target. Here we investigate the manipulation of fatty acid synthesis (FAS) by West Nile and Zika viruses, and the application of chemical inhibitors of FAS to restrict replication in Vero cells and human and mouse macrophages. We found overall that FAS is integral to the replication of these viruses in an immune and non-immune cellular background, but inhibiting different enzymatic activities along the FAS pathway yielded not only differences in viral restriction, but also had distinct effects on cellular processes. With one inhibitor in particular, orlistat (an FDA approved compound for cancer treatment), we made some unexpected observations which could give us insight into the specificity of fatty acid utilization by these viruses, and has implications regarding off-target effects of this drug which may be broadly relevant to research using this inhibitor

Salmonella Typhimurium induces cIAP1 degradation to promote death in macrophages

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Salmonella Typhimurium is a gastrointestinal pathogen that infects both humans and animals. In humans, this bacterium induces gastroenteritis with symptoms of diarrhoea, nausea, vomiting, and fever, and can cause systemic disease in immunocompromised individuals. *S.* Typhimurium infects epithelial cells and macrophages intracellularly, enabled by two specialised Type III Secretion Systems (T3SSs) which translocate effector proteins directly into the host cell cytosol. These effector proteins exert a range of pathogenic activities, including manipulation of innate immunity and programmed cell death processes[1]. Our research shows that wild type *S.* Typhimurium induces the degradation of cellular inhibitor of apoptosis protein 1 (cIAP1), an important host cell adaptor of tumour necrosis factor receptor 1 (TNFR1) signalling and inhibitor of apoptotic cell death. Degradation of cIAP1 was associated with functional *Salmonella* Pathogenicity Island 1 (SPI-1) T3SS effector translocation, and was not prevented by pan-caspase, proteasomal or lysosomal inhibitors. Consistent with cIAP1-mediated inhibition of apoptosis, we observed strong association between loss of cIAP1 and increased cellular cytotoxicity. Anti-cIAP1 immunoblot detected a low molecular weight peptide following *S.* Typhimurium infection, suggesting that a SPI-1 effector may cleave cIAP1 during infection. Current work combines several molecular and *in vitro* techniques to explore the cIAP cleavage mechanism, and determine the responsible SPI-1 effector protein. Future work will assess the involvement of cIAP proteins in overall susceptibility to *Salmonella* infection *in vivo*. We hypothesise that cIAP1 depletion is induced by a *S.* Typhimurium SPI-1 effector in order to promote host cell death, and potentially dissemination of the bacterium.

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Identification and characterisation of the pH-dependent membrane-targeting saltwater crocodile defensin CpoBD13

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Crocodilians are an order of ancient reptiles that have adapted throughout evolution to inhabit microbial-laden environments. Despite commonly receiving wounds during territorial disputes, the likelihood of developing a systemic infection is rare for these animals, indicating a potent immune system. Defensins, a class of cysteine-rich cationic host defence peptides, contribute to the innate immunity of all eukaryotes. These peptides, which permeabilise microbial cell membranes through the direct binding of negatively charged phospholipids, have been well characterised in humans[1] and plants[2], however, the defensins of reptiles are poorly understood.

In this study, to better define the structure-function of crocodilian defensins, *Crocodylus porosus* (saltwater crocodile) β-defensin 13 (CpoBD13) was recombinantly expressed in the methylotrophic yeast *Pichia pastoris*. CpoBD13 was shown to inhibit the growth of the pathogenic fungus *Candida albicans* through the permeabilisation of the cell's plasma membrane. Phospholipid-binding experiments revealed that CpoBD13 specifically bound the membrane lipid phosphatidic acid (PA). The protein structure of CpoBD13 in complex with PA was determined using X-ray crystallography and revealed that protein-lipid interactions were mediated by arginine and histidine residues. Membrane permeabilisation assays at a range of physiologically relevant pH levels showed that the antifungal activity of CpoBD13 was greater at pH <6.0 due to the increase in charge, and therefore the affinity for PA, accredited to the protonation of the peptide's histidine residues.

These results indicate that the membrane-targeting mechanism, established in the studies of human and plant defensins, has been evolutionarily conserved in the crocodilian defensin CpoBD13. This study has also uncovered that CpoBD13's ability to bind PA and permeabilise fungal cell membranes is regulated by changes in pH, an ability which has not been observed in previous defensin studies.

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The identification of addiction to a human kinase inhibitor in Plasmodium falciparum

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Malaria parasites have become resistant to all current therapeutics, necessitating the development of novel treatment strategies. Host-Directed Therapy is a promising approach, as it deprives pathogens of the most direct pathway to resistance, namely the selection of genotypes encoding mutated targets under drug pressure. Previous studies have identified that Plasmodium falciparum relies on the activation of host erythrocyte protein kinases for its own proliferation and survival, in particular the mitogen-activated protein kinase kinase 1 (MAPKK1 or MEK1). Trametinib, a highly selective MEK1 inhibitor approved to treat melanoma, has shown inhibition of parasite proliferation in vitro with low nanomolar potency, consistent with the observation that MEK activity is required for parasite survival. Unexpectedly, P. falciparum can rapidly gain resistance to Trametinib, showing a 100-fold increase in the IC50. Fascinatingly, some of these parasites display not only resistance but also dependency to Trametinib, with optimal growth at a concentration of 200nM (10-fold above the parasites wildtype IC50). We have now shown that this dependency phenotype is lost rapidly following Trametinib removal, raising interesting questions regarding the molecular basis for this phenotype. This work provides novel insights into the complexity of host-pathogen interactions between human erythrocytes and P. falciparum.

The mechanisms of *P. aeruginosa* OMV biogenesis alters their cargo composition and biological functions.

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Outer membrane vesicles (OMVs) released by Gram-negative bacteria are nanoparticles that perform numerous bacterial functions contributing to bacterial survival. This includes their roles in predation and antimicrobial activity due to the bactericidal cargo contained within OMVs. The production of OMVs by bacteria occurs via two main mechanisms, the budding of OMVs from the cell surface during bacterial growth and the formation of OMVs after prophage induced explosive cell lysis. We previously determined that bacterial growth stage can affect the composition and biological functions of OMVs, however it is currently unknown whether the mechanism of OMV biogenesis can also determine OMV cargo composition and function. Therefore, in this study we examined whether the production of OMVs by budding or by explosive cell lysis could determine their cargo composition and their subsequent antimicrobial activity against Gram-negative and Gram-positive bacteria.

OMVs were isolated from three *Pseudomonas aeruginosa* strains, PAO1 the wild-type strain which produces OMVs by both budding and explosive cell lysis, PAO1Δ*lys* which produces OMVs by budding only, and PAO1Δ*lys* pJN105*lys* which produces OMVs predominately by explosive cell lysis. We compared the production and cargo composition of OMVs produced by all three *P. aeruginosa* strains to identify any changes due to their mechanism of biogenesis. Additionally, we determined that OMVs produced by explosive cell lysis could significantly inhibit *P. aeruginosa* growth whilst OMVs produced by budding from the cell membrane could not inhibit *P. aeruginosa* growth. However, OMVs could significantly inhibit the growth of *Staphylococcus aureus* irrespective of their mechanism of biogenesis. We are currently investigating the proteome of OMVs produced by the three *P. aeruginosa* strains to determine if their mechanism of biogenesis alters the packaging of bactericidal proteins into OMVs and therefore their composition.

Overall, these results suggest that the mechanisms of OMV biogenesis can determine their antimicrobial activity against *P. aeruginosa* and this may be due to changes in the packaging of bactericidal cargo into OMVs when produced by different mechanisms of biogenesis. Therefore, our data provides insight into how OMV biogenesis can regulate their cargo composition and subsequent biological functions.

Understanding natural immunity to pre-erythrocytic *P. vivax* proteins in a longitudinal cohort

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There is currently no licensed vaccine against *Plasmodium vivax,* the second most prevalent malaria parasite, despite global efforts. Further understanding of natural immunity to *P. vivax* is necessary to identify vaccine candidates. The aim of our current study was to assess natural immunity to novel proteins of the pre-erythrocytic stage of the parasite life cycle, which precedes the symptomatic erythrocytic stage. We measured total IgG antibody responses against 11 pre-erythrocytic *P. vivax* proteins in a cohort of 34 individuals with clinical *P. vivax* infections from Tha Song Yang, Thailand. Measurements were made at 8 timepoints across 8 months post-diagnosis, with no recurrent *P. vivax* infections during follow-up as determined by PCR. We observed increases in IgG antibody responses to all 11 proteins post infection, peaking 2 weeks post-diagnosis before decreasing and reaching the seronegative baseline by 12 weeks. There was a trend towards higher antibody responses in individuals with previous self-reported malaria infection across all timepoints. In addition, older individuals (≥15 years old) showed a significantly higher antibody response at all time points when compared to younger (<15 years old) individuals. High antibody responses were observed for all proteins in at least some individuals of the cohort, showing that immunity may develop variably for these pre-erythrocytic proteins following natural *P. vivax* infection. We also observed a tendency for individuals to show similar levels of immunity to the whole panel of pre-erythrocytic proteins, with high IgG antibody response to one protein being a strong predictor of antibody response to the others. Further investigation in a larger cohort will reveal more insights into natural immunity against these pre-erythrocytic proteins. These findings will aid in the search for novel vaccine targets for *P. vivax*.

To vaccinate or not to vaccinate? COVID-19 vaccine intentions amongst priority groups in Victoria

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To optimise uptake of COVID-19 vaccines, communication strategies must address the information needs and concerns of groups prioritised to receive and deliver vaccination, including healthcare workers, older adults and adults with comorbidities. This survey-based study aimed to understand the vaccine intentions and informational needs of people initially prioritised for COVID-19 vaccination in Victoria.

This project was part of a mixed-methods study supported by the Victorian Government. An online survey was completed by Victorian adults who were either 1) a healthcare or aged/disability care worker ("healthcare workers"), or 2) aged ≥70 years or 18-69 years with comorbidities ("prioritised public"). Using custom items and items adapted from the World Health Organization Behavioural and Social Drivers of Immunisation COVID-19 vaccine survey, we assessed intention to vaccinate, information needs, and behavioural drivers of COVID-19 vaccine uptake. Descriptive statistics and relative risk measures were used to identify associations between intention to vaccinate, vaccine confidence and demographic variables.

A total of 2588 healthcare workers and 1975 prioritised public members completed the survey from February-March 2021, during the initial phases of the vaccine rollout. We found that 78% of healthcare workers intended to receive a COVID-19 vaccine, with highest intention amongst medical doctors (94%, n=174) and lowest amongst personal support workers (58%, n=69). Intention among nurses was 77% (n=2173). Intention to vaccinate among people aged ≥70 was 90% (n=920) and among people aged 18-69 with comorbidities 84% (n=908). Predominant concerns were related to vaccine safety, side effects and efficacy. The most trusted sources of information were medical professionals and scientists/researchers.

Findings from the overall mixed-methods study were used to develop a set of communication recommendations to support the COVID-19 vaccine rollout in Victoria.

Association of prenatal antibiotics and mode of birth with otolaryngology surgery in offspring: A national data linkage study

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Publish consent withheld

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Changes in infection-related hospitalizations in children following pandemic restrictions: an interrupted time-series analysis of total population data

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Infectious diseases are a leading cause of hospitalization during childhood. The various mitigation strategies implemented to control the coronavirus disease (COVID-19) pandemic could have additional, unintended benefits for limiting the spread of other infectious diseases and their associated burden on the health care system.

We conducted an interrupted time-series analysis using population-wide hospitalization data for the state of Victoria, Australia. Infection-related hospitalizations for children and adolescents (aged <18 years, total source population ~1.4 million) were extracted using pre-defined International Classification of Diseases 10th Revision Australian Modification (ICD-10-AM) codes. The change in weekly hospitalization rates (incidence rate ratio, IRR) for all infections following the introduction of pandemic-related restrictions from 15 March 2020 was estimated.

Over 2015–19, the mean annual incidence of hospitalization with infection among children less than 18 years was 37 per 1000 population. There was an estimated 65% (95% CI 62-67%) reduction in the incidence of overall infection-related hospitalizations associated with the introduction of pandemic restrictions. The reduction was most marked in younger children (at least 66% in those less than 5 years of age) and for lower respiratory tract infections (relative reduction 85%, 95% CI 85-86%).

The wider impacts of pandemic mitigation strategies on non-COVID-19 infection-related hospitalizations are not fully understood. We observed marked and rapid decreases in hospitalized childhood infection. In tandem with broader consequences, sustainable measures, such as improved hand hygiene, could reduce the burden of severe childhood infection post-pandemic and the social and economic costs of hospitalization.

Dynamics of gut-resident lymphocytes

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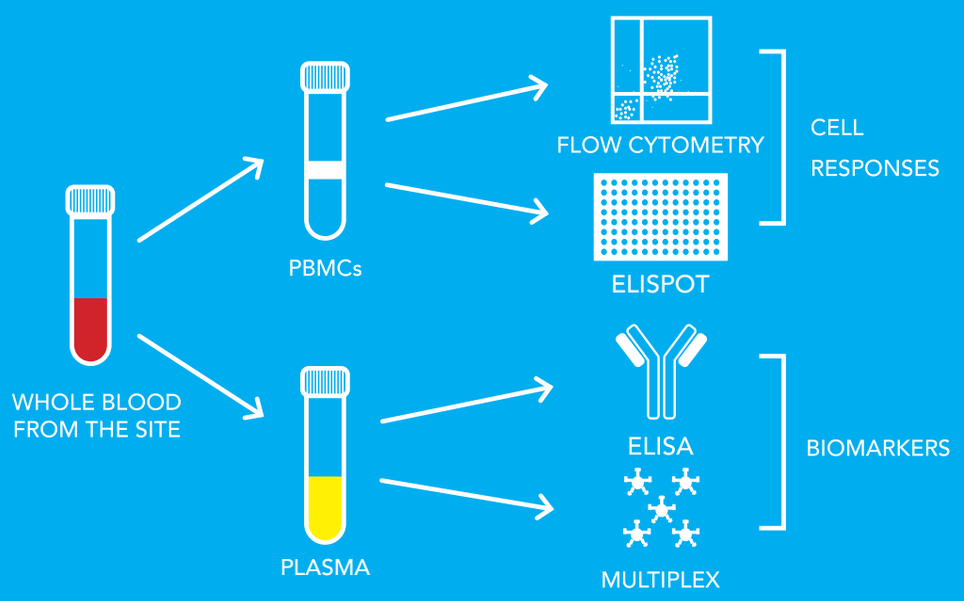
Tissue-resident memory T (T_{RM}) cells are noncirculating lymphocytes that mediate frontline immune defense in barrier tissues such as the skin or intestine. $CD8^+$ T_{RM} cells reside within the epithelial layer of these tissues and can be exposed to an array of inflammatory stimuli across their lifespan. While $CD8^+$ T_{RM} cells have been shown to be highly durable within the skin niche, those within the small intestine intraepithelial layer (SI-IEL) appear to decline following chronic stimulation or heightened microbial exposure. However, the mechanisms regulating T_{RM} cell decay are not known, nor whether T_{RM} cell depletion is a permanent phenomenon or if circulating precursor T cells can replenish the intestinal niche. Using a depletion method that is specific to tissue-resident lymphocytes in various organs, we show that antigen-specific $CD8^+$ SI-IEL T_{RM} are permanently lost from the intestinal niche, while other resident lymphocytes such as $TCR\gamma\delta^+$ and $CD8\alpha\alpha^+$ cells locally proliferate to original numbers. Using CRISPR-editing tools, we further demonstrate that the loss of bystander $CD8^+$ SI-IEL T_{RM} after chronic infection can be attributed to their recognition of danger signals within tissue environments. These findings indicate that durability of $CD8^+$ T_{RM} cells in the intestine can be affected during tissue adaptations and understanding how T_{RM} precursor cells can replenish the niche is crucial for mucosal vaccine design to establish long-lasting immune memory.

ABSTRACTS
SESSION 4

THE ENTIRE CRUX WORKFLOW
FOR YOUR PD/BIOMARKER REQUIREMENTS

Crux Biolabs



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The good, the bad and the ugly: The functional IgA response in convalescent COVID-19 patients

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Mutations in the receptor binding domain (RBD) (e.g.N501Y) of SARS-CoV2 has resulted in emergence of variants of concern (alpha). Following infection, virus-specific antibodies are generated that can neutralise and clear the virus via Fc effector functions (phagocytosis). The importance of IgG antibodies for protection and control of SARS-CoV2 has been extensively reported. In comparison, other antibody isotypes including IgA have been poorly characterized, especially to variants. Here we endeavoured to determine the functional contribution of plasma IgA from convalescent COVID-19 subjects.

IgA and IgG was purified from the plasma of convalescent COVID-19 patients (n=58) and healthy controls (n=25). IgA and IgA+IgG depleted plasma fractions were also collected. SARS-CoV2-specific antibody responses were characterized via multiplex assay. Neutralization was assessed via a multiplex ACE2-RBD binding inhibition assay. Samples were also characterized for their Fc functional capacity using a THP-1 cell bead-based phagocytosis assay and a cell association assay. Multivariate analysis was used to compare purified antibody binding to different RBD mutants.

Convalescent patients induced SARS-CoV2-specific IgG (100%) and IgA (91.38%) with 85.19% of patients able to inhibit ACE2-RBD binding. IgA depletion from plasma significantly increased neutralization (median=62.12%, $p=0.0013$) compared to plasma (median=39.62%). Interestingly, purified IgG and IgA exhibited differential antibody binding to 15 RBD mutants e.g. alpha (N501Y) ($p<0.05$) and neutralization to 5 mutants ($p<0.05$). Finally, IgA depletion resulted in similar Fc function as plasma, however, IgA+IgG depletion drastically reduced the phagocytosis ($p<0.0001$) and cell association ($p<0.0001$) compared to plasma.

We show SARS-CoV2-specific IgA responses are induced in most convalescent COVID-19 individuals, with negligible Fc functional capacity in comparison to IgG. Furthermore, potent IgA neutralisation was observed within a small subset of these individuals. Surprisingly, depletion of IgA from plasma increased neutralizing capacity of plasma in certain individuals, suggesting that IgA may block the binding of other neutralising antibody isotypes. Notably, purified IgG and IgA displayed differential binding to RBD mutants, such as alpha variant, suggesting convalescent antibody class switching could impact the capacity of plasma to neutralise different COVID variants. Understanding the mechanisms behind IgA neutralization and IgA mediated blocking is warranted to provide insights for improved vaccination and antibody therapies.

High frequency human MLKL mutation causes innate immune response defects and hematopoietic dysfunction in CRISPR-cas9 generated mouse model.

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Programmed cell death has long been implicated in the progression of human disease. Recently, there has been a major focus on the inflammatory lytic form of programmed cell death, necroptosis, in human pathogen responses. Necroptotic signaling is mediated by the terminal executioner protein, pseudokinase mixed lineage kinase domain-like (MLKL), and its upstream activating kinase, receptor interacting protein kinase 3 (RIPK3). These integral necroptotic proteins have been comprehensively revealed as potent drivers and suppressors of human disease in pre-clinical murine models. The investigation of MLKL's role in human disease within the 'real world' of diverse environmental challenges and genetic backgrounds is limited. Here, we present investigations of a high frequency missense polymorphism in human MLKL that is carried by 2-3% of the general population. This gene variant encodes a serine to proline substitution at position 132 within MLKL's regulatory brace region and is enriched in trans with similar MLKL polymorphisms in a cohort of Chronic Recurrent Multifocal Osteomyelitis patients. Primary patient cells heterozygous for *MLKL*^{S132P} and exogenous expression systems in immortalized cell lines were examined for their response to necroptotic and inflammatory stimuli *in vitro.* To study the potential disease modulating effects of MLKL^{S132P}, on a systemic level, we have generated a mouse model that expressed the mouse equivalent variant, *Mlkl*^{S131P}. *Mlkl*$^{S131P/S131P}$ mice exhibit innate immune cell defects in the bone marrow at steady state. Following challenge with *Salmonella*, Mlkl$^{S131P/S131P}$ mice exhibited impeded bacterial clearance and innate immune cell defects in peripheral blood. Furthermore, *Mlkl*$^{S131P/S131P}$ mice were susceptible to bone marrow failure characterized by hematopoietic dysfunction following sublethal irradiation or transplantation. Our work highlights that this human MLKL polymorphism may be an important modulator of disease progression under everyday environmental challenge and raises important questions about the historical circumstances that have led to its high frequency.

Durability of B-cell memory to SARS-CoV-2 infection and vaccination

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Background: Lasting immunity following SARS-CoV-2 infection or vaccination is questioned because serum antibodies decline in convalescence. However, functional immunity is mediated by long-lived memory T and B (Bmem) cells, which we hypothesise are more accurate markers of long-term immunity.
Objective: To determine the immunophenotype and durability of SARS-CoV-2-specific Bmem cells in individuals after infection or vaccination for SARS-CoV-2.
Methods: Recombinant Spike receptor binding domain (RBD) and Nucleocapsid (NCP) proteins were produced for ELISA-based serology, and biotinylated for fluorescent tetramer formation to identify SARS-CoV-2-specific Bmem cells by flow cytometry. Cells were obtained from 29 convalescent COVID-19 patients and repeat samples were taken from individuals up to one-year post-infection. In addition, samples were collected from healthy adults immunised with the Pfizer mRNA (n=30) and AstraZeneca vector (n=37) SARS-CoV-2 vaccines at three time points: pre-vaccination, 1-month post-prime and 1-month post-boost.
Results: All recovered COVID-19 patients had serum IgG that specifically recognised recombinant RBD and NCP proteins, with levels declining beyond 20 days post-infection. Vaccination induced anti-RBD antibodies, which were increased after boost, whereas no anti-NCP antibodies were formed. In recovered COVID-19 patients, RBD- and NCP-specific Bmem cell numbers peaked after 50 days and remained stable at 1.25-170 cells/ml of blood (0.008-0.1% of total B cells) in all patients for >240 days post-infection. RBD- and NCP-specific Bmem cells predominantly expressed IgM or IgG1. Individuals immunized with the Pfizer mRNA vaccination generated RBD-specific Bmem cells ranging from 16-85.4 cells/ml blood 1-month post-boost and also predominantly expressed IgM or IgG1.
Conclusion: Detailed immune profiling revealed durable RBD- and NCP-specific Bmem cells in COVID-19 convalescent individuals. We will now quantify the serological and antigen-specific Bmem cell response in individuals immunized with AstraZeneca vector vaccination. This will allow us to compare the generation of durable immunological memory between natural infection and vaccination, as well as between mRNA and vector-based SARS-CoV-2 vaccinations. This could inform on the need for future booster vaccinations and levels of protection to emerging variants of concern.

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The value of antibody avidity in malaria vaccine responses

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Malaria remains a major global health burden and, with increasing drug resistance in parasites and insecticide resistance in their mosquito vector, an effective vaccine that induces long-term immunity is critical. But in recent Phase III clinical trials, the most advanced malaria vaccine candidates induced only short-lived immunity. The development of vaccines capable of eliciting long-lasting protection is impeded by an absence of clear immune markers that signify a sustained response. While antibodies are crucial in the immune response to malaria, a high magnitude following vaccination guarantees neither clinical protection nor persistent antibody levels. Therefore, antibody characteristics and functions must be further investigated to identify properties that are associated with long-lasting protection and can be targeted in ongoing vaccine development. The binding strength, or avidity, of antibodies is a valuable serological marker in many diseases but in malaria it has shown inconsistent association with parasite exposure and protection. The role of high avidity antibodies has been further obscured by highly variable experimental designs. In this work, the avidity of antibodies targeting key malaria vaccine antigens was considered in relation to antibody function and maintenance. Avidity was examined using multiple experimental approaches in samples from malaria-naïve adults as well as young children residing in malaria endemic regions. In these cohorts, high antibody avidity did not improve functional properties that play a role in mediating immunity, such as the ability to bind complement proteins or Fcγ-receptors, but high avidity was associated with greater persistence of IgG over time. The association between high antibody avidity and maintenance was supported by observations in mouse models comparing various malaria vaccine formulations. This work suggests that the presence of high avidity antibodies after vaccination may be a useful marker of a persistent antibody response and may support the development and testing of improved malaria vaccines.

Molecular and functional mechanisms underlying age-related changes in influenza virus-specific CD8$^+$ T-cells across human lifespan

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Influenza viruses remain a constant global threat, causing significant morbidity and mortality. Although age is the major factor in determining disease duration and outcome during seasonal epidemic and pandemic outbreaks, the underlying mechanisms that drive age-related changes and disease severity are not well understood. A robust CD8$^+$ T-cell response plays a key role in protection against novel influenza virus strains and subtypes. CD8$^+$ T-cell receptors (TCRs) can recognize conserved influenza proteins, resulting in broad cross-reactivity across distinct influenza viruses. This makes them an attractive target for universal influenza vaccine strategies. As memory CD8$^+$ T-cells gradually change throughout human lifetime, we investigated how TCR composition and diversity relate to CD8$^+$ T-cell responses across immunologically-distinct phases of human life.

We combined *ex vivo* detection of influenza-specific CD8$^+$ T cells using peptide-HLA tetramers with single-cell multiplex-nested RT-PCR to analyse paired TCR$\alpha\beta$ clonotypes directed against the most prominent human influenza epitope, HLA-A*02:01-M1$_{58\text{-}66}$ (A2$^+$M1$_{58}$) in cord blood, children, adults and elderly individuals. We linked the TCR clonotype dynamics across different ages to the magnitude and phenotype of the A2$^+$M1$_{58}$-specific CD8$^+$ T-cells.

Our data show that frequency and phenotype of the A2$^+$M1$_{58}^+$-specific CD8$^+$ T-cells changes across human lifetime. Furthermore, the A2$^+$M1$_{58}^+$-specific TCR$\alpha\beta$ clonotypes in children and adults differ to those in cord blood and elderly. The optimal TCR$\alpha\beta$ repertoire found in children and adults is dominated by the public TRAV27-TRBV19 clonotype, which is absent in cord blood and is replaced by a private TCR$\alpha\beta$ signature which are clonally expanded and include broader usage of TRAV-TRBV gene segments with shorter and/or longer CDR3-loops in the elderly.

Overall, our study indicates that the changes in frequency and phenotype of the influenza virus-specific CD8$^+$ T-cells go hand-in-hand with changes in the TCR$\alpha\beta$ clonal composition, which together affect the overall strength of the virus-specific CD8 T-cells. These findings suggest that priming T-cell compartments at different stages of life may influence the clonal composition and diversity of responding TCR repertoires against viral infections.

Design of an Australian Facilities Survey on Management of Polioviruses and potentially infectious materials (PIMs)

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Poliovirus is the longest ongoing Public-Health Emergency of International Concern since 2014. The WHO had launched the Global Polio Eradication Initiative (GPEI) in 1988. Under its containment stage, after being certified as polio-free, every country needs to survey all its laboratories to check for presence of potentially infectious materials (PIMs). This paper identifies hindrances to GPEI and success factors for containment. Key Informant Interviews were conducted with Malaysia and Singapore, who have completed their PIM survey, to draw on good practices and challenges. Canada, UK and US were also consulted as their survey was ongoing/planned. The research revealed that worldwide, PIM containment survey processes are widely varied and complicated for even high-income countries.

Containment faces further hurdles in humanitarian situations; children administered with the Oral Polio Vaccine (OPV) excrete live attenuated virus for months, spread through the oral-faecal route. Rarely, the virus reverts to the paralytic form called circulating vaccine-derived poliovirus (cVDPV). Of the 25 countries with cVDPV outbreaks in the start of 2021[1], 18 were classified as humanitarian situations[2]. Additionally, Pakistan and Afghanistan also have wild polio cases. Therefore, improving vaccination coverage and WASH (water, sanitation and hygiene) are crucial. However, violence prevents polio workers from accessing vulnerable children in humanitarian situations, hindering PIM surveys.

Since March 2020, efficient and timely execution of PIM surveys has been further hampered by the COVID-19 pandemic, which caused many countries including the UK to postpone or suspend their ongoing polio management programs, and OPV administration services[3]. For instance, the WHO polio program in Africa has diverted 60-70% of its resources towards COVID-19 response[4].

Therefore, this paper calls for and provides a simplified template for PIM surveys worldwide. We suggest their execution should be a joint public health initiative between the WHO, local health clinics and NGOs, linking global public health to the humanitarian sector as a “transformative way of working”.

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P3-Mumbubvax intervention adaptation for general practitioners: a qualitative interview study

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Background

During pregnancy, expectant parents make key decisions about vaccines. The main driver of maternal vaccine uptake is provider recommendation. General practitioners are usually the first point of contact and highly accessed sources of vaccine information for pregnant women. However, current maternal vaccination coverage for influenza and pertussis is sub-optimal.

A multicomponent intervention package (P3-MumBubVax) has been designed for midwives, but interventions to support GPs' vaccine discussions are limited. This qualitative study explored Australian GPs' attitudes, practices and educational needs to inform adaptation of the P3-MumBubVax intervention for primary care.

Method

We conducted semi-structured interviews with 30 GPs to explore their attitudes towards recommending maternal vaccines, vaccine communication approaches and training preferences. Data were analysed using thematic template analysis to inform intervention design.

Results

Participants saw advising pregnant women about maternal vaccines as a very important feature. Participants emphasized that concerned pregnant women were generally apprehensive of vaccine safety and potential side effects. Maternal vaccines were discussed using several discussion techniques such as sharing information, presumptive communication, sharing personal experiences and involving the partner. GPs preferred convenient, interactive training with examples and up-to-date maternal vaccine resources.

Discussion

Vaccination was central to the GP's role and built rapport led to additional opportunities to discuss maternal vaccines by use of several discussion techniques. GPs preferred convenient training opportunities and highlighted the need for consistent maternal vaccine resources.

The findings from this study were used to adapt the P3-MumBubVax intervention. It offers GPs tailored vaccine resources, online communication training and interactive quizzes for individual or group learning.

Antibody responses to *P. falciparum* transmission-stage antigens in participants following a human experimental malaria infection study

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Background: Malaria is a devastating disease caused by the *Plasmodium* parasite, infecting over 200 million people each year. Despite the advancement of the RTS,S pre-erythrocytic vaccine to Phase IV clinical trials, the vaccine efficacy declined rapidly, rendering it unsuitable to achieve sustained malaria elimination. Transmission-blocking vaccines aim to induce functional antibodies within the human host to transmission-stage parasites to reduce malaria transmission throughout a population. However, transmission-stage immunity in humans remains poorly understood, impeding transmission-blocking vaccine progression.

Materials/Methods: This study utilised serum samples and clinical data collected from a human experimental malaria infection study uniquely designed to understand transmission-stage immunity in malaria-naïve participants. Participant serum samples at multiple time points were measured for antibody responses to key transmission-stage antigens, including functional antibodies that bind to Fc-gamma receptors and fix complement C1q. Antibodies were then correlated to clinical data in the study that measured parasite transmission and replication.

Results/Implications: Study participants induced high IgG, IgM and IgG2 responses to transmission-stage antigens. However, there were minimal cytophilic IgG1 and IgG3 subclasses detected, resulting in minimal functional antibody responses. Unfortunately, correlations between antibodies and clinical data did not achieve statistical significance. These findings suggest that despite a high magnitude of antibodies induced to transmission-stage antigens, these were not associated with reducing parasite transmission and replication. This pilot study design provided insights for future studies that can further our understanding of transmission-stage immunity and consequently contribute to effective transmission-blocking vaccine development to achieve sustained malaria elimination.

Functional and transcriptional differences in monocytes from children with obesity compared to children of healthy weight

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Background and Aim – Cardiometabolic risk accrues across the entire life course and childhood is a key epoch for effective prevention. Obesity in childhood is the most prevalent modifiable risk factor for later cardiovascular disease (CVD). Inflammatory biomarkers and capacity are increased in adults with obesity, but childhood data are scarce. We aimed to investigate (i) innate immune cell activation in children with and without obesity ; and (ii) whether weight loss impacts the innate immune inflammatory phenotype.

Methods - The innate immune phenotype of PBMCs from 33 children with obesity (BMI z-score>2.5) and 22 children of healthy weight (-1.5<BMIz<1.5, sex, age and pubertal stage matched) was characterized by high dimensional flow cytometry, ex vivo stimulation assays with subsequent 27-plex cytokine measurements, and transcriptome analysis using RNA sequencing. Children with obesity participated to the Royal Children's Hospital Weight Management Service (median 5 years) and at follow-up, PBMCs were obtained again as well as anthropometric data and subclinical cardiovascular phenotypes.

Results - Flow cytometric analysis showed marked differences in cell composition between children with obesity and children of healthy weight. Specifically, children with obesity have significant changes in B cells, NK cells and monocyte subsets, including increased expression of monocyte activation markers and an increased cytokine production capacity. Transcriptomic analysis of monocytes showed upregulation of immunometabolic pathways and downregulation of viral effector pathways. Effects of weight loss on these immune parameters and correlations with preclinical CVD phenotypes are currently being analysed.

Conclusions - Monocytes from children with obesity have a pro-inflammatory phenotype compared to children of normal weight indicative of a trained immune phenotype. Heightened inflammation may contribute to increased CVD risk later in life and may offer opportunities for early intervention.

ABSTRACTS
SESSION 6

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The emerging role of lipid droplets acting as modulators for innate immune signalling

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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, however, their role in innate immune pathways, and the antiviral response remains largely unknown.

Work by our lab has demonstrated LDs are upregulated during viral infection, and that this upregulation contributes to an enhanced interferon response from the infected cell, indicating for the first time that the LD contributes to an effective immune response, however the mechanism of this is unknown. Here, we describe for the first time that there are several critical key antiviral signalling molecules that localise to the LD during this response. We have optimised techniques to isolate pure lipid droplets from primary immortalised astrocyte cells before and following activation of viral RNA signalling pathways. Proteomic analysis has revealed there was 83 significantly upregulated proteins on LDs following stimulation with 10% of the significantly enriched proteins being associated with the interferon response. Of these, MX1, RIG-I, STAT1 and STAT2 were significantly upregulated on LD fractions at both 8 and 24hrs following RNA viral mimic stimulation. As many significantly upregulated proteins identified on the LD are currently labelled as cytoplasmic proteins, further work was required to validate their interaction with LDs. To confirm the localisation of these signalling proteins to the LD, a technique was designed to perform fluorescent confocal microscopy on isolated fluorescently stained lipid droplets to probe for the identified immune proteins; and this, along with western blotting, has confirmed the localisation of these proteins to LDs.

Here, we demonstrate that there are important antiviral immune signalling proteins that localise to the LD following viral mimic stimulation, perhaps indicating that the LD can act as a signalling platform for signalosome formation to aid host immunity. The mechanism by which these proteins localise to the LD and the function of this is still being explored by our laboratory.

Variations in microRNA length: A new source of disease biomarkers?

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MicroRNAs (miRNAs) were originally posited to offer a new suite of disease biomarkers due to their ubiquitous expression in biofluids. To date, their utility in this regard has, however, been limited by a redundancy of dysregulated miRNAs in disease, and problems with miRNA normalisation. In this work, we reasoned that considering miRNA length variants could significantly increase their repertoire by 5-10 fold, to increase disease specificity and their use as biomarkers.

Previous research by our laboratory demonstrated that miRNA length variations can be observed during cell infections (Nejad et al. 2018). In the current work, we investigated whether such length variations could be used to distinguish between different cell types and cell treatments. Relying on bioinformatic analyses of public datasets, we demonstrated that variation of miRNA length can also be observed in response to various stimuli, independent of infections. Analyses of miRNA length variations between 7 blood cell subsets in a cohort of 43 patients confirmed the robustness of these variations between cell types, and across patients, supporting the rationale that these variations are related to biological function. We are currently working to confirm their utility as biomarkers of cellular responses in large cohorts of patients, while establishing a broad atlas of miRNA variations, with the objective to define disease-specific length variations. This research has the potential to revolutionise the use of miRNAs as disease biomarkers.

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Cyclopropyl amide antimalarials act by disrupting *Plasmodium falciparum* pyrimidine metabolism

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The spread of *P. falciparum* parasites resistant to all available antimalarial therapies is threatening the future treatment of malaria. Therefore, the discovery of new antimalarial drugs with novel mechanisms of action is vital. To aid this discovery, Medicines for Malaria Venture have started Libre, an open source drug discovery program, and have identified several new series of compounds for development. One of these series is the cyclopropyl amide aryl piperazines. These compounds display promising blood and liver stage activity and low toxicity against mammalian cells, but lack information about their mode of action, which would help guide their future development. Pulsed exposure assays showed the cyclopropyls have a slow rate of killing (>5 h) and are most active against trophozoite stage parasites. Our untargeted metabolomics studies were conducted on MACS purified mid trophozoite stage parasites that were incubated with the cyclopropyls MMV1804508 (IC50 = 225 nM) or MMV1804742 (IC50 = 835 nM) for 5 h at 5x IC50. The inactive cyclopropyl, MMV1803903, and atovaquone were also included as controls. This untargeted analysis revealed that the active cyclopropyls perturbed pyrimidine biosynthesis, resulting in a significant ($p<0.05$) increase in carbamoyl aspartate (81-fold) and dihydroorotate (25-fold) and depletion of downstream pyrimidines, such as UMP (0.5-fold) compared to untreated parasites. This metabolic signature is similar to that seen for atovaquone, a known parasite cytochrome bc1 inhibitor, indicating a similar mode of action for cyclopropyls and atovaquone. We further confirmed this mechanism in cross resistance studies with the atovaquone resistant parasite line, SB1-A6, which was completely resistant to both cyclopropyls tested (IC50 > 10 μM). Taken together, these data indicate that the cyclopropyl series act by inhibiting the parasite mitochondrial bc1 complex. Future development of this series will be guided by these findings.

Influenza infection of human macrophages

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Attachment and entry of influenza A virus (IAV) into epithelial cells, a primary target for infection in the respiratory tract, is well characterised. However, infectious entry of IAV into airway macrophages, the first immune cells to respond to respiratory pathogens, is not clearly defined. In epithelial cells and macrophages, IAV hemagglutinin (HA) mediates virion attachment to the cell surface through recognition of sialic acid expressed on glycoproteins and glycolipids. Macrophages also express pattern recognition receptors, including C-type lectin receptors (CLR), which recognise microbial carbohydrate structures. Our laboratory has shown that IAV utilises specific CLRs, such as the mannose receptor (MMR), galactose-type lectin (MGL) and Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN), for infectious entry of IAV into macrophages.

Herein, we investigated the role of CLRs and sialic acid in mediating IAV infection of primary human macrophages. We showed that monocyte-derived macrophages (MDMΦ) isolated from peripheral blood express MMR and stimulation with IL-4 resulted in upregulation of DC-SIGN, but not MGL. We observed a slight increase in IAV infection of IL-4 stimulated compared to unstimulated MDMΦ, using H1N1 and H3N2 representative strains. In addition, blockade of mannose-specific CLRs (MMR and DC-SIGN) by pre-treatment with mannan only partially reduced IAV infection. This suggests the presence of galactose-specific CLR or CLR-independent entry pathways for IAV during infection of MDMΦ. To dissect out the relative contribution of CLRs and sialic acid for IAV infectious entry into human macrophages, we engineered IAV variants with (i) varying numbers of glycosylation sites on the HA head and (ii) a preference for sialic acid expressed in either a α2,3- or α2,6-linked conformation. Modulation of receptor preference for α2,3- or α2,6-linked sialic acid did not alter the ability of the respective IAV variant to infect MDMΦ. Intriguingly, the addition of two glycosylation sites to the HA head of PR8, led to a decrease in infection of human MDMΦ. This is in contrast to findings in murine macrophages, where additional glycosylation confers increased infectivity to the PR8 strain. It is possible that glycans near the receptor binding site influence sialic acid binding strength of the HA. Future work will implement these techniques to elucidate the role of CLRs and sialic acid during IAV infection of human alveolar macrophages (AMΦ).

Single-cell analysis of αβ versus γδ T cell development

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T cells develop in the thymus, where they acquire a range of distinct functional identities. T cells are divided into the αβ or γδ lineages. Progenitors that enter the thymus remain multipotent, with the potential to differentiate into a range of cell types. T lineage identity is then fixed early in T cell development. Later, γδ T cells branch off, while αβ development continues onto additional stages. Studies over the years have defined, at a population level, many of the specific genes that have to be activated or silenced as T cell progenitors differentiate toward either lineage. However, it is still unknown whether this combination of activation/silencing actually occurs in each and every cell as they differentiate. To investigate this, single-cell RNA-sequencing (scRNA-seq) was employed to re-assemble *de novo* a model of the early stages in T cell development based on the transcriptional profiles of individual cells. >20,000 CD4-CD8- double negative (DN) and γδ thymocytes were analysed using Chromium 10x scRNA-seq over three runs. Hierarchical clustering revealed that early thymocyte populations are much more complex than the standard view of T cell development. Assembly of these thymocyte populations into developmental trajectories based on gene expression suggested that the decision to differentiate into αβ versus γδ T cells occurs at a much earlier stage than the current model and that distinct DN1 and DN2 sub-populations are specific to either the αβ or γδ developmental pathways. To confirm that specific sub-populations are restricted to either αβ or γδ lineages, they were sorted and analysed for lineage outcomes in OP9-DL1 cultures. We show that lineage is already restricted in DN1 thymocytes, which is considered the earliest stages of T cell development. Thus, γδ developmental appears to occur in a parallel with αβ development and not a branch as previously thought.

Evading the host immune response in neuronal grafts using immune-cloaked human stem cell-derived midbrain dopamine progenitors

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Clinical trials involving the transplantation of dopaminergic (DA) progenitors into the brain of Parkinson's Disease (PD) patients requires prolonged, systemic delivery of immune-suppressive drugs to prevent graft rejection. An alternative approach is use donor cells that will avoid detection by the host immune system. While the use of a patient's own stem cells or allogenic (HLA-matched) cells is optimal, it remains largely unfeasible due to high costs, necessary QC and associated labor (Lanza et al., 2019). To circumvent such conundrum, we propose to use an 'immune-cloaked' pluripotent stem cell (PSC) line – capable of evading detection by the host system. A mouse and a human 'immune-cloaked' PSC line were created by forced expression of 8 immunomodulatory genes (CCL21, PDL1, FASL, HLA-G, Serpinb9, CD47, CD200, MFGE8) that interfere with the activity of several immune cell types, including T-cells, NK-cells, antigen presenting cells and macrophages (Harding et al., 2019). Subcutaneous grafts of these mouse 'immune-cloaked' PSC were shown to generate ectopic tissues in HLA-mismatch hosts and evade long term immune detection (Harding et al., 2019). To date, only the efficiency of mouse 'immune-cloaked' cells has been demonstrated in healthy animals and therefore assessment of the therapeutic potential of human cloaked cells in healthy and disease models is needed. Moreover, while mouse cloaked cells can differentiate efficiently into cardiomyocytes in vivo (Harding et al., 2019), the capacity of human cloaked cells to generate different cell types has not been investigated. Advancing on these findings, we are now examining the capacity of human cloaked cells to (1) be differentiated into bona fide ventral midbrain DA neurons in vitro, (2) promote functional recovery following transplantation into an athymic rat model of PD (to demonstrate the functionality of the donor cells) and (3) evade immune detection in a humanised mouse model of PD (to confirm efficiency of the cloaking).

Defining the mechanisms of action of antibodies against Plasmodium vivax malaria

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Effective vaccines against both *Plasmodium falciparum* and *P. vivax* will be essential to achieve malaria elimination goals globally. There have been great strides in developing *P. falciparum* vaccines, substantially less progress has been made for developing *P. vivax* vaccines. This is partly due to the lack of knowledge of key immune functions that are naturally acquired to *P. vivax* infection. Antibodies play an essential role in malaria immunity and can target antigens to activate the complement system, mediate binding to Fcγ-receptors expressed by immune cells and promote opsonic phagocytosis. However, currently we have limited knowledge on functional activities of acquired antibodies to *P. vivax*.

Here, we quantified functional mechanisms of antibodies to *P. vivax* acquired from natural exposure. We evaluated a cohort of children (n=37; ages 5-14), resident in a malaria-endemic region of PNG, for antibody responses to 4 *P. vivax* antigens - AMA1, MSP3α and MSP1-19, and the two allelic forms of CSP. Specifically, we quantified the acquisition of IgG and cytophilic subclasses, measured the ability of antibodies to mediate complement fixation and Fcγ-receptor binding (types I, IIa and IIIa). We additionally used an established flow cytometry-based method to detect antibody-mediated opsonic phagocytosis of antigen coated beads by THP-1 monocyte cells.

Children poorly acquired antibodies with complement fixation activity to all antigens tested, whereas antibodies to MSP3α and AMA1 antigens were able to bind to Fcγ receptors. Substantial opsonic phagocytosis activity with THP-1 cells was only observed with MSP3α coated beads. Limited functional activity to the other antigens may be explained by the lower acquisition in this cohort of IgG cytophilic subclasses, IgG1 and IgG3, which were correlated with functional antibody responses. Further studies will investigate these responses in other cohorts, the neutrophil-mediated phagocytosis and other antigen targets of functional antibodies.

Our findings suggest that antibodies to *P. vivax* antigens can mediate a range of effector functions, including opsonic phagocytosis, and acquired functional antibodies target some antigens more effectively. This contributes to our knowledge of *P. vivax* immunity and will be valuable for the development of effective vaccines.

Profiling the antibody response 6 years following reduced-dose quadrivalent HPV vaccination in adolescent Fijian girls

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Prophylactic human papillomavirus (HPV) vaccines protect against persistent HPV infection, cervical pre-cancerous lesions and cancer. Although a two-dose schedule separated by 6 months is currently recommended for boys and girls under the age of 15, emerging data suggest that a single dose of the bivalent (2vHPV) or quadrivalent (4vHPV) HPV vaccine generates similarly protective antibody responses. Neutralising antibodies induced by vaccination are thought to be the primary mechanism of protection against infection. However, previous studies in animal and *in vitro* models suggest that there may be additional mechanisms of antibody-mediated protection apart from neutralisation, particularly at low antibody concentrations. This includes Fragment crystallizable-mediated effector functions such as opsonophagocytosis, cellular cytotoxicity and immune cell degranulation. At present, very little is known about the characteristics of the antibody response following reduced-dose HPV vaccination.

In this study, we will examine the antibody profile of girls who previously received 1 or 2 doses of 4vHPV 6 years earlier, compared to those who received 3 doses. In 2015, a prospective cohort study of 200 Fijian girls (aged 15-19 years at the commencement of the study) previously vaccinated with 0, 1, 2 or 3 doses was conducted, with equal numbers of girls in each dose group. Blood was taken before and 28 days following a single dose of 2vHPV. To measure antibody subclasses (total IgG, IgG2, IgG3, IgG4, IgA1, IgA2 and IgM) specific to oncogenic HPV genotypes (HPV16, 18, 31, 33, 45, 52 and 58) in serum, a multiplex microsphere-based immunoassay was employed. Preliminary analyses of a subset of the study samples (N=80; 20/group) show significant increases in total IgG, IgG1 and IgG3 to all HPV genotypes post-2vHPV. Further analyses are currently ongoing to measure HPV-specific IgM, IgA1, IgA2, IgG2 and IgG4 before stratification by dose group. Profiling the antibody response to HPV vaccination will aid our understanding of the immunological mechanisms of the prophylactic HPV vaccines and provide an explanation for the robust protection offered by single-dose vaccination.

Investigating the antimalarial activity of novel heterospirocyclic compounds in drug-resistant parasites

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Malaria remains a significant global health burden and a leading contributor to global mortality rates, particularly in Africa and South-East Asia. Despite its prevalence, an efficient vaccine has yet to be developed. The rise of antimalarial drug resistance and plateau in new therapeutic trials, create an urgent demand for new treatment options. A focus on novel antimalarial drugs with underrepresented chemical scaffolds is vital for antimalarial development and to combat drug resistance. We have synthesised a new class of 3D-spiroheterocycle compounds with chemical connectivities never previously synthesised or explored. We hypothesise the unique and pronounced 3D architecture of these molecules allows them to interact with biological domains otherwise inaccessible to relatively flat structures, leading to increased efficiency. We have previously shown that two spirocyclic compounds (C25 and C26) induce death of the most lethal malaria parasite *P. falciparum,* within 48hrs and present IC_{50} values in the low micromolar range. Recently, we have shown that both compounds present no toxicity in kidney- (HEK293) and hepatic- (HepG2 and Huh-7) derived humans cell lines, even at 20µM concentration, when compared to the chemotherapeutic positive control cisplatin. Further, we have shown that both compounds have a killing effect on multiple drug-resistant *P. falciparum* strains, including artemisinin-sensitive Cam3.II^{rev}, artemisinin-resistant Cam3.II^{R539T} and Cam3.II^{C580Y}, and multi-drug resistant W2mef parasite lines. Untargeted metabolomics analysis indicates that C25 perturbs pyrimidine biosynthesis by inhibition of the *de novo* pyrimidine biosynthesis pathway. Further analysis is ongoing to identify the specific target(s) of C25 and whether cross-resistance is observed with other drugs that inhibit this pathway. Parasites resistant to C25 and C26 have been generated in 3D7 and Dd2 genetic backgrounds and whole genome sequencing will be performed on the resulting resistant clones. This biological information will allow the synthesis of refined derivatives with more potent antimalarial activity.

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Multi-Pronged CAR-T Cells to eliminate Cutaneous T Cell Lymphoma

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Inflammation dependent differentiation of two distinct VAT Treg populations shape systemic metabolism

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Visceral adipose tissue (VAT) is a multifaceted organ that regulates systemic metabolism through energy storage and endocrine functions. Inflammation, induced by dietary changes or ageing, however, hamper VAT function and can lead to metabolic disease. The VAT is rich in Foxp3+ regulatory T (Treg) cells, which are recruited by inflammatory mediators and contribute to the control of VAT inflammation. Enrichment of Treg cells, however, is sexually divergent and as we have shown, imprinted by inflammatory cues and sex hormones (Vasanthakumar et al. Nat. Immunol. 2015, Vasanthakumar et al. Nature 2020). Precisely how inflammatory conditions are linked to VAT physiology and Treg cell homeostasis is, however, unclear.

Here we uncover that female and male VAT show distinct inflammatory signatures. These differences in cytokine landscape promoted the differentiation of phenotypically distinct VAT Treg cells in males and females which conform two major populations, ST2+ and ST2- VAT Treg cells. We further show that the differentiation of these VAT Treg cell populations is driven by separate molecular pathways that complement the known transcriptional regulators of VAT Treg differentiation, Blimp1 and PPARg (Cipolletta et al. Nature 2012, Vastanthakumar et al. Nature 2020). Functionally, both VAT Treg cell populations are required to control VAT inflammation and preserve organismal glucose homeostasis. Hence, this study for the first-time establishes how distinct population of Treg cells with unique molecular requirements regulate VAT inflammation and organismal metabolism in a sex-specific manner.

MAIT cells expand in the absence of NKT and γδT cells

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Unconventional T cells, namely MAIT, NKT, and γδT cells, recognise non-peptide antigens using T cell receptors (TCRs) of limited diversity, and exhibit rapid, 'innate-like' functions. In particular, MAIT cells recognise microbial riboflavin metabolites via their invariant *Trav1-Traj33*$^{+}$ TCRα chain and potently secrete IL-17 and IFN-γ upon activation. Notably, MAIT cells comprise 0.1-10% of circulating T cells in humans, the cause of this wide variance in frequency being poorly understood. Recent evidence has suggested that MAIT, NKT, and γδT cells may be regulated by similar environmental or genetic factors and may reside within a shared niche.

We showed that NKT cell-deficient mice have increased MAIT cells and demonstrated that this increase was due to the loss of NKT cells rather than their restricting element, CD1d. MAIT cells were also markedly increased in γδT cell-deficient mice and expand further in NKT/γδT cell double-deficient mice. Expanded MAIT cells phenotypically and functionally resembled their wildtype (WT) counterparts. As MAIT cells were increased in the thymus, we analyzed *Trav1-Traj33* transcripts within developing thymocytes and showed that overall *Trav1-Traj33* transcript levels were increased 2-fold within γδT- and NKT/γδT-deficient thymocytes relative to WT controls. As γδT-deficient mice harbor a modified TCRδ locus, our findings imply that TCRα rearrangement may be altered in these mice, potentially manifesting in greater rearrangement of distal *Trav* gene segments like *Trav1* and increased intrathymic generation of MAIT cells. However, increases in peripheral MAIT cells exceeded increases in the thymus, indicating that MAIT cells may compete with peripheral NKT and γδT cells for similar homeostatic factors and expand in their absence. Accordingly, we show that adoptively transferred MAIT cells underwent more proliferation within NKT/γδT-deficient hosts relative to WT controls.

Together, our findings highlight a shared niche in which MAIT, NKT, and γδT cells co-exist and compete for common homeostatic factors. Importantly, these findings provide insights into factors regulating MAIT cell levels and cautions the interpretation of studies on NKT and γδT cells using NKT- or γδT-deficient mice, respectively, due to previously unappreciated increases in MAIT cell levels and potential alterations in TCRα chain rearrangement in γδT-deficient mice.

ABSTRACTS
SESSION 8

Increased immunopathology and perturbed immune dynamics during influenza virus and arbovirus co-infection

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Infection with more than one pathogen, in sequence or concurrently, occurs commonly in nature and can substantially affect immune responses. Reports on co-infections often note poorer health outcomes and increased pathogen burdens compared to single infections. Viral co-infections can affect individual antiviral responses, reduce protection, and enhance immunopathology. Limited information exists on the outcome of co-infection with influenza and non-respiratory viruses. As influenza is prevalent worldwide, its geographical distribution overlaps with that of many arboviruses. To explore the interaction between these two types of virus infection, we established a C57BL/6 mouse model of co-infection using Semliki Forest Virus (SFV), a neurotropic arbovirus, and Influenza A virus (IAV). Adult C57BL/6 mice were infected with IAV only (respiratory infection); SFV only (systemic infection followed by encephalitis); or sequentially co-infected on day 8 post-primary infection (either SFV→IAV or IAV→SFV). Viral, inflammatory and immunological analyses were performed on day 7 following either single infection (IAV; SFV) or co-infection (SFV→IAV; IAV→SFV). In the SFV→IAV co-infection group, we observed more severe disease. This was linked to an exacerbated lung cytokine storm and delayed viral clearance in co-infected animals, resulting in more severe lung pathology. Moreover, we found altered trafficking of immune responses, particularly IAV-specific $CD8^+$ T cells being redirected to the brain in SFV→IAV co-infection. These data provide new insights into how co-infection with viruses which cause predominantly either lung or brain disease each alter the immune response and disease outcome of the other. Improved fundamental knowledge on how viral infections interact to affect the course of immune responses, could be of a direct relevance to improved disease management programs, specialist treatments and optimisation of vaccination strategies.

Structural Basis of Coronavirus E protein interactions with human PALS1 PDZ domain

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Abstract

SARS-CoV-2 infection leads to coronavirus disease 2019 (COVID-19), which is associated with severe and life-threatening pneumonia and respiratory failure. However, the molecular basis of these symptoms remains unclear. SARS-CoV-1 E protein has previously been shown to interfere with the control of cell polarity in human epithelial cells by binding to the PDZ domain of PALS1, a key component of the Crumbs polarity complex. We now show that the C-terminal PDZ binding motifs of SARS-CoV-1 and SARS-CoV-2 bind the PALS1 PDZ domain with 28.4 and 22.8 mM affinity, whereas the related sequence from MERS-CoV did not show any binding. We then determined the crystal structures of PALS1 PDZ domain bound to both SARS-CoV-1 and SARS-CoV-2 E protein PDZ binding motifs. Our findings establish the structural basis for SARS-CoV-1/2 mediated subversion of Crumbs polarity signalling, and serve as a platform for the development of small molecule inhibitors to disrupt SARS-CoV-1/2 mediated disruption of polarity signalling in epithelial cells.

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Influenza A virus causes maternal and fetal pathology via innate and adaptive vascular inflammation

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Introduction: Seasonal and pandemic influenza is a disease of global significance and in pregnant women, severe maternal illness and foetal complications ensue by enigmatic mechanisms. Major fetal complications develop despite a lack of vertical transmission of the influenza A virus [1,2]. Hence, to discern the mechanisms that prompt these major foetal complications, we developed specific aims targeting maternal cardiovascular function.

Aims: We aimed to determine whether influenza infection led to maternal vascular dysfunction and the accompanying foetal pathology, and to characterize the role of the innate and adaptive immune system in influenza induced vascular dysfunction.

Methods: Eight-to-twelve-week old time-mated pregnant (E12 gestation) and non-pregnant C57BL/6 female mice were intranasally infected with a moderate-pathogenic H3N2 IAV strain (HKx31; 10^4 PFU) or with PBS. Mice were culled 3 and 6 days post-infection, for tissue collection. Innate and adaptive immune inflammatory profiles of the aorta and placenta were determined by qPCR or flow cytometry. Maternal thoracic aorta vascular function was assessed via myography in response to endothelium-dependent and independent smooth muscle relaxants, acetylcholine (Ach) and sodium nitroprusside (SNP), respectively.

Results: We have demonstrated a significant and dynamic alteration in the maternal vascular landscape that underpins maternal and foetal pathology to IAV infection. IAV infection that causes only a mild local lung inflammatory response in non-pregnant female mice, resulted in contrast, in significant virus dissemination to the aorta in pregnant mice. The dissemination of virus in the aorta was associated with a significant increase in pro-inflammatory and anti-viral mediators and the influx of $Ly6C^{low}$ and $Ly6C^{high}$ monocytes, neutrophils and T cells, in what we define as a "Vascular Storm". Moreover, the vascular storm was associated with elevated levels of the adhesion molecules ICAM and VCAM, as well as pattern recognition receptor TLR7 in the vascular wall. We also report that maternal influenza infection elicited hypoxia in the placenta and foetal brain as well as placental growth retardation and intrauterine growth restriction. In contrast, IAV infection in non-pregnant mice caused no alterations in endothelial function or vascular inflammation.

Conclusion: These results indicate that IAV infection during pregnancy drives a significant cardiovascular event in pregnant mothers, which likely suppresses critical blood flow to the placenta and foetus resulting in hypoxia. This study provides a fundamental mechanistic insight and a new paradigm into how the immune system drives maternal and foetal pathologies during pregnancy to respiratory viruses such as influenza A virus, which normally do not result in transplacental infection.

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Pomalidomide as an immunomodulatory agent to enhance NK cell anti-HIV immunity

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Chronic HIV infection is characterised by dysfunction of key immune effector cells including $CD8^+$ T cells and NK cells that persist despite successful viral suppression by antiretroviral therapy (ART). Here, we investigated pomalidomide, an immunomodulatory drug licensed for the treatment of multiple myeloma and Kaposi's Sarcoma, to augment anti-HIV immune responses through enhancing NK cell cytotoxicity.

Pomalidomide was assessed at a therapeutically relevant concentration *ex vivo* in PBMC from uninfected donors and ART-suppressed people with HIV (PWH). Direct NK cytotoxicity was assessed by co-culturing pomalidomide-treated PBMC with the MHC-I devoid K562 cell line. We next established a novel assay where pomalidomide pre-treated NK cells were co-cultured with *in vitro* HIV-infected autologous $CD4^+$ T cells carrying an EGFP-reporter. The effect of pomalidomide on direct NK cell killing of productively HIV-infected $CD4^+$ T cells was quantified from the reduction in GFP^+HIV-infected cells. Antibody dependent cellular cytotoxicity (ADCC) was assessed using the 8E5 cell line, with 50% of cells containing a single defective provirus and expressing p24 and envelope. Pomalidomide pre-treated NK cells and 8E5 were cultured with anti-HIV immunoglobulin or an isotype control, with HIV-specific ADCC measured as a relative reduction in $p24^+$ 8E5 cells.

Treatment with pomalidomide in both HIV-negative donors and PWH significantly enhanced NK killing of K562 cells, in both HIV-negative donors and PWH, with 34.7% (95% CI 23.8-51.05; P=0.0002) greater cytotoxicity in PWH, relative to DMSO. Concurrently, $CD56^{bright}CD16^+$ NK cell subset was expanded in the presence of pomalidomide. Pomalidomide treatment significantly enhanced direct NK-mediated killing of *in vitro* HIV-infected autologous $CD4^+$ T cells. In contrast, pomalidomide did not enhance anti-HIV ADCC.

These results show that pomalidomide can enhance direct NK cell cytotoxicity and augment killing of HIV-infected cells, and may be employed in therapeutic strategies to eliminate or control the persisting HIV reservoir.

Snotwatch: When data go viral

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Polymerase chain reaction (PCR) testing has become the favoured viral detection testing method in recent years. Multiplex PCR data can be used to analyse viral patterns of multiple viruses over time and place.[1] Spatiotemporal data analysis, an emerging area that allows analysis of data that have both a space and a time component, is optimal for the kind of population-level data that PCR testing provides. The global response to the COVID-19 pandemic best demonstrates how PCR results can be used in conjunction with a range of geographic information systems (GIS) to track disease spread in near-real time.[2] This is one application of spatiotemporal analysis techniques.

Our project, 'Snotwatch', aims to extend the uses of spatiotemporal analysis from single variable to multi-variable. Where most previous spatiotemporal analyses consisted of surveillance studying respiratory PCR Data, we aim to analyse the relationship of viral circulation to health outcomes of interest. Looking at both the space and time component of the data, our project will create a novel statistical model for assessing *relationships* between independent datasets of respiratory virus circulation and various clinical outcome presentations using primary care and hospital encounter datasets. We will study viral incidence patterns in relation to the clinical incidence of asthma exacerbations, febrile seizures, Kawasaki disease, acute myocardial infarctions, and chilblains.

To date, we have conducted a proof-of-principle ecological study of presentations of febrile seizures and respiratory viruses detected at Monash Health hospitals and laboratories from 2010-2019. Associations were studied temporally and geo-temporally through mixed effects Poisson regression analysis. Febrile seizure incidence peaked in June-September. Temporal analysis showed febrile seizure associations with Human metapneumovirus, Influenza A, Influenza B and Respiratory syncytial virus (RSV) ($p<0.01$ for all). Geo-temporal analysis supported the association between febrile seizures and Influenza A, Influenza B and RSV (1.06, 1.03 and 1.07 risk ratio respectively, $p<0.01$). We have begun applying this same methodology to chilblain diagnoses in a general practice setting in relation to COVID-19 circulation but are yet to obtain results.

Further application, assessment and modification of this statistical model will be the focus of this project. This model has implications for predictive modelling using real-time viral data, which can subsequently inform public health policy.

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Mouse Mx1 inhibits HSV-1 at a Late stage in the Virus Replication Cycle.

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The Myxoma (Mx)-family proteins are interferon-inducible GTPases and some Mx proteins are known to be potent inhibitors of influenza A virus (IAV) and other RNA viruses. Recent evidence suggests that human Mx proteins can also modulate some herpesviruses infections however little is currently known regarding the ability of mouse Mx proteins to inhibit herpesvirus infections. Herein, we have used a doxycycline (dox)-inducible system to demonstrate that expression of mouse (m)Mx1, but not mMx2, in a murine airway epithelial cell line results in potent inhibition of IAV, as well as HSV-1 and HSV-2. Confocal microscopy confirmed dox-inducible mMx1 was expressed in the nucleus whereas inducible mMx2 localised to the cytoplasm. Subsequent studies have focussed on investigating the antiviral activity of mMx1 against HSV-1. First, primary lung fibroblasts generated from mice expressing a functional endogenous Mx1 protein were shown to mediate potent inhibition of HSV-1 replication compared to fibroblasts from control animals. Second, we generated cells expressing mMx1 mutants known to abrogate anti-IAV activity, namely T69A (abrogates GTPase activity and GTP binding) and R614E (results in relocalisation of mMx1 from nucleus to cytoplasm) and used these cells to demonstrate that restriction of IAV and HSV-1 replication was lost following expression of either mMx1 mutant. Third, we have used a labelled HSV-1 where green fluorescence protein (GFP) is driven from the gB promoter (γ1 gene, expressed in small amounts before genomic replication) and red fluorescence protein (RFP) from the gC promoter (γ2 gene, expression absolutely dependent on genomic replication) to demonstrate that mMx1 potently inhibits expression of HSV-1 late genes that are dependent on genomic replication. Finally, we demonstrated that mice with a functional endogenous mMx1 were protected from the development of sever skin lesions and had lower virus titre in the skin and dorsal root ganglia in the mouse model of cutaneous HSV-1 infection.

Illuminating the chemical space for MR1-restricted ligands: a binding assay using fluorescence polarisation

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Studies into T-cell-mediated immunity have largely been focused on understanding peptide presentation by Major Histocompatibility Complex (MHC) and their recognition by αβ T-cell receptors (αβ TCRs). By contrast, our understanding of non-peptide T-cell-mediated immunity in humans is limited. Mucosal-associated Invariant T-cells (MAIT cells) represent a significant population of T-cells in mucosal tissues and peripheral blood, and play a critical role in early immune responses to microbial infection.

MAIT cells recognise MHC class I-related (MR1) presenting small microbial vitamin B metabolites. The two major metabolites are pterin-based ligand 6-formylpterin (6-FP), a photodegradation product of folic acid (vitamin B_9); and pyrimidine-based ligand 5-OP-RU, being derived from microbial biosynthesis of riboflavin (vitamin B_2). The relative plasticity of the MR1 binding groove has recently led to the exploration of chemical space for novel MR1-restricted antigens. However, there is currently no method to quantify the affinity of a ligand bound to MR1. In addition, the current pipeline from initial hit to structural evidence is highly time- and resource-intensive.

Here, we have developed an MR1 binding assay using fluorescence polarisation (FP) technology that is able to quantify the affinity of MR1 ligands as well as provide a facile method of screening for novel ligands. We have demonstrated that the FP competitive binding assay is a suitable tool for quantifying the IC_{50} for MR1-restricted ligands in the nM to mM range, with affinity of the ligand correlating well with the number of polar interactions between MR1 and ligand. We have also determined the structure of a novel dietary antigen, ethylvanillin, bound to MR1. Through FACS, we were able to determine that ethylvanillin is an inhibitory molecule that can upregulate MR1 on the surface of cells. We have demonstrated that the FP competitive binding assay greatly accelerates the rate at which novel MR1 ligands may be identified and from these results, this developed tool will greatly contribute to both the chemical space of MR1-restricted ligands and to our knowledge on the biology of the MAIT-MR1 axis.

Lipid composition is altered in immune cells and impacts susceptibility to ferroptosis

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The cellular lipidome is comprised of thousands of individual lipid species each belonging to a number of structurally distinct lipid classes. Lipids influence numerous aspects of cellular physiology, with differences in lipid composition between cell types contributing to cell-specific functionality. Our laboratory has recently characterised the cellular lipidome of the human and mouse immune systems. One of the most striking effects we observed was a marked variance in the levels of polyunsaturated fatty acid (PUFA)-containing phospholipids (PL) between different immunes cell types. This was particularly intriguing as these lipid species have recently been identified as the key executioners of ferroptosis, a newly discovered form of cell death. Accordingly, we hypothesised that the distinct PUFA-PL composition of different immune cell types would dictate susceptibility to ferroptosis. Therefore, we treated immune cells isolated from murine bone marrow with ML210, an inhibitor of GPX4, the major ferroptosis suppressing pathway. Cell viability analysis revealed that T and B cells, cells with the highest abundance of pro-ferroptotic PUFA-PLs, were the most susceptible to ferroptosis. Importantly, ML210-induced cell death in T and B cells was prevented by treatment with inhibitors of ferroptosis. In contrast, neutrophils and monocytes, cells with the lowest abundance of pro-ferroptotic PUFA-PLs, were resistant to ferroptosis. Excitingly, supplementation with oleic acid and PE(18:0/18:1) re-modelled the cellular lipidome of T cells, protecting them from ferroptotic cell death. Meanwhile, neutrophils supplemented with arachidonic and docosahexaenoic acids demonstrated increased sensitivity towards ferroptosis. Overall, we identify that the susceptibility of immune cells to ferroptosis is governed by their cellular lipid composition. This work establishes the foundation for future *in vivo* work and promising therapeutic approaches for diseases associated with ferroptosis and immune cells.

Immune responses in the respiratory tract and blood of COVID-19 patients reveal mechanisms of disease severity

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Although the respiratory tract is the primary site of SARS-CoV-2 infection, respiratory immune responses are understudied and urgently needed to understand mechanisms underlying COVID-19 disease pathogenesis. We collected paired longitudinal blood and respiratory tract samples (endotracheal aspirate, sputum, or pleural fluid) from hospitalized COVID-19 patients and non-COVID-19 controls. Cellular, humoral and cytokine responses were analysed and correlated with clinical data. SARS-CoV-2-specific IgM, IgG and IgA antibodies were detected using ELISA and multiplex assay in both the respiratory tract and blood of COVID-19 patients, although a higher receptor binding domain (RBD)-specific IgM and IgG seroconversion level was found in respiratory specimens. SARS-CoV-2 neutralization activity in respiratory samples was detected only when high levels of RBD-specific antibodies were present. Strikingly, cytokine/chemokine levels and profiles greatly differed between respiratory samples and plasma, indicating that inflammation needs to be assessed in respiratory specimens for the accurate assessment of SARS-CoV-2 immunopathology. Diverse immune cell subsets were detected in respiratory samples, albeit dominated by neutrophils. Importantly, we also showed that dexamethasone with/without remdesivir treatment did not affect humoral responses in blood of COVID-19 patients. Overall, our study unveils stark differences in innate and adaptive immune responses between respiratory samples and blood and provides important insights into effect of drug therapy on immune responses in COVID-19 patients.

ABSTRACTS
SESSION 10

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A structural basis underpinning biased T cell receptor recognition of an immuno-dominant HLA-A2 restricted epitope from the SARS-CoV-2 spike protein

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Human Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is responsible for the ongoing worldwide COVID19 pandemic, having caused over 233 million infections to date. CD8+ T cells play a crucial role in establishing adaptive immunity against SARS-CoV-2 infection. Several CD8+ T cell epitopes with different HLA restrictions have been identified in SARS CoV-2, but the molecular basis underlying T cell receptor (TCR) recognition of SARS CoV-2 epitopes is unknown. In individuals carrying the common HLA A*201 allomorph, the spike protein derived epitope $S^{269–277}$ is the most immuno-dominant epitope. TCRs of $S^{269–277}$ responsive CD8+ T cells are characterised by biased TRAV12 gene usage. To understand the molecular mechanism behind TRAV12 bias, we expressed, refolded and purified three TRAV12-1 TCRs, hereby named NR1A, NR1C and NR1D and one TRAV12-2 TCR, named NR2F, which bound to HLA-$A2^{S269–277}$ complex with low μM affinity. We solved the crystal structure of the HLA-$A2^{S269–277}$ binary complex, and subsequently, a ternary complex NR1C TCR with HLA-$A2^{S269–277}$. We found that NR1C TCR and HLA-$A2^{S269–277}$recognition was dominated by TRAV12-1 germline-encoded residues and conserved sequence motifs located in CDR3α and CDR3β loop regions. The NR1C TCR made extensive interactions with the $S^{269–277}$ peptide, suggesting that TRAV12-1 TCRs are sensitive to substitution of epitope residues. We subsequently investigated the cross-reactivity of TRAV12 TCRs for $S^{269–277}$ variants and epitopes from other closely related coronaviruses. Surface plasmon resonance and tetramer studies showed that TRAV12 TCRs cross-react poorly with these homologous epitopes. In summary, the study defined the molecular mechanism behind the biased CD8+ T cell recognition of the immuno-dominant HLA-$A2^{S269–277}$ epitope and provided a scaffold for understanding cross-reactivity and potential viral escape in the HLA-$A2^{S269–277}$ restricted T cell response.

Discrete tissue microenvironments instruct diversity in resident memory T cell function and plasticity

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Tissue-resident memory T cells (T_{RM}) are non-recirculating cells that exist throughout the body and mediate local protection, therefore harnessing T_{RM} functionality is an advantageous strategy for bolstering durable immunity. However, the influence of local microenvironmental imprinting on T_{RM} functionality and fate in remains incomplete. Here, we chart phenotypic and transcriptional T_{RM} heterogeneity between sites and find that the different environments in which these cells differentiate dictate T_{RM} function, durability and malleability. Using organ transplantation and T_{RM} transfer experiments, we uncover TGF-β as the major driver of functional heterogeneity between epithelial and non-epithelial tissues. We found that the absence of TGF-β signaling engendered $CD103^-$ T_{RM} with increased proliferative potential, enhanced function, and trans-differentiation capacity compared to their TGF-β-responsive $CD103^+$ T_{RM} counterparts. Thus, despite common requirements for T_{RM} development, tissue adaptation of these cells confers discrete functional properties that affect T_{RM}-mediated secondary immune responses. Therefore, exploiting these mechanisms will inform new immunotherapy strategies to bolster site-specific T_{RM}-mediated immunity.

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Christo, S.N., Evrard, M., Park, S.L. *et al.* Discrete tissue microenvironments instruct diversity in resident memory T cell function and plasticity. *Nat Immunol* **22,** 1140–1151 (2021). https://doi.org/10.1038/s41590-021-01004-1

INCREASED BASAL AND INDUCED PHOSPHOINSITOL-3-KINASE SIGNALLING IN B- AND T-CELLS OF HEALTHY ADULTS CARRYING THE PTPN22 R620W MUTATIONS

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Background: The non-synonymous common variant c.1858C>T (p.R620W) in *PTPN22* is a risk variant for autoimmunity. Furthermore, prevalence is higher in patients with predominantly antibody deficiency (PAD), a primary immunodeficiency with increased incidence of autoimmunity. PTPN22 is a phosphatase that restricts signal transduction downstream of B- and T-cell receptors. Whether this mutation enhances or impairs PTPN22 function is currently disputed. Here, we addressed this by studying phosphoinositol-3-kinase (PI3K) signalling in B- and T-cells from healthy adults with and without the c.1858C>T variant.

Methods: Peripheral blood was collected from 63 healthy adults for Sanger sequencing for *PTPN22* c.1858C>T variant identification, detailed B- and T-cell immunophenotyping, and *in vitro* stimulation of antigen receptor signalling for S6 phosphorylation.

Results: 6/63 healthy controls were heterozygous for *PTPN22* c.1858C>T, at an allele frequency of 4.8%. Heterozygous carriers had significantly higher $CD27^+IgM^+IgD^+$ B-cell numbers. Carriers expressed higher CD19 and lower CD21 levels on transitional and naïve-mature B-cells, and higher CD8 levels on cytotoxic T-cells. Additionally, their naïve B-, $CD4^+$ and $CD8^+$ T-cells exhibited higher basal phosphorylated-S6 levels. Finally, antigen receptor stimulation resulted in higher phospho-S6 levels in B-cells (anti-IgM) and T-cells (anti-CD3) compared to individuals without the variant.

Conclusion: Here, we showed for the first time that PTPN22 R620W results in increased PI3K signalling in B- and T-cells, demonstrating that the mutation inhibits the repressive function of PTPN22. PI3K signalling in lymphocytes is tightly balanced with increased activity resulting from *PIK3CD* and *PIK3R1* mutations leading to antibody deficiency and autoimmunity. Our results indicate that this variant has similar effects but to a milder degree, which would explain the predisposition to autoimmunity. Thus, a common variant can directly impact an individual's immune profile. Future studies, will address the functional consequences of this variant in PAD patients and whether this functions as a first genetic hit for developing disease.

Circulating microRNA biomarkers enable accurate identification of COVID-19 patients

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COVID-19 continues to extensively impact every facet of our lives. Central to our efforts to slow the spread of SARS-CoV-2 is accurate and sensitive diagnostic tests. Unfortunately, news reports continually highlight examples of individuals initially deemed uninfected, only to test positive later, contributing to the spread throughout the community. Conventional diagnostic tests directly target the viral genome, which may not be readily detectable in the early, particularly pre-symptomatic, stages of infection. Host-encoded microRNAs (miRNA) have recently shown exciting promise as biomarkers of infection as they change rapidly in response to stimuli and are easily detectable within biofluids. While other host responses, especially inflammatory cytokines, have been extensively studied, the host-encoded miRNA response to SARS-CoV-2 remains poorly defined.

Here, we used next generation sequencing to profile the circulating miRNAs from 10 COVID-19 patients, sampled longitudinally, and compared these with age and gender matched controls. We found 55 differentially expressed miRNAs (FDR adjusted p-value < 0.05) during early-stage disease, with several miRNAs having previously established roles in inflammation. We then employed a multivariate machine learning approach to identify a three-miRNA biomarker signature (miR-423-5p, miR23a-3p, miR-195-5p) that identifies COVID-19 cases with 99.9% accuracy, 99.8% sensitivity, 99.8% recall, and a receiver operator characteristic area under the curve (ROC AUC) of 1. This signature is associated with the early stages of COVID-19; as the patients recovered, their samples clustered with the healthy controls. Further validation was conducted in a ferret model, where these three miRNAs identified SARS-CoV-2 infection with 99.7% accuracy, and distinguished SARS-CoV-2 infection from influenza A (H1H1) infection and uninfected animals with 95% accuracy.

This study, recently published in PLoS Pathogens [1], gives insights into the host miRNA response to SARS-CoV-2 infection and highlights the use of these molecules as circulating biomarkers for the detection of COVID-19. This biomarkers signature has great potential to compliment existing diagnostic tests and help to identify infections that may otherwise be missed.

1. Farr, R.J., et al., *Altered microRNA expression in COVID-19 patients enables identification of SARS-CoV-2 infection.* PLoS Pathog, 2021. **17**(7): p. e1009759.

Single-cell landscape of tissue-resident memory T cell development

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Tissue-resident memory T (TRM) cells exist throughout the body where they are poised to mediate local immune responses against infections and cancer. Although studies have defined a common mechanism of residency independent of location, there is likely to be a level of specialization that adapts TRM cells to a given tissue. We explored changes occurring in chromatin accessibility during TRM cell formation in different tissues to reveal the developmental trajectory and associated genetic regulators involved in the establishment of this immune population. Analyses of scATAC-seq profiles from LCMV- and HSV-specific CD8+ T cells identify changes in gene accessibility of liver and skin TRM cells and reveal both exclusive and common chromatin regulators involved in the residency program. UMAP analysis of peak accessibility display clustering of cells in a time-sensitive and tissue-specific fashion, indicating heterogeneity of effector cells and TRM populations across organs and at different times post infection. Reconstruction of cellular developmental trajectories display a common pathway for circulating memory T cells found in the liver and the spleen with late divergence involved in the formation of effector and central memory T cells. Contrarily, liver TRM cells display a unique trajectory indicating the most epigenetic disparity when compared to the other memory subsets. Finally, by integrating deviations in DNA-binding motifs and gene activity scores, we identified transcription factors critical for skin and liver TRM development that will be explored through genetic engineering to explore whether they regulate TRM formation in a tissue-specific manner.

Abstract title: IL-23 in arthritic and inflammatory pain development

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Background

The cytokine, interleukin-23 (IL-23), can be critical for the progression of inflammatory diseases, including arthritis, and is often associated with T lymphocyte biology. However, little is known about the role(s) of IL-23 in arthritic and inflammatory pain development and its dependence with other inflammatory cytokines, for example, tumour necrosis factor (TNF), granulocyte macrophage-colony stimulating factor (GM-CSF) and CCL17, which all have been implicated in arthritic pain development.

Methods

To assess the requirement of IL-23 for arthritic and inflammatory pain development, *Il23p19* gene deficient (IL23p19$^{-/-}$) and wild type (WT) mice were induced with zymosan-induced arthritis (ZIA), zymosan-induced inflammatory pain or cytokine (TNF, GM-CSF or CCL17)-driven arthritis. To assess the ability for IL-23 to induce pain and its requirement for other cytokines and eicosanoid activity, IL-23-induced inflammatory pain was elicited in WT, *Tnf*$^{-/-}$, *GM-CSF*$^{-/-}$ and *Ccl17*$^{E/E}$ mice or in WT mice treated with or without cyclooxygenase inhibitors. Experiments were approved by The University of Melbourne Animal Ethics Committee and complied with the GSK Policy on the Care, Welfare and Treatment of Animals.

Results

We report here, using *Il23p19*$^{-/-}$ mice, that innate immune (zymosan)-driven arthritic pain and optimal disease development require IL-23, as does zymosan-induced inflammatory pain. We found that exogenous TNF-, GM-CSF- and CCL17-driven arthritic pain and disease, as well as inflammatory pain driven by each of these cytokines, are all IL-23 dependent. Supporting this connection, it was found conversely that IL-23 can induce inflammatory pain with a requirement for each of the other cytokines and cyclooxygenase activity.

Conclusion

These findings indicate a role for IL-23 in innate immune-mediated arthritic and inflammatory pain with potential links to TNF, GM-CSF, CCL17 and eicosanoid function.

Chromate stress dysregulates *Pseudomonas aeruginosa* molybdenum homeostasis

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Pseudomonas aeruginosa is a ubiquitous, opportunistic human pathogen responsible for a range of human diseases, including pneumonia, urinary tract, and intestinal tract infections, and causes significant morbidity in immunocompromised individuals. *P. aeruginosa* thrives in anaerobic and microaerophilic environments, such as the mucoid enrich lung environment of individuals with cystic fibrosis, generating energy via the dissimilatory nitrate reduction. Respiration using nitrate occurs via the molybdenum cofactor enzymatic complex NarGHI. Molybdenum is a group IV element that is essential to all forms of life. In bacteria molybdenum is acquired in the oxyanion form, molybdate, via the high-affinity ATP-binding cassette transporter ModBC and the solute binding protein ModA. Here, we investigated the biochemical and structural properties of *P. aeruginosa* ModA to define its role in molybdenum homeostasis. Biochemical analyses of recombinant ModA revealed that it was permissive for interaction with the group VI oxyanions, chromate (CrO_4^{2-}), molybdate (MoO_4^{2-}), and tungstate (WO_4^{2-}), and not restricted to its physiological ligand. X-ray crystallographic analyses of ModA-metal complexes showed that all oxyanions bound at the high-affinity metal binding site within the protein with minimal apparent structural differences. We then investigated the role of ModA in *P. aeruginosa* susceptibility to chromate intoxication due to increasing industrial interest in the group IV metal as a basis for novel antimicrobial materials. Analysis of a *modA* deletion strain revealed that susceptibility to chromate intoxication and cellular accumulation of the metal were not affected by loss of *modA*. Unexpectedly, exposure to sub-lethal chromate stress increased cellular accumulation of molybdenum in the wild-type strain and, to a lesser extent, the Δ*modA* strain. This indicates that the molybdate uptake is predominantly driven by ModA, but that secondary import pathway(s) for the metal are also present in *P. aeruginosa*. The observed response to chromate intoxication further suggests that a molybdenum starvation response is induced, most likely attributable to the molybdenum-sensing metalloregulator ModE. Collectively, this work advances our understanding of the biophysical properties of ModA and reveals that although chromate stress dysregulates molybdenum homeostasis, chromate toxicity is not due to inhibition of molybdate uptake.

Analysis of the Dynamics and Composition of Lipid Droplets During Viral Infection

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The role of lipid droplets (LDs) in innate immune pathways remains relatively undescribed, however, recent research by us and others suggests that LDs may underpin the outcome of several signalling pathways.

We can now demonstrate that LDs are transiently upregulated as a host repose to multiple viral infections, both *in vitro* and *in vivo*, with this upregulation of LDs driving an increase in type I and III interferon. To understand if LDs enhance these interferon responses through a bystander effect in infected cells, we generated GFP and mCherry CRISPR/Cas9 fluorescently labelled LD cell lines. A co-culture of these two cell lines revealed for the first, that LDs were able to cross between cells. Live-time microscopy trafficking analysis of individual LDs revealed that during viral infection, LD velocity and distance travelled increased significantly as early as 2 hrs post activation of antiviral signaling pathways, and remained upregulated for 72 hrs. As LDs are known to interact with other organelles to transfer lipids and proteins, we developed techniques to isolate pure LDs from cells to analyse their lipidome and proteome before and following activation of antiviral pathways. Lipidomic analysis revealed significant changes within the lipid profile of LDs themselves, but not in whole cell lysates. Additionally, an upregulation of long chain fatty acids, and bioactive lipid mediator precursors were observed in virally driven LDs. Significant changes were also seen in the LD proteome, with 83 proteins upregulated 24 hrs post infection, including multiple antiviral proteins, and members of the early innate antiviral signaling pathways, demonstrating for the first time that the LD may act as a signaling platform during an effective antiviral response.

We believe that LDs play vital roles in facilitating the magnitude of the early antiviral immune response, in particular the production of IFN following viral infection. Here, we characterise the changing lipidome and proteome of LDs during an early antiviral response for the first time. This data represents a paradigm shift in our understanding of the molecular mechanisms which coordinate an effective antiviral response by implicating LDs as a critical signaling organelle.

SARS-CoV-2 infection in children does not necessitate establishment of adaptive SARS-CoV-2-specific immunological memory

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Children are at lower risk of developing severe COVID-19, however the underlying immune mechanisms are understudied. While children's innate immunity can drive rapid resolution of SARS-CoV-2 infection, establishment of SARS-CoV-2-specific T-cell and B-cell memory in mild COVID-19 children is unexplored. We recruited a household cohort to understand SARS-CoV-2-specific adaptive B-cell, $CD4^+$ and $CD8^+$ T-cell immune responses in PCR-positive children at one month after mild SARS-CoV-2 infection, in comparison to their mothers. We analysed SARS-CoV-2-specific B-cell and T-cell responses directly *ex vivo* using Spike- and Receptor Binding Domain (RBD)-specific B-cell probes, six SARS-CoV-2 T-cell HLA class-I tetramers (A1/ORF1a$_{1637}$, A2/S$_{269}$, A3/N$_{361}$, A24/S$_{1208}$, B7/N$_{105}$, B40/N$_{322}$) and one class-II tetramer (DPB4/S$_{167}$). Despite high PCR-seropositivity, low RBD and nucleocapsid seroconversion rates in SARS-CoV-2 PCR-positive children were observed. Our in-depth profiling of epitope-specific T-cell responses at quantitative, phenotypic and clonal levels found that only children who seroconverted had prominent memory B-cell and T-cell profiles. These children had high magnitude of SARS-CoV-2-specific T-cells displaying memory phenotypes and prevalent T cell receptor motifs, which were not observed in RBD IgG^- but PCR^+ children. This suggests that seroconversion but not PCR-positivity defines establishment of adaptive SARS-CoV-2-specific immunological memory in children. SARS-CoV-2-specific $CD8^+$ and $CD4^+$ T-cell responses in RBD IgG^+ children were comparable to their mothers, with more prominent tetramer-specific T-cell responses being associated with seropositivity rather than PCR status alone. Our study suggests that COVID-19 vaccination of children with mRNA vaccines could be a major advantage in terms of establishing T-cell and B-cell immunological memory.

Repeated *Plasmodium falciparum* infection in humans drives the clonal expansion of an adaptive γδ T cell repertoire

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Repeated *Plasmodium falciparum* infections drive the development of clinical immunity to human malaria. However, immunological mechanisms underpinning this response are only partially understood. γδ T cells have been linked to clinical protection from malaria, but how adaptive-like Vδ1$^+$ T cells respond to repeated *P. falciparum* infections is unclear. We investigated the impact of repeated *P. falciparum* infections on γδ T cell subsets and the γδ T cell receptor (TCR) repertoire in humans. We studied peripheral blood samples from Australian children, Malian children naturally exposed to malaria, and U.S. adults serially challenged with four repeated controlled human malaria infections (CHMIs). Clonally expanded cytotoxic Vδ1$_{effector}$ T cells were a major component of the γδ T cell compartment in Malian children compared to malaria-naïve Australian children. Sequential CHMIs initiated a robust innate-like Vδ2$^+$ T cell response to three *P. falciparum* infections in malaria-naïve U.S. adults, however, these cell frequencies declined between infections and were not sustained after the fourth infection. In contrast, Vδ1$^+$ T cell frequencies increased after repeated infection and correlated with clinical immunity to malaria. Populations of Vδ1$_{naive}$ T cells differentiated into cytotoxic Vδ1$_{effector}$ cells concomitant with waves of clonal selection after each infection. Finally, Vδ1$^+$ T cells from individuals with a history of malaria were licensed for *in vitro P. falciparum* parasite reactivity. Together, our study indicates that repeated *P. falciparum* infections drive the clonal expansion of an adaptive γδ T cell repertoire and establishes a role for Vδ1$^+$ T cells in the human immune response to malaria.