Victorian Infection & Immunity Network

Young Investigator Symposium

Friday 14th November 2025
Monash Institute of Pharmaceutical Sciences
Royal Parade, Parkville

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Welcome to the 2025 VIIN Young Investigator Symposium

Dear Colleagues and Friends,

On behalf of the members of the Victorian Infection and Immunity Network's Executive Committee, we are pleased to welcome you to the 2025 Young Investigator Symposium.

Special welcome to our keynote speakers: Dr Marcel Doerflinger of Walter and Eliza Hall Institute of Medical Research, and Dr Nancy Wang of Murdoch Children's Research Institute & University of Melbourne.

This is the 18th year that the VIIN has convened a symposium for young investigators. We are delighted to be seeing you all in-person again at this year's event.

We are indebted to many who have been vital to bringing about the 2025 meeting, namely:

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

Aaron Brice, ACDP, CSIRO **(Co-Chair)**
Stella Liong, RMIT University **(Co-Chair)**
Thomas Angelovich, RMIT University
Sarah Ashley, Murdoch Children's Research Institute,
Marine Besnard, Centre for Inflammatory Diseases, Monash University
Dimitra Chatzileontiadou, LIMS, La Trobe University
Michelle Chonwerawong, Hudson Institute of Medical Research
Sandra Chishimba, Burnet Institute & Uni. Melbourne
Sam Davis, CSIRO
Emily Eriksson, Walter and Eliza Hall Institute
Isabelle Foo, Doherty Institute, University of Melbourne
Paul Gill, Immunology, Monash University
Robson Kriiger Loterio, Burnet Institute
Kevin Lee, Medicine at RMH, University of Melbourne
Christophe Macri, Bio21 Institute, Uni. Melbourne
Kitty McCaffrey, Hudson Institute of Medical Research
Damian Oyong, Burnet Institute
Louis Perriman, MCRI & FECRI, Federation University
Ashleigh Rogers, Microbiology, Monash BDI
Sampa Sarkar, RMIT University
Natalia Sampaio, Hudson Institute of Medical Research
Simona Seizova, Walter and Eliza Hall Institute
Ghizal Siddiqui, MIPS, Monash University
Helen Stölting, Biochem. and Mol. Biol., Monash BDI
Isabella Stuart, Doherty Institute, University of Melbourne
Caroline Soliman, R&D, CSL Innovation & Uni. Melbourne
Praveena Thirunavukkarasu, Biochem., Monash BDI
Ryan Toh, Murdoch Children's Research Institute
Naveen Vankadari, Bio21 Institute, Uni. Melbourne
Kayla Wilson, Doherty Institute, University of Melbourne
Jinxin Zhao, Microbiology, Monash BDI

Madeleine Wemyss, VIIN Project Officer

- The 65+ Session chairs and judges for oral presentations, Science Bites and posters, which is a reflection of the ongoing popularity of this event. Thanks to each for your time and expertise.
- The sponsors and advertisers for this symposium. Your support is more and more important every year to the success of this event.
- The 17 Academic Institutions and government agencies that support VIIN annually through financial contributions. Without your support, our activities would cease.

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

Sincerely,

Prof Richard Ferrero and Prof Gilda Tachedjian, Co-Convenors of VIIN

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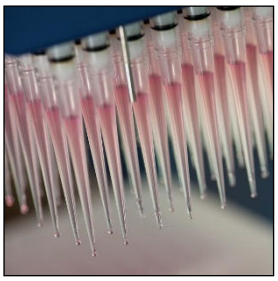
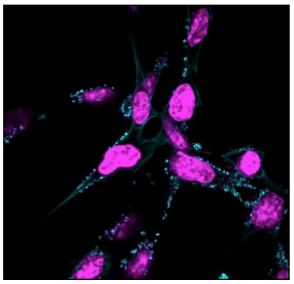
https://viin.org.au/event/viin-young-investigator-symposium-2025

Victorian Infection & Immunity Network

Uniting infection and immunity researchers in academia and industry for better health

The VIIN's mission is to promote and grow an inspiring, inclusive network that champions infection and immunity research and education for impactful discoveries and practical health outcomes. VIIN provides networking and development opportunities to Victorian infection and immunity researchers through free annual events and the annual Lorne Infection and Immunity Conference.


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

VIIN Contributors include:

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For enquiries contact: info@viin.org.au

The Hartland Oration at Lorne I&I

Professor Elizabeth Hartland was VIIN Co-Convenor between 2009 and 2017. Professor Hartland had key roles in co-convening the Lorne Infection and Immunity Conferences (2012-2017), the VIIN Industry Alliance (2012-2014) and the VIIN Young Investigator Symposia (2009 – 2016). Together with former Co-Convenor Professor Paul Hertzog, Liz oversaw the roll-out of VIIN's current 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 Prize. This oration will be delivered at the Lorne Infection and Immunity Conference from 2018 onwards. The Hartland Orator for 2026 will be selected at this VIIN Young Investigator Symposium and will be the post-doctoral researcher giving the best oral presentation. This outstanding young researcher will receive free registration to the 2026 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:

- Best Oral Presentation by a Student or RA
- Science Bites prizes (3 minute oral presentations)
- Poster prizes
- Best question prizes
- People's Choice Awards – stay tuned!

Program-at-a-Glance 2025

FRIDAY 14 NOVEMBER		
Time	**Session**	**Location**
08:15 – 08:50	**Registration** (Includes transit time to Lecture Theatres)	Sisson's Foyer in Cossar Hall
08:50 – 09:00	**Welcome and Acknowledgement of Country**	Lecture Theatre 1
09:00 – 09:45	**Session 1: Oral Presentations** **Theme: Infection Mechanisms and Host Responses**	Lecture Theatre 1
09:45 – 10:20	**Session 2: Keynote Speaker, Dr Marcel Doerflinger – Laboratory Head, Walter and Eliza Hall Institute**	Lecture Theatre 1
10:20 – 11:00	**Morning Tea** (Includes transit time from and to Lecture Theatres)	Cossar Hall
11:00 – 11:40	**Session 3: Oral Presentations** **Theme: Diagnosing and Targeting Disease**	Lecture Theatre 1
11:40 – 12:30	**Session 4: Science Bites I**	Lecture Theatre 1
12:30 – 13:40	**Lunch and Poster Session I** (Includes transit time from and to Lecture Theatres)	Cossar Hall
13:40 – 14:20	**Session 5: Oral Presentations** **Theme: Adaptive and Cellular Immunity**	Lecture Theatre 1
14:20 – 14:55	**Session 6: Science Bites II**	Lecture Theatre 1
14:55 – 15:30	**Session 7: Keynote Speaker, Dr Nancy Wang – Laboratory Head, Murdoch Children's Research Institute**	Lecture Theatre 1
15:30 – 16:10	**Afternoon Tea** (Includes transit time from and to Lecture Theatres)	Cossar Hall
16:10 – 16:40	**Session 8: Science Bites III**	Lecture Theatre 1
16:40 – 17:20	**Session 9: Oral Presentations** **Theme: Responses to Infection and Treatment**	Lecture Theatre 1
17:20 – 17:30	**Thanks and Announcements**	Lecture Theatre 1
17:30 – 18:45	**Evening Networking and Poster Session II** (Includes transit time from Lecture Theatres)	Cossar Hall
18:45 – 19:00	**Prizes, Acknowledgements and Conclusion** – Including Hartland Oration Prize winner for 2025 **Presented by VIIN Co-Convenors:** **Prof Gilda Tachedjian and Prof Richard Ferrero**	Cossar Hall

Keynote Speakers

Dr Marcel Doerflinger, Walter and Eliza Hall Institute

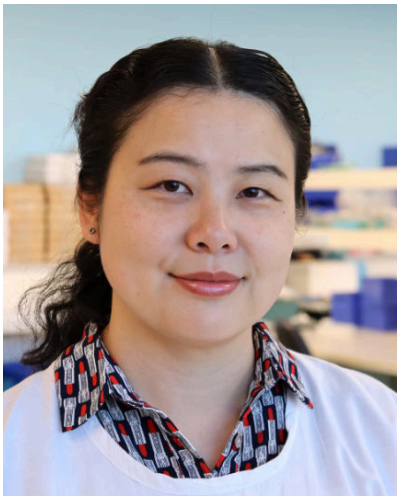
How to become a Laboratory Head …for Dummies (myself)

Marcel Doerflinger is a Bellberry-Viertel Senior Medical Research Fellow and Laboratory Head in the Infection and Global Health Division at WEHI. His research focus is on understanding the regulatory processes of programmed cell death and inflammation at the host-pathogen interface of infection. His team's projects leverage basic discovery biology and advanced in vivo models of disease for the development of novel therapeutic strategies towards translational outcomes for high impact infections including Tuberculosis, HIV, HTLV-1 and (long) COVID.

Marcel has over 50 career publications, including first and last-author manuscripts in prestigious journals including Cell, Immunity, PNAS and Nature Comms. His collaborative network includes institutes across Australia, Japan, Europe and the US and the pre-clinical disease models used in his lab have supported the initiation of two international clinical trials and enabled 2x patents.

When he is not found in the lab (or more often the office these days), he likes to explore Victoria's and Australia's National Parks, campgrounds and bushwalks or is looking for the next scuba diving adventure.

Dr Nancy Wang, Murdoch Children's Research Institute



From mouse to human: harnessing CD4+ T cell-mediated immunity against *Salmonella*

Nancy Wang is a Ruth Bishop Fellow and Team Leader at the Murdoch Children's Research Institute. Nancy is an immunologist and bacteriologist with expertise in bacterial pathogenesis, vaccinology and T cell-mediated immunity. Her recent research highlight includes the discovery of a novel collection of T cell antigens in typhoid fever, with translational potential for better prevention, detection and management of the disease

Nancy completed her PhD at the University of Melbourne with Dr Tom Brodnicki and Dr Odilia Wijburg in 2013. She spent her postdoc years under the mentorship of Prof Dick Strugnell at the University of Melbourne & Peter Doherty Institute for Infection and Immunity, during which time she developed an interest in understanding the mechanism of cellular immunity that confers protection against clinically significant bacterial pathogens, especially *Salmonella*.

Between 2016-2019, Nancy spent extended periods visiting and working with international collaborators at the Wellcome Trust Sanger Institute, the Weatherall Institute of Molecular Medicine and the Oxford Vaccine Group and the University of Oxford, and the LIMES Institute at the University of Bonn. Her research programs have been funded by the Australian Research Council, and various UoM initiatives including commercialisation. In August 2024, Nancy was named the inaugural Ruth Bishop Research Fellow at the Murdoch Children's Research Institute. She now leads a research team investigating disease mechanisms of typhoid fever and other salmonellosis, with the hope to improve future vaccine design, surveillance and diagnostics.

Notes

Health & Safety Precautions

Although prevalence of COVID-19 and other respiratory infections in the community currently remain stable at relatively low levels, we encourage all delegates to continue to take sensible precautions before attending this year's Symposium.

Please:

- **Stay home if unwell:** We will refund your deposit in the case of illness. Virtual access options will also be available to registered delegates.
- Wear a mask: VIIN encourages and supports all delegates who choose to wear closely-fitting face masks in order to manage risk when attending the Symposium. A small number of masks will be available at the registration desk if required.
- Wash hands and sanitise frequently: Hand sanitiser will be available at registration and in the Cossar Hall.
- If you are concerned about being exposed to airborne respiratory viruses while eating or drinking, please feel free to use the courtyard during the refreshment breaks

Photography and Social Media

Photographs will be taken during the Symposium for use on the VIIN website and social media. Please let the photographer know if you don't want your photograph taken.

Social Media: We encourage you to join the conversation on BlueSky, LinkedIn or X by including #YoungVIIN2025 and #viinyis in your posts. Please follow us @theviin.bsky.social where we will be live posting Symposium highlights.

Please remember:

(1) **Follow the VIIN's Social Media Policy**, available at the link here https://viin.org.au/docs/viin-policy-on-social-media-v2-final-240524.pdf or via the QR code →

(2) **Have permission! Please only post about presentations where speakers or poster presenters have explicitly given permission**, either verbally at the time of the presentation or by the use of a symbol on their slides or poster that conveys their wishes.

(3) **Don't capture or transmit any 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.

Wi-Fi Login

Guests from participating institutions can access the eduroam network. Alternatively, register and connect using Monash Guest Wi-Fi: https://www.monash.edu/esolutions/network/guest-wifi

Sponsors

We are fortunate to have the support of several companies at our Symposium, some of whom are in attendance as representatives of their company. Please greet our Sponsor representatives and ask about their products and services during each networking session.

Catering

Thank you to our caterers, Cinnamon Grove Café & Catering, for providing another fantastic menu at this year's Symposium.

Do you have special dietary requirements? Please visit the Registration Desk or ask our friendly catering team for information about where to collect your special meal. These will be individually packaged and identified by delegate name. Halal and vegetarian options have been provided as part of our standard menu.

Venue and Registration

This year's Symposium is once again hosted by Monash Institute of Pharmaceutical Sciences, located at 381 Royal Parade, Monash University Parkville Campus. A big thank you to our venue for their continued support!

On arrival, please register at the desks located in the foyer in Sissons Building, to your left as you face the courtyard. Posters, displays and catering will be available throughout the day in the adjoining Cossar Hall. Bathrooms are located in the Lecture Theatre block and in Cossar Hall. Talks will be held in Lecture Theatre 1, with Lecture Theatre 2 available as an alternative location for any delegates who would like to make use of this space (including parents and those with accessibility needs). Lecture Theatres are accessed through the courtyard, heading in the direction of Mile Lane.

Drink tickets: At registration, please collect your name tag and drinks coupons. These drinks coupons must be retained during the symposium and exchanged during the Evening Networking and Poster Session for beverages of your choice.

Storage of posters: Please store your poster at the back of Cossar Hall, ensuring it is not a trip hazard. See Venue Map on next page for your reference.

Awards and Prizes

People's Choice Awards: QR codes and links to access the People's Choice voting forms will be available via the master slides at the end of each session, as well as displayed in the Cossar Hall during Poster Sessions. We encourage you to vote for your favourite presentations and posters throughout the Symposium – you could be the one to decide our award winners!

Best Question Prizes: Prizes are up for grabs for Best Question! To participate, please remember to identify yourself verbally by stating your name and affiliation, before beginning your question.

Post-Event Survey

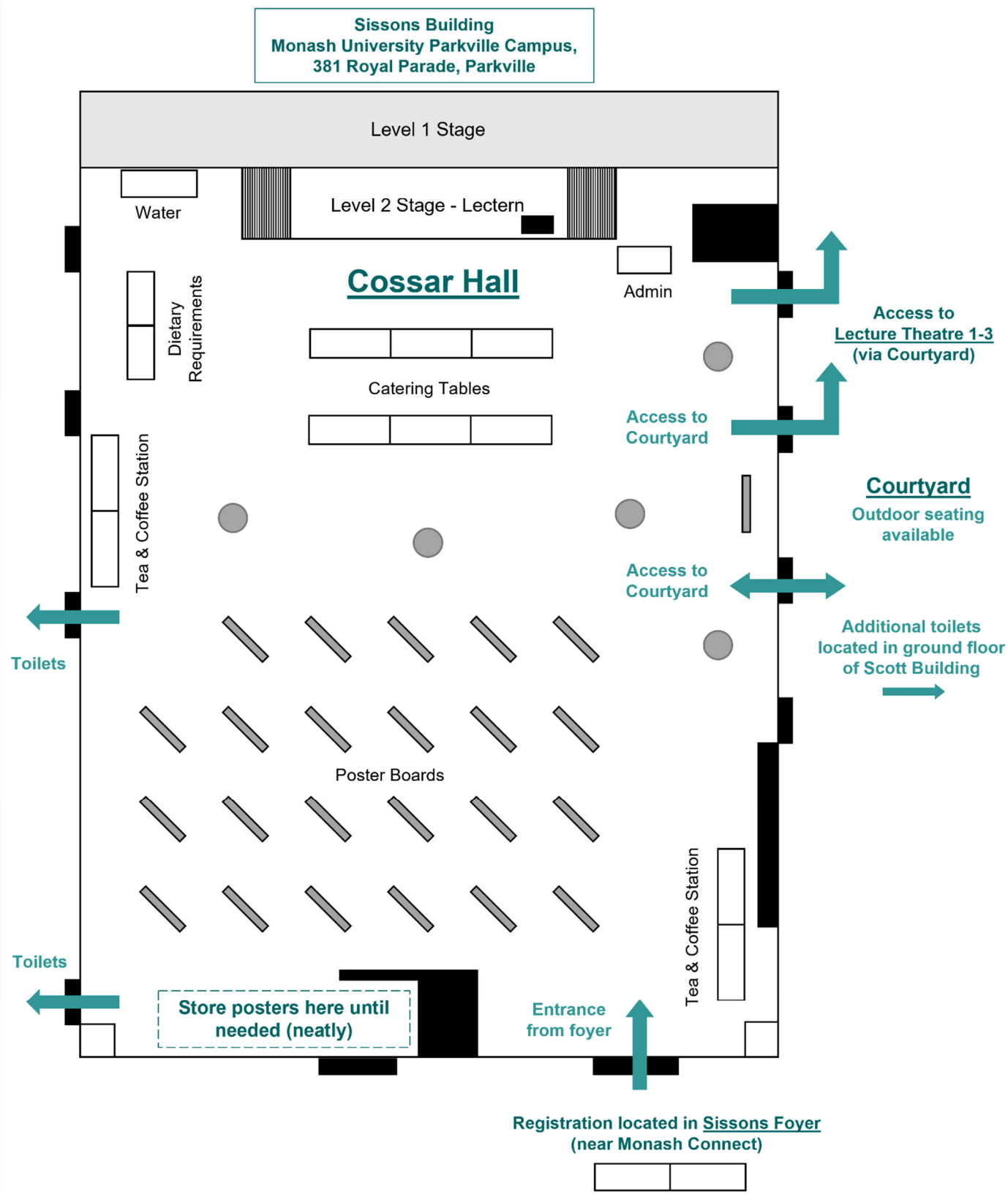
Please provide us with your feedback regarding this year's Symposium using our post-event survey form, accessible at the link below or via the QR code: https://forms.gle/WpA5Z7NRit7uhtGH9

Your experiences of the event will help inform our planning for 2026, and are invaluable for making the Young Investigator Symposium an annual success – Thank you!

Delegates List

Please see final pages of this booklet for a list of registered delegates in attendance at the 2025 VIIN Young Investigator Symposium.

Venue Map


Sissons Building
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Tea & Coffee Station
Access to Lecture Theatre 1-3 (via Courtyard)
Access to Courtyard
Courtyard
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Access to Courtyard
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Format	Spectral	Conventional	Relative brightness	Spillover (1 = low, 4 = high)	Alternative to
Blue laser					
RB545	✓		●●○○	1	(Unique position)
RB613	✓	✓	●●●●	2	PE-CF594, PE-Dazzle™ 594, BB630-P2 or StarBright™ Blue 615
RB670	✓	✓	●●●●	2	PE-Cy5, PerCP, BB660-P2 or StarBright™ Blue 675
RB705	✓	✓	●●●●	2	PerCP-Cy5.5, BB700 or StarBright™ Blue 700
RB744	✓	✓	●●●●	1	BB755-P
RB780	✓	✓	●●●●	1	PE-Cy7 or BB790-P
RB824	✓	✓	●●●○	1	PE/Fire™ 806 or StarBright™ Blue 810
Yellow-green laser					
RY586	✓	✓	●●●●	1	PE
RY610	✓	✓	●●●○	1	PE-CF594 or PE-Dazzle™ 594
RY655	✓	✓	●●●●	3	PE-Cy5 or StarBright™ Yellow 665
RY703	✓	✓	●●●●	2	PE-Cy5.5 or StarBright™ Yellow 720
RY743	✓	✓	●●●●	2	PE/Fire™ 744
RY775	✓	✓	●●●●	2	PE-Cy7 or StarBright™ Yellow 800

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Icahn School of Medicine at Mount Sinai, USA

Prof Michael Gale Jnr
University of Minnesota, USA

A/Prof Robert Moon
London School of Hygiene and Tropical Medicine, UK

Prof Dana Philpott
University of Toronto, Canada

Prof Jacques Ravel
University of Maryland, USA

Prof Anita Sil
University of California San Francisco, USA

Prof Hedda Wardermann
DFKZ, Germany

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Friday 14 November 2025

Friday 14 November: 8.15 am – 7.00 pm

Time	Session	Location
08:15 – 08:50	**Registration** (Includes transit time to Lecture Theatres)	Sisson's Foyer in Cossar Hall
08:50 – 09:00	**Welcome and Acknowledgement of Country** **Chairs: Aaron Brice and Stella Liong**	Lecture Theatre 1
09:00 – 09:45	**Session 1: Oral Presentations** **Theme: Infection Mechanisms and Host Responses** **Chairs: Simona Seizova and Naveen Vankadari**	Lecture Theatre 1
09:00	**Defining how macrophages drive OLAH-mediated life-threatening influenza disease** Deborah Gebregzabher, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
09:10	**Determinants of innate immune cytokine responses in pre-school children** Rutger Röring, Infection, Immunity and Global Health, Murdoch Children's Research Institute; Early Career Researcher	
09:20	**Proteasome dependent degradation of type I interferon signalling factors during *Shigella flexneri* infection** Anita Chaulagain, Centre for Innate Immunity and Infectious Diseases (CiiiD), Hudson Institute of Medical Research & Monash University; PhD Student	
09:30	**Structural basis of DNA sliding clamp-mediated long-range gene silencing on a multi-drug resistance plasmid** Thomas McLean, Infection and Global Health, Walter and Eliza Hall Institute of Medical Research; Early Career Researcher	
09:45 – 10:20	**Session 2: Keynote Speaker – Dr Marcel Doerflinger** **Laboratory Head, Walter and Eliza Hall Institute** **Chairs: Emily Eriksson and Simona Seizova**	Lecture Theatre 1
09:45	**How to become a Laboratory Head…for Dummies (myself)** Dr Marcel Doerflinger Laboratory Head, Infection and Global Health Division, Walter and Eliza Hall Institute of Medical Research	
10:10	**Q&A**	
10:20 – 11:00	**Morning Tea** (Includes transit time from and to Lecture Theatres)	Cossar Hall
11:00 – 11:40	**Session 3: Oral Presentations** **Theme: Diagnosing and Targeting Disease** **Chairs: Christophe Macri and Sampa Sarkar**	Lecture Theatre 1
11:00	**A Rapid Point-Of-Care Self-Test For Liver Disease** Angus Watson, Life Sciences, Burnet Institute; Research Assistant	
11:10	**Evidence for repurposing pirfenidone to treat viral exacerbations of COPD based on a novel precision-cut lung slice model** Julia Chitty, Department of Pharmacology, Monash Biomedicine Discovery Institute, Monash University; Early Career Researcher	

Time	Session	Location
11:20	**The voltage dependent ion channel is an essential mitochondrial protein in malaria parasites** Mitchell Trickey, Monash Institute of Pharmaceutical Sciences (MIPS), Monash University; Early Career Researcher	
11:30	**Repurposing human medicines as novel antimalarials against *Plasmodium falciparum* malaria** Khoi Nguyen, Burnet Institute & Department of Microbiology and Immunology, University of Melbourne; Masters Student	
11:40 – 12:30	**Session 4: Science Bites I** **Chairs: Damian Oyong and Ghizal Siddiqui**	Lecture Theatre 1
11:42	**Validation of Solvent Proteome Profiling for Antimalarial Drug Target Deconvolution** Yijia (Jessica) Ji, Monash Institute of Pharmaceutical Sciences (MIPS), Monash University; Research Assistant	
11:45	**Expansion microscopy reveals how Kelch 13 mutations impair feeding in artemisinin resistant *Plasmodium* parasites** Long Huynh, Department of Biochemistry and Pharmacology, University of Melbourne; PhD Student	
11:48	**Dual inhibition of Plasmepsins IX and X in *Plasmodium falciparum* sporozoites inhibits development within *Anopheles stephensi* mosquitoes** Elena Lantero Escolar, Infection and Global Health, Walter and Eliza Hall Institute of Medical Research; Early Career Researcher	
11:51	**Development of next-generation mRNA vaccines against malaria** Jiaqi Shi, Life Sciences, Burnet Institute; Masters Student	
11:54	**PfPTRAMP-CSS as a multi-stage malaria vaccine target** Pailene Lim, Infection and Global Health, Walter and Eliza Hall Institute of Medical Research; PhD Student	
11:57	**Enhanced immunogenicity in mouse by recombinant BCG prime and protein boost based on latency antigen Rv1733c and/or reactivation antigen RpfE** Mitra Ashayeripanah, Department of Microbiology, Shahid Beheshti University, Iran; Early Career Researcher	
12:00	**Multi-Dot/Icm effector deletion mutants reveal distinct host transcriptional responses in *Legionella pneumophila* infected macrophages** Rachelia Raissa Wibawa, Centre for Innate Immunity and Infectious Diseases (CiiiD), Hudson Institute of Medical Research; Early Career Researcher	
12:03	**Reprogramming Bacterial Autotransporters: Turning Pathogenesis into Therapeutics** Kaitlin Clarke, La Trobe Institute for Molecular Science (LIMS), Department of Biochemistry and Chemistry, La Trobe University; PhD Student	
12:06	**Bacterial membrane vesicles promote horizontal gene transfer in *Helicobacter pylori*** Christopher McCrory, Peter Doherty Institute for Infection and Immunity, Department of Microbiology and Immunology, University of Melbourne; Early Career Researcher	
12:09	**Identification of immunomodulatory proteins in *Helicobacter pylori*-derived extracellular vesicles** Kavitha Wilson Rajaratnam, Centre for Innate Immunity and Infectious Diseases (CiiiD), Hudson Institute of Medical Research; Honours Student	

Time	Session	Location
12:12	**Light-induced circadian rhythm disruption protects the host from *Clostridioides difficile* infection** Desirel Ng, Department of Microbiology, Monash Biomedicine Discovery Institute, Monash University; PhD Student	
12:15	**AIMing for *Streptococcus pyogenes*-specific memory T cells before and after experimental human challenge** Sabrina Bush, Department of Immunology, Monash University; Research Assistant	
12:18	**Barbet: A Deep Learning Approach to Bacterial Taxonomic Classification** Wytamma Wirth, Peter Doherty Institute for Infection and Immunity, University of Melbourne; Early Career Researcher	
12:30 – 13:40	**Lunch and Poster Session I** (Includes transit time from and to Lecture Theatres) **See below for more information**	Cossar Hall
13:40 – 14:20	**Session 5: Oral Presentations** **Theme: Adaptive and Cellular Immunity** **Chairs: Paul Gill and Dimitra Chatzileontiadou**	Lecture Theatre 1
13:40	**Revealing dose and repeat effects of a novel Influenza vaccine on humoral immunity** Ludivine Grzelak, Peter Doherty Institute for Infection and Immunity, Department of Microbiology and Immunology, University of Melbourne; Early Career Researcher	
13:50	**Targeted Depletion of CCR6+ Cells Elicits Robust Therapeutic Effects Across Multiple Autoimmune Diseases** Md Jahangir Alam, Department of Physiology, Monash Biomedicine Discovery Institute, Monash University; Early Career Researcher	
14:00	**Single-Cell Analysis Reveals the Precise Timing of T Lineage Commitment in the Thymus** Dhruti Parikh, Department of Medicine, University of Melbourne & St Vincent's Institute; PhD Student	
14:10	**Targeted Regulatory T Cells to Treat Autoimmune Small Vessel Vasculitis** Elean Tay, Department of Medicine, Monash University; PhD Student	
14:20 – 14:55	**Session 6: Science Bites II** **Chairs: Louis Perriman and Natalia Sampaio**	Lecture Theatre 1
14:22	**Immunomodulatory Lipid Nanoparticles to Treat Experimental Glomerulonephritis** Dian Hasanah, Centre for Inflammatory Diseases, Department of Medicine, Monash University; PhD Student	
14:25	**Harnessing MR1 Antigen Presentation with a Ligand-Restricted Nanobody for Immune Manipulation** Hisham Shakir, Peter Doherty Institute for Infection and Immunity, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
14:28	**Suppressing the Suppressors – New Antibody Strategies in Cancer Immunotherapy** Galih Adhyatma, Department of Physiology, Monash Biomedicine Discovery Institute, Monash University; PhD Student	

Time	Session	Location
14:31	**Pre-existing humoral immunity and comorbidity-associated inflammatory signatures shape responses toward seasonal influenza vaccination in Australian First Nations people** Morgan Skinner, Peter Doherty Institute for Infection and Immunity, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
14:34	**Influenza A virus drives TLR-dependent inflammatory responses in non-small cell lung cancer** Isabella Rosano, School of Health and Biomedical Sciences, RMIT University; Honours Student	
14:37	**Beta-blockade reshapes the tumour immune landscape to enhance chemotherapy control of triple-negative breast cancer progression** Md Amir Hossain, Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences (MIPS), Monash University; PhD Student	
14:40	**Selecting β-synuclein epitopes for a targeted regulatory T cell therapy for progressive multiple sclerosis** Grace Osmond, Centre for Inflammatory Diseases, Monash University; Honours Student	
14:43	**Designing Improved Diagnostic Assays for IBDV Detection in Australia** Jesse Thomas, CSIRO & School of Life and Environmental Sciences, Deakin University; PhD Student	
14:47	**Development of a rapid point-of-care test for active syphilis** Patrick Bajan, Burnet Diagnostics Initiative, Burnet Institute; Research Assistant	
14:55 – 15:30	**Session 7: Keynote Speaker – Dr Nancy Wang** **Laboratory Head, Murdoch Children's Research Institute** **Chairs: Isabelle Foo and Kevin Lee**	Lecture Theatre 1
14:55	**From mouse to human: harnessing CD4+ T cell-mediated immunity against *Salmonella*** Dr Nancy Wang Ruth Bishop Fellow and Team Leader, Infection, Immunity and Global Health, Murdoch Children's Research Institute & Peter Doherty Institute for Infection and Immunity, Department of Microbiology and Immunology, University of Melbourne & Department of Paediatrics, Melbourne Medical School, University of Melbourne	
15:20	**Q&A**	
15:30 – 16:10	**Afternoon Tea** (Includes transit time from and to Lecture Theatres)	Cossar Hall
16:10 – 16:40	**Session 8: Science Bites III** **Chairs: Michelle Chonwerawong and Caroline Soliman**	Lecture Theatre 1
16:12	**Investigation of microbe associated antibody cross-reactivity in SARS-CoV-2 Nucleocapsid immune response** Joshuah Fialho, School of Health and Biomedical Sciences, RMIT University; PhD Student	
16:15	**Bispecific IgG4 display epitope-dependent ability to bind and mediate Fc effector functions against SARS-CoV-2** Carissa Aurelia, Peter Doherty Institute for Infection and Immunity, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
16:18	**First molecular insight into HLA-C contribution to COVID-19 outcome** You Min Ahn, Department of Biochemistry and Chemistry, La Trobe University; PhD Student	

Time	Session	Location
16:21	**Defining molecular gene profiles that underpin superior antiviral CD8+ T cell immunity in nonagenarians and centenarians** Tejas Menon, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
16:24	**A High-Dimensional Flow Cytometry Profiling on Peripheral Blood Mononuclear Cells of Transitioning Transgender Individuals** Den Celestra, Murdoch Children's Research Institute & Department of Paediatrics, University of Melbourne; Masters Student	
16:27	**Decoding chronic urinary tract infections: A predictive multi-omic trio of bladder transcriptomics, urinary metabolomics, and the gut microbiome** Theresa Nguyen, Department of Immunology, Monash University; PhD Student	
16:30	**Altered gastrointestinal permeability and immune profiles in the Shank3B-/- mouse model of autism and modulation by the microbial metabolite-sequestering drug, AB-2004** Vic Lin, School of Health and Biomedical Sciences, RMIT University; PhD Student	
16:33	**Macrophage heterogeneity influences cellular response to HIV infection and latency modulation** Laura Rikard-Bell, Life Sciences, Burnet Institute; PhD Student	
16:36	**Selective viral modulation of IFN-regulated genes to supress antiviral genes while permitting pro-viral gene expression** Alebachew Kebede, Department of Microbiology, Monash Biomedicine Discovery Institute, Monash University; PhD Student	
16:40 – 17:20	**Session 9: Oral Presentations** **Theme: Responses to Infection and Treatment** **Chairs: Ash Rogers and Sarah Ashley**	Lecture Theatre 1
16:40	**Macrophages play critical roles in MR1 antigen presentation and regulation of MAIT cell immunity against bacteria** Xiaoyue Zhang, Peter Doherty Institute for Infection and Immunity, University of Melbourne; PhD Student	
16:50	**Memory B cells and plasmablasts expand following experimental infection with *Streptococcus pyogenes* in humans** Holly Fryer, Department of Immunology, Monash University; PhD Student	
17:00	**Dynamic Single-Cell Microfluidics Uncovers Heterogeneous Mechanisms of Response to Anti-PD-1 Therapy** Wei-Che Chang, Integrated Photonics and Applications Centre (InPAC), RMIT University; PhD Student	
17:10	**Pro-apoptotic Agents Reduce Chronic HIV Infection *in vivo*** Le Wang, Infection and Global Health, Walter and Eliza Hall Institute of Medical Research; PhD Student	
17:20 – 17:30	**Thanks and Announcements** **Chairs: Aaron Brice and Stella Liong**	Lecture Theatre 1
17:30 – 18:45	**Evening Networking and Poster Session II** (Includes transit time from Lecture Theatres) **See below for more information**	Cossar Hall
18:45 – 19:00	**Prizes, Acknowledgements and Conclusion** – Including Hartland Oration Prize winner for 2025 **Presented by VIIN Co-Convenors:** **Prof Gilda Tachedjian and Prof Richard Ferrero**	Cossar Hall

Poster Session I: 12.30 pm – 1.40 pm

12:30 – 13:40	**Lunch and Poster Session I** (Includes transit time from and to Lecture Theatres) **Poster judging to be finalised by 13:20**	Cossar Hall
Poster Number	**Poster Details**	
1	**Mechanism-based modelling of antibiotic effects on *Pseudomonas* strains with different resistance mechanisms** Alice Terrill, Monash Institute of Pharmaceutical Sciences (MIPS), Monash University; PhD Student	
2	**Exploring the hepatoprotective role of glucagon** Ashish Foollee, Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute, Monash University; PhD Student	
3	**Exploring a possible role for immune-responsive gene 1 (IRG1) in modulating influenza A virus replication** Shirley Lin, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
4	**Evaluation of Neglected Tropical Diseases exposure in an urban population cohort in Quito, Ecuador** Belen Prado-Vivar, Walter and Eliza Hall Institute of Medical Research & Department of Infectious Diseases, University of Melbourne; PhD Student	
5	**Cross-serotypically conserved epitope recommendations for a universal T cell-based dengue vaccine** Syed Faraz Ahmed, Peter Doherty Institute for Infection and Immunity & Department of Electrical and Electronic Engineering, University of Melbourne; Early Career Researcher	
6	**Exploring the natural resistance of juvenile abalone to haliotid herpesvirus (HaHV-1) infection: The role of algae** Jacinta Agius, Department of Microbiology, Anatomy, Physiology and Pharmacology, La Trobe University; PhD Student	
7	**Cedar virus and Salt Gully virus are capable of viral mRNA editing during henipavirus infection** Emily Dowling, Australian Centre for Disease Preparedness (ACDP), CSIRO & Deakin University; PhD Student	
8	**Exploring SAMHD1 as a cross-species restriction factor against Herpesvirus in cattle and humans** Yongyan Xia, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
9	**Beyond Storage: Lipid droplets as novel extracellular communicators in flavivirus infection** Irumi Amarasinghe, La Trobe Institute for Molecular Science (LIMS), La Trobe University; PhD Student	
10	**Transport of Japanese Encephalitis Virus neutralising monoclonal antibodies across the blood-brain barrier** Rachel Evans, Peter Doherty Institute for Infection and Immunity, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
11	**Characterisation of novel bat pararubulavirus identified in Australian *Pteropus* bat urine** Abbey Pay, Health and Biosecurity, CSIRO & Life and Environmental Sciences, Deakin University; Honours Student	

12:30 – 13:40	**Lunch and Poster Session I** (Includes transit time from and to Lecture Theatres) **Poster judging to be finalised by 13:20**	Cossar Hall
Poster Number	**Poster Details**	
12	**Cancer-related gene expression in bat retrovirus infection** Nancy Wilson, Life Sciences, Burnet Institute; Honours Student	
13	**Development of an *ex vivo* airway epithelium model to study bat innate immune responses** Mithun Das, Health and Biosecurity, CSIRO; Early Career Researcher	
14	**Breaking the Barrier: The IL-33-ILC2 axis drives severe urinary tract infections** Ashleigh Paschek, Department of Immunology, Monash University; PhD Student	
15	**Structural basis of biofilm formation by the oral pathogen *Treponema denticola*** Bindusmita Paul, Department of Biochemistry and Pharmacology, University of Melbourne; PhD Student	
16	**Structural and functional characteristics of *Legionella* effectors targeting mitochondria** Brenda (Yee Wa) Lim, School of Chemistry, University of Melbourne; PhD Student	
17	**Subcellular localisation of RNA-binding effectors from *Legionella pneumophila*** Daniela Lambrechts, Centre for Innate Immunity and Infectious Diseases (CiiiD), Hudson Institute of Medical Research & Monash University; Honours Student	
18	**Prior *S. aureus* colonisation impacts future immune responses to invasive *S. aureus* infections** Jessica Braverman, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
19	**Detection and Characterization of Antimicrobial Resistance Genes in Mothers and Infants During the First Year of Life** Aseel Al-Araji, School of Medicine, Deakin University; PhD Student	
20	**Disruption of mecR impairs MecA-mediated cephamycin resistance in *Clostridioides difficile*** Georgia-Rose Gilmore, Department of Microbiology, Monash Biomedicine Discovery Institute, Monash University; PhD Student	
21	**Simple sequence repeats power *Staphylococcus aureus* adaptation** Ashleigh Hayes, Department of Microbiology and Immunology, University of Melbourne; Research Assistant	
22	**Aztreonam plus ciprofloxacin synergistically kills resistant *Pseudomonas aeruginosa* strains** Charlotte Picton, Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences (MIPS), Monash University; PhD Student	
23	**Investigating the immunomodulatory and clinical effects of fermentable fibre intervention to reduce symptoms of Long COVID** Joel Jefi Varghese, Department of Immunology, Monash University; Honours Student	
24	**Investigating the impact of diet on invasive fungal infection outcomes** Antara Ghoshal, Monash Biomedicine Discovery Institute, Monash University; PhD Student	

12:30 – 13:40	**Lunch and Poster Session I** (Includes transit time from and to Lecture Theatres) **Poster judging to be finalised by 13:20**	Cossar Hall
Poster Number	**Poster Details**	
25	**Genetic dissection of *Toxoplasma* differentiation** Amalie Jayawickrama, Infection and Global Health, Walter and Eliza Hall Institute of Medical Research; PhD Student	
26	**Ecological Surveillance of *Mycobacterium ulcerans* (MU) in Urban Possums and Development of a CRISPR-SHERLOCK MU Assay** Pranab Paul, CSIRO & School of Medicine, Deakin University; PhD Student	
27	**Dissecting Host Manipulation by *Cryptosporidium*** Lena Chng, Infection and Global Health, Walter and Eliza Hall Institute of Medical Research; PhD Student	
28	**Investigating the role of Kelch-13 during sexual development in *P. falciparum*** Sophie Collier, Department of Biochemistry and Pharmacology, University of Melbourne; Early Career Researcher	
29	**Immune profiling of severe and fatal influenza infections in First Nations patients with multimorbidities** Lilith Allen, Peter Doherty Institute for Infection and Immunity, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
30	**Investigating Antibody Responses to Endothelial Protein C Receptor Binding *Plasmodium falciparum* Erythrocyte Membrane Protein 1 Associated with Severe Malaria in Children** Daniel Getacher Feleke, Peter Doherty Institute for Infection and Immunity, Department of Infectious Diseases, University of Melbourne; PhD Student	
31	**The Effects of N6 methyladenosine on the Proteome of the Malaria Parasite *Plasmodium falciparum*** Lakvin Fernando, Department of Biochemistry and Pharmacology, University of Melbourne; PhD Student	
32	**Mechanistic insights into repurposed compounds as potential antimalarials** Chris Taylor, Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences (MIPS), Monash University; PhD Student	
33	**Predicting the impact of antimalarial resistance in West African parasites** Rachael Yong, Life Sciences, Burnet Institute & School of Medicine, Deakin University; PhD Student	
34	**Impacts of apicoplast-targetting antibiotics on dihydroartemisinin activation in *Plasmodium falciparum*** Zoe Tregloan-Dunn, Bio21 Institute, University of Melbourne; Undergraduate Student	
35	**Investigating primary and recall humoral immune responses in malaria and the role of type I interferon signalling** Agustiningsih Agustiningsih, Life Sciences, Burnet Institute; PhD Student	
36	**Investigating the role of Kelch 13 protein in *Plasmodium falciparum* gametocytes** Haowen Deng, Department of Biochemistry and Pharmacology, University of Melbourne; PhD Student	
37	**Investigating the activation of new permeability pathways in *Plasmodium falciparum* infected erythrocytes** Nadine Djunaedi, Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences (MIPS), Monash University; PhD Student	

12:30 – 13:40	**Lunch and Poster Session I** (Includes transit time from and to Lecture Theatres) **Poster judging to be finalised by 13:20**	Cossar Hall
Poster Number	**Poster Details**	
38	**Antibody-dependent activation of NK cells as a potential correlate of protection from placental malaria: Insights from Malawi and PNG cohorts with varying infection status at enrolment** Yvonne Dube, Department of Infectious Diseases, University of Melbourne; PhD Student	
39	**Identification of B cell epitopes in serological exposure markers for improved *Plasmodium vivax* surveillance** Hanqing Zhao, Infection and Global Health, Walter and Eliza Hall Institute of Medical Research; PhD Student	
40	**Unfolding malaria parasite biology: targeting protein disulphide isomerases to block *Plasmodium* invasion and transmission** Senna Steen, Life Sciences, Burnet Institute & Monash University; PhD Student	
41	**γδ T cell receptor recognition of CD1d in a lipid-independent manner** Michael Rice, Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute, Monash University; Early Career Researcher	
42	**Runx3 acts as a rheostat for CD8+ tissue-resident memory T cell formation in the liver** James Dosser, Peter Doherty Institute for Infection and Immunity, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
43	**Exploration of small intestine and colon plasma cell survival heterogeneity** Youngmin Lee, Department of Immunology, Monash University; Honours Student	
44	**Investigating T cell responses to a nested peptide** Georgia Dow, Department of Biochemistry and Chemistry, La Trobe University; PhD Student	
45	**Immune Cell Robbery: Tracking Dendritic Cell-to-B Cell Material Transfer** Laura Almagro, Bio21 Institute & Department of Biochemistry and Pharmacology, University of Melbourne; PhD Student	
13:20	**Judging to be finalised**	
13:40	**[Return to main program above]**	

Poster Session II: 5.30 pm – 6.45 pm

17:30 – 18:45	**Evening Networking and Poster Session II** (Includes transit time from Lecture Theatres) **Poster judging to be finalised by 18:20**	Cossar Hall
Poster Number	**Poster Details**	
46	**Evaluating cell:cell antiviral protection by *Wolbachia* in *Aedes aegypti* cells** Katrina Ibay, Life Sciences, Burnet Institute; PhD Student	
47	**Investigating the mechanism of viral control in HIV-infected individuals** Jamie Tuibeo, La Trobe Institute for Molecular Science (LIMS), La Trobe University; PhD Student	
48	**Understanding viral and host determinants that influence infection outcomes for influenza A virus in human macrophages** Kevin Tang, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
49	**Differential temporal immune responses to influenza A virus and PRR stimulation in lung epithelial versus macrophage cells** Ameanah El-Hennawi, School of Health and Biomedical Sciences, RMIT University; PhD Student	
50	**Identifying novel antibody biomarkers of Asian-Pacific *Anopheles* vector bite exposure for malaria transmission surveillance** Ashleigh Heng-Chin, Life Sciences, Burnet Institute; PhD Student	
51	**Defining conserved sites of vulnerability on the Influenza B HA Using Human Monoclonal Antibodies** Yee-Chen Liu, Peter Doherty Institute for Infection and Immunity, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
52	**Modelling a microphysiological system to mimic pig lymph nodes** Dijina Swaroop, Australian Centre for Disease Preparedness (ACDP), CSIRO; Early Career Researcher	
53	**Lymph node entrapment of CD8+ T cells underlies lymphopenia in severe influenza disease** Aira Cabug, Peter Doherty Institute for Infection and Immunity, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
54	**Further insights into asymptomatic outcome after SARS-CoV-2 in HLA-B*15:01+ individuals** Lawton Murdolo, Department of Biochemistry and Chemistry, La Trobe University; PhD Student	
55	**Selective Endocytosis-mediated Omicron S1-RBD Internalization Revealed by Reconstitution of ACE2-S1-RBD Interaction on Micropatterned Membrane Substrates** Angelin Philip, College of Health and Life Sciences, Hamad Bin Khalifa University, Qatar; Early Career Researcher	
56	**Preclinical Evaluation of Cytokine-Adjuvanted MVA Vaccines Encoding SARS-CoV-2 Antigens** Wanxiaojie (Jessie) Xie, Department of Microbiology and Immunology, University of Melbourne; Early Career Researcher	
57	**Leveraging RNA-based approaches to develop new therapies and discovery tools to overcome current treatment limitations for HTLV-1** Lena Scherer, Infection and Global Health, Walter and Eliza Hall Institute of Medical Research; PhD Student	

17:30 – 18:45	**Evening Networking and Poster Session II** (Includes transit time from Lecture Theatres) **Poster judging to be finalised by 18:20**	Cossar Hall
Poster Number	**Poster Details**	
58	**The investigation of the T cell receptor diversity of skin-resident and circulating CD8+ T cells in psoriasis patients** Jingran Ye, Monash Biomedicine Discovery Institute, Monash University; PhD Student	
59	**Persistent low antigen in chronic infections sustain a potent CD8⁺ effector T cell response which leads to severe immunopathology** Sining Li, Peter Doherty Institute for Infection and Immunity, University of Melbourne; PhD Student	
60	**A Novel Targeted Regulatory T Cell Therapy for HLA-DR3-associated Systemic Lupus Erythematosus** Jessica Whui Yuin Wu, School of Clinical Sciences at Monash Health, Monash University; PhD Student	
61	**Understanding the Development of Terminally Exhausted T cells** Kwan Ho Leung, Peter Doherty Institute for Infection and Immunity, University of Melbourne; Masters Student	
62	**γδ T cell-derived IL-4 initiates CD8 T cell immunity** Shirley Le, Peter Doherty Institute for Infection and Immunity, University of Melbourne; PhD Student	
63	**Ly108 Preserves CD8⁺ T Cell Fitness Under Chronic Antigen Stimulation** Louis Lee, Peter Doherty Institute for Infection and Immunity, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
64	**miRNA expression of bovine CD4+, CD8+ and γδ T cells** Georgia Kennedy, Australian Centre for Disease Preparedness (ACDP), CSIRO & Charles Sturt University; PhD Student	
65	**Mechanisms of Antigen Presentation and CD8 T cell Priming by mRNA Vaccines** Atefeh Yaftiyan, Peter Doherty Institute for Infection and Immunity, University of Melbourne; PhD Student	
66	**Crohn's associated invariant T cells recognise small benzofuran-sulfonate molecules with self-lipids on CD1d** Alison White, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
67	**Harnessing the immunotherapeutic potential of MAIT cells with a novel MAIT TCR nanobody** Ryan Harper, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
68	**Identification of the skin cells specialised in MR1 antigen presentation to MAIT cells** Atieh Mousavizadeh, Peter Doherty Institute for Infection and Immunity, University of Melbourne; PhD Student	
69	**Identification of checkpoint markers, cancer pathways and cancer genes in a mouse model of chronic colitis: Implications for new cancer biomarkers** Ramya Ephraim, School of Health and Biomedical Sciences, RMIT University; PhD Student	

17:30 – 18:45	**Evening Networking and Poster Session II** (Includes transit time from Lecture Theatres) **Poster judging to be finalised by 18:20**	Cossar Hall
Poster Number	**Poster Details**	
70	**Bispecific antibody approach to harness dendritic cells in cancer immunotherapy** Marco Velázquez Delgado, Monash Biomedicine Discovery Institute, Monash University; Masters Student	
71	**Loss of expression of the tumour surveillance protein NLRC5 in chief cells undergoing transdifferentiation in *Helicobacter pylori*-associated carcinogenesis** Xiaohu Zhao, Centre for Innate Immunity and Infectious Diseases (CiiiD), Hudson Institute of Medical Research & Monash University; PhD Student	
72	**Cancer-Microbiome interface: Novel Phage Therapy by Targeting *Fusobacterium nucleatum* in Gastrointestinal Cancer** Birhanu Jemere, Olivia Newton-John Cancer Research Institute (ONJCRI) & La Trobe University; PhD Student	
73	**Sex-specific immune resilience to DSS-induced colitis in the Nlgn3R451C mouse model of autism** Angela Renata Jimenez-Perez, School of Health and Biomedical Sciences, RMIT University; PhD Student	
74	**Functional Assessment of the NOD2 Signalling Pathway in Patients with Inborn Errors of Immunity** Ebony Blight, Department of Immunology, Monash University; PhD Student	
75	**Temporal proteomic profiling reveals novel IRAK3 interactors in Toll-like receptor signalling** Cassandra Cianciarulo, Holsworth Biomedical Research Centre, La Trobe University; PhD Student	
76	**The protective effect of breastfeeding on infant inflammation: a mediation analysis of the plasma lipidome and metabolome** Toby Mansell, Inflammatory Origins, Murdoch Children's Research Institute; Early Career Researcher	
77	**Immune Cell Necroptosis Restrains Cutaneous Inflammation: Implications for Therapeutic Targeting** Holly Anderton, Inflammation, Walter and Eliza Hall Institute of Medical Research; Early Career Researcher	
78	**Structural Insights into KvrA: A MarR Homologue that Senses and Responds to Copper Stress** Stephanie Penning, La Trobe Institute for Molecular Science (LIMS), La Trobe University; PhD Student	
79	**The *Burkholderia* T6SS-5 and oxidative stress responses during infection** Jiaxing Yang, Hudson Institute of Medical Research & Department of Molecular and Translational Science, Monash University; PhD Student	
80	**Investigating the role of O-GlcNAcylation in regulating Dendritic cell maturation** Bjorn Wee, Department of Biochemistry and Pharmacology, University of Melbourne; PhD Student	
81	**Role of Ubiquitination in *Toxoplasma* Differentiation** Karan Singh, Infection and Global Health, Walter and Eliza Hall Institute of Medical Research; PhD Student	

17:30 – 18:45	**Evening Networking and Poster Session II** (Includes transit time from Lecture Theatres) **Poster judging to be finalised by 18:20**	Cossar Hall
Poster Number	**Poster Details**	
82	**Understanding metabolic sensing during *Toxoplasma* differentiation** Amber Simonpietri, Infection and Global Health, Walter and Eliza Hall Institute of Medical Research; PhD Student	
83	**Mapping protein complexes to reveal new functions in the malaria parasite** Zhaochun Li, Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences (MIPS), Monash University; PhD Student	
84	**Characterising monoclonal antibodies to placental malaria antigen, VAR2CSA** Vivin Kokuhennadige, Peter Doherty Institute for Infection and Immunity, Department of Infectious Diseases, University of Melbourne; PhD Student	
85	**Cross-reactive Immunity to VAR2CSA Antigen in Placental Malaria** Pragya Kumar, Department of Medicine at Royal Melbourne Hospital, University of Melbourne; Masters Student	
86	**Uncovering the mechanism of action of second generation bis-triazines, a potent new class of antimalarials** Jennifer Le, Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences (MIPS), Monash University; PhD Student	
87	**Improving the capacity of vaccine-induced antibodies to arrest the growth of *Plasmodium falciparum*** Alysha Literski, Burnet Institute & School of Translational Medicine, Monash University; PhD Student	
88	**Antibody-dependent neutrophil phagocytosis of *Plasmodium falciparum* infected erythrocytes is mediated by FcγRIIa** Maria Saeed, Department of Infectious Diseases, University of Melbourne; PhD Student	
89	**Metabolic Tracing in *P. falciparum* Using a Stable Isotope Labelling Strategy** Junwei Tang, Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences (MIPS), Monash University; PhD Student	
90	**Hydrogel Loaded with Endometrial Mesenchymal Stem Cells Alleviates Birth Injury in a Sheep Model** Ayenew Abawa, Hudson Institute of Medical Research & Department of Obstetrics and Gynaecology, Monash University; PhD Student	
18:20	**Judging to be finalised**	
18:45 – 19:00	**Prizes, Acknowledgements and Conclusion** **Presented by VIIN Co-Convenors:** **Prof Gilda Tachedjian and Prof Richard Ferrero**	Cossar Hall

ABSTRACTS
SESSION 1
Oral Presentations

Defining how macrophages drive OLAH-mediated life-threatening influenza disease

Deborah Gebregzabher[1*], Xiaoxiao Jia[1], Jeremy Crawford[2], Svenja Fritzlar[1], Alice Trennery[1], Hayley Mcquilten[1], Thi Nguyen[1], Lukasz Kedzierski[1], Jason Mackenzie[1], Patrick Reading[1], Sarah Londrigan[1], Paul Thomas[2,5], Jianqing Xu[3], Zhongfang Wang[1,4], Brendon Chua[1], and Katherine Kedzierska[1,5]

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[3]Shanghai Public Health Clinical Centre and Institutes of Biomedical Sciences, Key Laboratory of Medical Molecular Virology of Education/Health, Shanghai Medical College, Fudan University, Shanghai, China
[4]State Key Laboratory of respiratory Disease and National Clinical Research Centre for Respiratory Disease, Guangzhou Medical University, Guangzhou, China
[5]Center for Influenza Disease and Emergence Response (CIDER), USA

Influenza A virus (IAV) infections represent a significant global health challenge, contributing to >500,000 deaths annually prior to the COVID-19 pandemic. Although severe disease predominantly affects high-risk individuals, a substantial proportion of hospitalized patients lack identifiable risk factors, indicating that host determinants of disease severity remain poorly understood. Our blood transcriptomics of A/H7N9-infected patients identified elevated expression of oleoyl-ACP-hydrolase (OLAH), an enzyme involved in fatty acid biosynthesis, in individuals that died. Subsequent studies in *olah*$^{-/-}$ mice demonstrated a role for OLAH in promoting severe influenza outcomes, with macrophages implicated as key mediators. In this study, we aimed to define the mechanisms by which macrophages drive severe OLAH-mediated influenza disease. Macrophages from *olah*$^{-/-}$ mice infected with IAV *in vitro* exhibited reduced viral antigen expression and production of inflammatory mediators compared to those from wild-type (WT) mice. Supplementation with oleic acid, the primary catalytic product of OLAH, restored viral antigen expression and cytokine responses in these cells. As oleic acid can inhibit IFITM3, an interferon-stimulated antiviral mediator, we examined its effects in *ifitm3*$^{-/-}$ macrophages. Unlike WT macrophages, *ifitm3*$^{-/-}$ macrophages demonstrated no changes in viral antigen expression upon oleic acid treatment, implicating IFITM3 inhibition as a mechanism by which oleic acid exacerbates IAV infection. To delineate the role of lipid metabolism, we used TOFA and C75 to inhibit fatty acid synthase (the enzyme complex incorporating OLAH) and Beauveriolide I and ML-262 to inhibit lipid droplet formation. Influenza infection in WT macrophages was inhibited by fatty acid synthase inhibitors TOFA and C75, with C75 also affecting *olah*$^{-/-}$ macrophages. In contrast, lipid droplet inhibitors reduced infection only in WT macrophages, indicating that OLAH-mediated disease severity relies on lipid droplet utilisation. Collectively, our findings provide key insights into the role of macrophages in OLAH-mediated severe influenza disease, potentially constituting a target for protection against life-threatening influenza.

Determinants of innate immune cytokine responses in pre-school children

Rutger Röring[1*], Toby Mansell[1], Rachel Morgan[2], Gabbi MacKechnie[1], Katherine Lange[1], Prue Huntington[1], Tong Chen[1], Minh Bhui[2], Thao Tran[2], Anne-Louise Ponsonby[1, 3], Fiona Collier[2], Mimi Tang[1], Petter Brodin[4, 5], Peter Sly[6], Melanie Neeland[1], Aaron Weinman[1], Jim Buttery[1], Boris Novakovic[1], Richard Saffery[1], Luba Sominsky[2], Peter Vuillermin[2], David Burgner[1], on behalf of the Barwon Infant Study Investigator Group

*[1]Murdoch Children's Research Institute, Parkville, Victoria, Australia; [2]Institute for Mental and Physical Health and Clinical Translation (IMPACT), School of Medicine, Deakin University, Geelong, Victoria, Australia; [3]The Florey Institute of Neuroscience & Mental Health, Parkville, VIC, Australia; [4]Department of Women's and Children's Health, Karolinska Institutet, 17165 Solna, Sweden; [5]Department of Immunology and Inflammation, Imperial College London, London, UK; [6]Child Health Research Centre, Children's Health and Environment Program, The University of Queensland, South Brisbane, Queensland, Australia; *presenting author*

The magnitude of innate immune responses varies considerably between individuals and is subject to modulation by host-intrinsic factors and environmental exposures. Inappropriately strong immune responses and chronic inflammation drive noncommunicable disease pathogenesis throughout the life course, starting in childhood. Optimising immune responses in early life is thus an attractive avenue for preventing disease in adulthood. This necessitates gaining a better understanding of the mechanisms and determinants of early-life immune responses (including trained immunity), a field that is currently data-scarce.

To that end, we studied the innate immune cytokine responses of healthy children. The whole blood of 290 healthy 4-year-old children from the Barwon Infant Study (BIS) was stimulated with 8 bacterial and viral ligands. We measured 13 cytokines/chemokines in the supernatants. We observed substantial inter-individual variation in cytokine response strength. The strength and type of cytokine response differed for each stimulus. To increase statistical power by reducing the number of parallel comparisons, we identified co-expressing cytokine-'modules' using unsupervised clustering ('CytoMod'). Although the modules were unique for each stimulus, TNF, IL-6, IL-1β, and IL-10 tended to cluster together, as did IFNα/IFNγ, and IL-8, MCP-1, VEGF-A, and PDGF-BB. We investigated associations between module response strength and a range of host factors and environmental exposures and correlates of inflammation. We found weak evidence for associations between cytokine responses and sex, adiposity, and exact age. Markers of systemic inflammation (hsCRP, glycoprotein-acetyls, neutrophil-lymphocyte-ratio) were strongly associated with cytokine production, and infection pressure in the community coincided with seasonal peaks in particularly antiviral responses. Furthermore, we identified a number of genomic variants ('cytokine-QTLs') that affected the production of individual cytokines upon stimulation with LPS, PGN, cGAMP, or Poly(I:C). Finally, we used the rich data available in BIS to test if cytokine response strength is related to pre-school wheeze, an indicator of early-life respiratory health that often precedes asthma.

In summary, we contributed to resolving the data-gap regarding childhood immune responses. We studied cytokine responses at 4 years in BIS, a unique, deeply phenotyped childhood cohort. We provide novel translational data that will serve as a platform for understanding drivers, mechanisms and consequences of childhood inflammation.

Proteosome dependent degradation of type I interferon signalling factors during *Shigella flexneri* infection

Anita Chaulagain[1,2*], Yogeswari Chandran[1,3], Jaclyn Pearson[1,2,4,5], Cristina Giogha[1,2], Elizabeth Hartland[1,2]

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Shigellosis, caused by *Shigella flexneri*, remains a significant global health threat, particularly to children in low-income regions. An important aspect of *Shigella* pathogenesis lies in its ability to subvert host immunity through a type III secretion system (T3SS), which injects effector proteins into host cells that manipulate critical innate immune signalling pathways. While type I interferons (IFNs) are traditionally linked to antiviral defence, recent studies highlight their role in bacterial infections. Recent work has suggested that *Shigella* interferes with type I IFN signalling during infection and that T3SS effectors modulate this pathway. However, the range of effectors contributing to the evasion of type I IFN responses and their host targets remain unclear.

To address this, we systematically screened type I IFN components during *Shigella flexneri* infection of HeLa cells and found that *Shigella* induces cleavage of STING and IRF3 which are key regulators of type I IFN signalling. To determine the mechanism, we tested several different inhibitors targeting host cell transcription, translation, lysosomal degradation and caspases. These treatments did not prevent cleavage during infection. However, inhibition of the proteasome blocked both STING and IRF3 cleavage, indicating that this process is dependent on the host cell proteasome and may represent a host-mediated mechanism during *Shigella* infection. We are currently investigating the proteasomal mechanism responsible for this process.

Our work will help understand the host-pathogen interactions that enable *Shigella* to subvert type I interferon signalling. Exploring this mechanism will help to develop strategies that prevent *Shigella* from subverting type I IFN responses, thereby retaining immune function and enhancing host defence against *Shigella* infection.

Structural basis of DNA sliding clamp-mediated long-range gene silencing on a multi-drug resistance plasmid

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The discovery of antibiotics in the 20th century was one of the most significant advances in global health. Since their introduction, antibiotics have saved millions of lives from bacterial infections and extended the average human lifespan by more than two decades. However, widespread overuse and misuse have driven the emergence of antimicrobial resistance (AMR). Today, the WHO recognises AMR as one of the most urgent threats to global public health and food security.

A major driver of AMR is the horizontal spread of antibiotic resistance genes carried on plasmids. These extrachromosomal DNA elements can move between cells, and even across species, via a process known as conjugation. This highly efficient mode of gene transfer underpins the rapid global dissemination of resistance traits among pathogenic bacteria.

Examples of long-range gene regulation in bacteria are rare and generally thought to involve DNA looping. We used a combination of X-ray crystallography, biophysics and single-molecule analysis to investigate the KorB-KorA system in *Escherichia coli*. We show that long-range gene silencing on the plasmid RK2, a source of multi-drug resistance across diverse Gram-negative bacteria, is achieved cooperatively by the DNA-sliding clamp protein KorB, and the clamp-locking protein KorA. We show that KorB is a CTPase clamp that can entrap and slide along DNA to reach distal target promoters up to 1.5 kb away. We resolved the tripartite crystal structure of a KorB-KorA-DNA co-complex, revealing that KorA latches KorB into a closed clamp state. DNA-bound KorA thus stimulates repression by stalling KorB sliding at target promoters to occlude RNA polymerase holoenzymes. Together, our findings explain the mechanistic basis for KorB role switching from a DNA-sliding clamp to a co-repressor and provide an alternative mechanism for long-range regulation of gene expression in bacteria.

By uncovering the structural and mechanistic basis of plasmid gene silencing by the KorB-KorA system, our work provides new insight into how resistance genes are regulated on broad-host-range plasmids. Understanding this novel mode of long-range gene repression deepens our knowledge of plasmid biology and highlights potential strategies for controlling the spread and expression of antibiotic resistance determinants.

ABSTRACTS
SESSION 3
Oral Presentations

A Rapid Point-Of-Care Self-Test For Liver Disease

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Background: Liver disease is estimated to affect 1.5 billion people worldwide and is responsible for over 2 million deaths annually. Alcohol consumption, drug-induced liver-injury (DILI), fatty liver disease, and viral hepatitis all impact liver health. Measurement of the enzyme and key biomarker alanine aminotransferase (ALT), specifically in the liver-isoform ALT1, is essential for disease diagnosis, monitoring, and treatment evaluation. Liver disease is generally monitored by pathology testing of venous blood. Rural or resource poor settings such as developing countries may have limited access to pathology services. Point-of-care testing provides immediate results and may allow for more regular monitoring of liver enzymes and urgent intervention to prevent long-term liver damage. This study aimed to develop a point-of care self-test to monitor changes in ALT1 levels.

Methods: Novel anti-ALT1 monoclonal antibodies were generated in alpacas and rabbits. These antibodies were conjugated with microparticles to detect ALT1, producing a quantifiable signal that was interpreted by a lateral flow reader. The test was optimised with blood spiked with in-house produced recombinant ALT1, and clinical samples of patients with chronic liver disease or healthy volunteers, to achieve optimal antibody, conjugate, and running buffer conditions.

Results: The point-of-care self-test is housed in a fully integrated AtomoRapid^{TM} Pascal cassette, and utilises an in-built safely lancet, blood collection unit, and buffer delivery system. The lateral flow assay detects ALT1 in 10 µL finger-prick or venous blood with a time to result of 20 minutes. The test result can be read in a lateral flow reader to quantify ALT1 levels between 0 and 5 ng (equivalent to ~ 0-500 IU/L) and shows high correlation with the standard of care enzymatic ALT test (Spearman r = 0.76).

Conclusions: We have successfully developed and optimised a quantitative and fully integrated lateral flow assay to detect ALT1 in finger prick and venous blood. The convenient and inexpensive test allows for more frequent ALT1 monitoring, provides access to testing in rural and resource poor settings, and provides a point of care screening tool for monitoring of liver health in viral hepatitis, drug-induced liver injury, and other causes of liver pathology.

Evidence for repurposing pirfenidone to treat viral exacerbations of COPD based on a novel precision-cut lung slice model.

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Background**:** Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death globally. Viral-induced acute exacerbations (AECOPD), a major contributor to mortality, are amplified by current standard-of-care therapy, glucocorticoids (GCS). Pirfenidone (PFD), an anti-inflammatory, anti-fibrotic drug approved for idiopathic pulmonary fibrosis, may offer a novel alternative.

Aim**:** To develop a novel *ex vivo* model of AECOPD using precision cut lung slices (PCLS), a unique organotypic platform that maintains 3D lung architecture and resident cells, and to determine whether PFD can reduce inflammation in this disease-relevant context.

Methods**:** PCLS from naïve mice were left untreated (vehicle) or treated with elastase (2.5 ng/ml) for 16 hrs to induce an emphysema-like phenotype. PCLS were washed, left for 24 hr, then exposed to the viral mimetic poly I:C (10 ng/ml) to mimic viral-induced inflammation in the absence or presence of PFD (500 uM) for up to 48 hr. PCLS and conditioned media were analysed for viability, histopathology, alveolar gene expression, and inflammatory cytokines.

Results**:** Elastase treatment caused emphysema-like changes in PCLS alveoli, including a 25% increased size (elastase *cf* control, $p<0.01$) and decreased gene expression (*Aqp5*, *Sftpc*, *Rage*, $p<0.01$). Elastase-induced increases in inflammatory gene expression (*Il-6*, *Il-8*, *Tnf*a, $p<0.05$) and cytokines (IL-6, KC, $p<0.01$) were amplified by poly I:C (1.5-fold for IL-6; 2.2-fold for KC, elastase+poly I:C *cf* elastase alone, both $p<0.01$) and prevented with PFD (genes *Il-6, Il-8;* mediators IL-6, KC, $p<0.01$).

Conclusion**:** We have established a novel *ex vivo* PCLS model mimicking key features of AECOPD, namely an emphysema-like phenotype, reduced alveolar gene expression and amplified inflammation. Inhibition of inflammation by PFD in this model identifies a promising therapeutic alternative for AECOPD treatment. Validation of these positive findings in virally-infected human PCLS to establish superior efficacy relative to GCS will accelerate clinical repurposing of PFD to limit lung damage in AECOPD.

The voltage dependent ion channel is an essential mitochondrial protein in malaria parasites

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Despite global gains combating malaria, the increasing incidence of antimalarial drug resistance to front line therapeutics demands new drugs with novel targets be developed. Potential targets for the design of therapeutic drugs include channel proteins that are critical for the movement of essential cargo within the parasite. Here, the essentiality of the voltage dependent anion channel (VDAC) was investigated in the deadliest species of malaria *P. falciparum*, via the generation of a transgenic parasite line harbouring a *glmS* ribozyme switch to permit conditional knockdown of *vdac*. Additionally, epitope tagging of *vdac* facilitated both its localisation and affinity purification. Knockdown of *vdac* led to a survival defect in the asexual red blood cell stages. Furthermore, the failure to generate conventional *vdac* knockouts in both *P. falciparum* and rodent malaria species *P. berghei,* indicated VDAC is essential for parasite survival. Immunofluorescent microscopy localised VDAC to the mitochondria, whilst mass spectrometry and proteomic analysis of immunoprecipitated VDAC and its associated proteins validated the localisation of VDAC and its interaction with mitochondrial proteins. Additionally, VDAC was shown to associate with proteins in close proximity to the endoplasmic reticulum, consistent with the presence of mitochondrial-endoplasmic reticulum contact sites. VDAC knockdowns were shown to sensitise parasites to mitochondrial target drug Atovaquone as determined by half maximal growth assays. This provided further indication for a role at the outer mitochondrial membrane, possibly involving the transport of intermediates in the pyrimidine biosynthesis pathway. Whilst the precise role of VDAC at the mitochondria requires further investigation, this channel protein is an essential and unique target for the future design of novel antimalarial therapeutics.

Repurposing human medicines as novel antimalarials against *Plasmodium falciparum* malaria

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Pathologically relevant *Plasmodium falciparum* blood stage parasites have developed drug resistance to nearly all available antimalarials, highlighting the critical need for novel therapeutics to eliminate malaria. However, traditional drug discovery processes are often challenged with lengthy pre-clinical and clinical studies, and required substantial financial investments before the treatments can reach the clinic. Here, we proposed that regulatory-approved human medicines can be repurposed as novel antimalarials.

We used nanoluciferase-expressing *P. falciparum* (Hyp1-Nluc) parasites to screen a library of 634 FDA-approved medicines and found compounds that exhibited antimalarial activity, yielding 48 compounds exhibiting >80% growth inhibition when used at 2μM over 72 hours. Afterwards, 41 compounds with harmful side effects, unfavourable pharmacokinetic or pharmacodynamic properties were eliminated, yielding 7 lead compounds with IC_{50} values between 65 – 505 nM. The leading candidates' timing of action were then determined through stage-specific growth inhibition assays, where each compound was treated with one of ring, trophozoite, or schizont stage Hyp1-Nluc parasites for four hours, then incubated without drug until the parasites reached trophozoite stage to quantify growth post-treatment. This experiment revealed three candidates – CDL036, CDL176, CDL226 – exhibit activity against all three stages of the *P. falciparum* blood cycle.

We also attempt to discover the functional target of CDL036 and CDL176 through a standard *in vitro* resistance selection protocol by treating clonal 3D7 *P. falciparum* parasites with the compound over three on/off cycle. While we were unsuccessful in selecting for resistance-conferring mutations for both candidates using this method, these results indicated that CDL036 and CDL176 are potentially refractory to resistance, making them viable as treatment options for clinical malaria

In conclusion, we have shown that CDL036 and CDL176 can exhibit multi-stage activity against blood stage *P. falciparum* parasites and are refractory against field resistance, making them highly promising candidates that can be deployed as novel antimalarials. Further investigation can employ alternative methods to identify these candidates' functional target to prioritise their development for future clinical use.

ABSTRACTS
SESSION 4
Science Bites I

Validation of Solvent Proteome Profiling for Antimalarial Drug Target Deconvolution

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Malaria remains a global health threat, with rising drug resistance accelerating the urgent need for new therapeutics. Target elucidation is a critical step in antimalarial drug discovery, enabling a deeper understanding of the molecular mechanisms of action of both existing and novel compounds. This study validates solvent-induced proteome profiling (SPP) as a proteomics-based approach for identifying drug-protein interactions in *Plasmodium falciparum*. SPP detects shifts in protein stability induced by ligand binding, allowing the identification of drug target/s without the need for compound modifications. Here, we successfully generated solvent denaturation curves for the *P. falciparum* proteome, and demonstrated the utility of SPP with five antimalarial compounds: pyrimethamine, atovaquone, cipargamin, MMV1557817 and OSM-S-106. In addition to measuring each compound's impact across the full denaturation curve, investigating protein levels at individual solvent percentages preserved specific stability changes that would otherwise be masked in pooled analyses performed by integral SPP. This strategy was critical for the identification of the cipargamin target, non-SERCA-type Ca^{2+}-transporting P-ATPase (*Pf*ATP4). Notably, we propose live-cell treatment SPP as a novel approach, demonstrating its ability to identify the validated target of pyrimethamine, bifunctional dihydrofolate reductase-thymidylate synthase (*Pf*DHFR), with high specificity. We also introduced the novel one-pot mixed-drug SPP, which enables the evaluation of multiple drugs within a single lysate and experimental setup. This alternative method simplifies the experimental workflow and includes positive controls to affirm the performance of the experiment. Overall, this study demonstrates that SPP can be successfully applied in both lysate and live-cell treatment conditions to elucidate drug targets in *P. falciparum,* as well as providing additional information regarding the mechanisms of drug action, offering insights for the optimisation of existing antimalarials and the development of novel therapies.

Expansion microscopy reveals how Kelch 13 mutations impair feeding in artemisinin resistant *Plasmodium* parasites

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Resistance to the frontline antimalarial, artemisinin, poses a critical threat to global malaria control efforts. Artemisinin resistance is predominantly driven by mutations in the gene encoding the Kelch 13 (K13) protein. K13 plays a crucial role in the regulation of specialised double-membraned invaginations termed the cytostome; the apparatus which facilitates haemoglobin uptake from the host red blood cell into the *Plasmodium* parasite. As parasites progress through their asexual life cycle, they form more cytostomes to increase haemoglobin uptake rates. Haemoglobin digestion supplies essential amino acids and creates space within the red blood cell to facilitate parasite growth, while also releasing haem-iron as a waste byproduct which is required for the activation of artemisinin. Parasites harbouring K13 mutations exhibit slower feeding rates and reduced haem levels, leading to decreased artemisinin activation and reduced parasite death. However, the precise mechanism by which K13 mutations impair parasite feeding remains unclear, limiting our ability to combat this emerging threat.

We propose that mutations in K13 reduce its stability and abundance, impacting the formation and maintenance of new cytostomes and thus parasite feeding. Using ultrastructure expansion microscopy coupled with super-resolution microscopy, we resolved K13 as distinctive ring-shaped structures ~160 nm in diameter, localising to the parasite periphery surrounding cytostome necks. We also performed live-cell lattice light-sheet microscopy, enabling systematic analysis of cytostome dynamics across the asexual life cycle in mutant and wild-type parasites using an automated machine learning pipeline. Critically, K13 mutant parasites formed new cytostomes at significantly slower rates than wild-type controls and exhibited a 4-hour developmental delay. Quantitative analysis revealed that K13 mutant parasites formed 18% fewer K13 rings than wild-type controls despite producing similar numbers of progeny merozoites. Additionally, mutants displayed aberrant cytostome morphologies, correlating with decreased haemoglobin uptake efficiency.

These findings fundamentally advance understanding of artemisinin resistance by providing the first mechanistic explanation for K13-mediated feeding defects. This mechanistic insight enhances our ability to predict resistance emergence and could inform molecular surveillance strategies and partner drug selection in artemisinin combination therapies, ensuring therapeutic efficacy against resistant parasites.

Dual inhibition of Plasmepsins IX and X in *Plasmodium falciparum* sporozoites inhibits development within *Anopheles stephensi* mosquitoes

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Plasmepsin IX (PMIX) and X (PMX) are essential aspartyl proteases in all Plasmodium spp. that cleave many proteins required for egress and invasion of host cells across the lifecycle. The dual PMIX and PMX inhibitor WM382 prevents merozoites egress and invasion of erythrocytes, blocks transmission to mosquitoes and completely attenuates the egress of liver merozoites and erythrocytic infection. Despite PMIX being expressed in oocyst and PMX in sporozoites, their function in these stages of the parasite life cycle remains unknown. Treatment of mice with WM382 4-hours prior to intravenous injection with P. berghei sporozoites had no effect on the rate of liver stage infection nor did it cause any delay in the prepatent period. Since PMIX and PMX are expressed in the mosquito stages, what is their function? They might have an important role in the sporozoite development within the mosquito vector. We tested this hypothesis by dosing An. stephensi mosquitoes with WM382 beginning at oocyst stages and this significantly reduced the number of sporozoites located within the salivary glands. This suggests that PMIX and PMX processes substrates required for sporozoite egress from oocysts and/or invasion of salivary glands, such as MAEBL and AMA1 that contain a putative PMIX/X cleavage site. We will present our up-to-date understanding of the function of these aspartyl proteases in P. falciparum sporozoites as they develop within the mosquito host.

Development of next-generation mRNA vaccines against malaria

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Malaria is primarily caused by the *Plasmodium falciparum* and *vivax* species and remains a global health burden, causing over 200 million cases and nearly 600 thousand deaths across 83 countries in 2023, the majority of which are young children. Currently, there are only two vaccines (RTS, S/AS01 and R21/Matrix-M) that are approved for use in young children. These vaccines express the *P. falciparum* circumsporozoite surface protein (*Pf*CSP) as adjuvanted virus-like particles. However, their modest and waning efficacy against clinical malaria emphasises the urgent need for novel approaches in vaccine designs, vaccine platforms, and delivery. The recent success of messenger RNA (mRNA) vaccines highlights the potential to harness this platform for highly efficacious next-generation malaria vaccines. The mRNA platform has shown to be more efficient and robust for construct design and multi-antigen formulation compared to conventional platforms, and could be used to target both *Plasmodium* species, unlike the current malaria vaccines.

We aimed to validate the RTS,S/R21 malaria vaccine antigen using the mRNA platform as proof-of-concept, and to evaluate a novel multi-species mRNA vaccine combining the RTS,S/R21 antigen and two leading *P. vivax* vaccine candidates. We vaccinated C57BL/6 mice with our RTS,S/R21 mRNA and the multi-species multivalent mRNA in a three-dose regimen (28 days apart). IgG levels were monitored over 9 months to examine the immunogenicity and durability of the immune response. Our preliminary data have shown that the RTS,S/R21 mRNA induces high IgG levels against *Pf*CSP, while the multi-species multivalent mRNA induces IgG responses to all three antigens.

In this work, we have demonstrated that the mRNA platform can be used to combine antigens and generate a vaccine against the two major *Plasmodium* species that cause malaria in humans. In future studies, we will further evaluate vaccine-induced IgG subclasses and antibody functional activities that are known to be important in malaria immunity. We will also investigate other leading malaria vaccine candidates and emerging mRNA platforms, such as self-amplifying RNA and circular RNA, to evaluate their superiority to conventional mRNA. This work will inform the next-generation malaria vaccine design and contribute to the global malaria control and elimination goal.

PfPTRAMP-CSS as a multi-stage malaria vaccine target

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Malaria continues to cause significant global health burden. The disease is caused by parasites from the *Plasmodium* species, of which *P. falciparum* accounts for almost all cases and more than 600,000 deaths annually. Current malaria vaccines only target the pre-erythrocytic stage of the parasite and induce short-lived antibody responses. Next-generation malaria vaccines that can inhibit multiple parasite life-cycle stages and species are highly desired to progress malaria elimination. In *P. falciparum*, the pentameric PTRAMP-CSS-Ripr-CyRPA-Rh5 (PCRCR) complex is essential for invasion into human red blood cells. Rh5, CyRPA and Ripr are leading blood-stage vaccine candidates that have been well characterised in comparison to PTRAMP-CSS. Although nanobodies, single heavy chain variable domains derived from alpacas, raised to PTRAMP-CSS have been shown to be growth inhibitory, these were only of moderate efficacy. Furthermore, the expression and function of the PCRCR complex in both the pre-erythrocytic and mosquito-stages of the parasite, and whether we could potentially prevent transmission of malaria using PCRCR specific biologics has not been explored. Here, we characterise an extensive panel of nanobodies raised to the PTRAMP-CSS complex that are more potent than any described to date. We identified three invasion inhibitory epitopes on CSS and two on PTRAMP that are highly conserved across *P. falciparum* strains. The crystal structures of all inhibitory nanobodies to PTRAMP-CSS and two non-inhibitory nanobodies to PTRAMP have been solved which has significant implications for structurally guided design of immunogens to the PCRCR complex. We have additionally engineered nanobody-Fc (Nb-Fc) fusion proteins and have found that PTRAMP-CSS specific Nb-Fcs are able to prevent transmission of the parasite in the mosquito stages. This highlights the potential of the PCRCR complex as a multi-stage vaccine target.

Enhanced immunogenicity in mouse by recombinant BCG prime and protein boost based on latency antigen Rv1733c and/or reactivation antigen RpfE

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Background: *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) is the only licensed vaccine against tuberculosis (TB), but it fails to prevent the establishment and reactivation of latent TB. Overexpression of latency and reactivation antigens by BCG may enhance its efficacy against latent infection and subsequent reactivation. This study aimed to construct recombinant BCG strains (rBCGs) overexpressing the latency antigen Rv1733c and/or resuscitation promoting factor E (RpfE).

Methods: Episomal (pJH222) or integrative (pJH223) plasmids–either empty or encoding *rv1733c* or *rpfE*–were transformed into a lysine auxotroph strain of BCG for lysine complementation. The resulting rBCGs were genetically and phenotypically characterized. Female BALB/c mice underwent a six-month vaccination regimen consisting of a homologous prime-boost immunisation with the rBCGs, followed by a heterologous boost with Rv1733c and/or RpfE recombinant proteins. Splenocytes from immunised mice were stimulated *ex vivo* with purified protein derivative (PPD) or the corresponding recombinant protein(s) to measure secreted cytokines.

Results: The rBCGs carrying episomal recombinant plasmids encoding *rv1733c* or *rpfE* elicited higher levels of antigen-specific interferon-gamma (IFN-γ) compared to empty-vector controls. rBCG strains with integrative constructs encoding either antigen induced higher levels of tumour necrosis factor alpha relative to the sham, wild-type BCG, and empty-vector groups. All rBCGs expressing either or both antigens generated more favourable IFN-γ to interleukin (IL)-10 ratios upon stimulation with their respective antigen(s) than with PPD. While two rBCGs elicited detectable IL-4 responses, the rBCGs expressing both antigens did not induce IL-4.

Conclusions: This study demonstrates promising immunogenicity of the latency antigen Rv1733c and the resuscitation antigen RpfE, highlighting their potential to enhance BCG efficacy through a tailored prime-boost vaccination strategy.

Multi-Dot/Icm effector deletion mutants reveal distinct host transcriptional responses in *Legionella pneumophila* infected macrophages

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Legionella pneumophila is the causative agent of Legionnaires' disease, an accidental and severe form of pneumonia. *L. pneumophila* replicates within alveolar macrophages by establishing an intracellular niche termed the Legionella containing vacuole (LCV). LCV biogenesis requires the Dot/Icm type IV secretion system that translocates more than 330 bacterial effector proteins into the infected host cell. Many Dot/Icm effectors manipulate host processes through novel mechanisms. Thus, the functional characterization of the effectors may aid in the understanding of host-pathogen biology, as well as the discovery of molecular tools that can be adapted in research or therapeutics. However, to date, only a minority of effectors have been fully characterized and redundancy among effector protein activities and host targets has complicated the characterization of individual effector proteins.

To explore the collective impact of Dot/Icm effectors on host cell processes, we created a library of 14 *L. pneumophila* genomic mutant strains lacking effector-rich regions (a total of 85 effector genes deleted) and used the multi-deletion mutants to interrogate macrophage transcriptional responses during infection. While none of the mutants exhibited a defect in intracellular replication in PMA-differentiated THP-1 cells, we observed distinct transcriptomic responses in macrophages infected with different mutant strains. Whereas wild-type *L. pneumophila* 130b triggered a strong inflammatory and cellular stress transcriptional response in macrophages, six mutants displayed a reduced interferon response compared to wild-type infection, suggesting an effector-triggered immunity. Further, one mutant displayed a marked decrease in the global expression of host heat shock response (HSR) genes. Through the creation of further sub-deletions, we identified the Dot/Icm effector kinase LegK4 as responsible for the induction of a global host HSR. We confirmed that the kinase activity of LegK4 induced a global HSR via the canonical transcription factor heat shock factor 1 (HSF1). Infection of THP-1 macrophages deficient in *Hsf1* revealed the importance of the host HSR to the early stages of *L. pneumophila* infection. Altogether, these results reinforce the utility of multi-gene deletions to identify Dot/Icm effector phenotypes and the importance of the host HSR to *L. pneumophila* infection.

Reprogramming Bacterial Autotransporters: Turning Pathogenesis into Therapeutics

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Autotransporters (ATs) are bacterial nanomachines that drive virulence through diverse mechanisms including immune evasion, adhesion, and biofilm formation [1-3]. Evolving alongside us for millennia, ATs represent a versatile molecular toolkit that bacteria use to manipulate human biology. Although widespread across many pathogens, functional studies have largely focused on Escherichia coli, leaving many ATs from clinically relevant species unexplored [4]. This project both exploits the wealth of accumulated knowledge for well-characterised ATs and uncovers new mechanisms for poorly characterised ATs.

Our multidisciplinary approach spans structural biology, biophysics, cell biology, and microbiology to both understand and exploit the molecular mechanisms by which ATs contribute to pathogenesis. First, we exploit the extensive knowledge of *E. coli* ATs to engineer the protein Pet for intracellular drug delivery. Second, we investigate under-characterised ATs from Bordetella pertussis, the causative agent of whooping cough.

We have reprogrammed Pet to deliver therapeutic peptides into epithelial cells, triggering targeted, cargo-dependent cell death. This proof of principle will lead the way into using this platform to deliver other peptide drugs, increasing their efficacy by delivering them directly into the cytoplasm. Meanwhile, our phylogenetic classification revealed that Vag8 and TcfA, two adhesins from *B. pertussis*, likely employ distinct mechanisms. Vag8 combines local host adhesion with remote immune suppression via complement inhibition, facilitated by its presence in outer membrane vesicles [5-6]. In contrast, TcfA is ungrouped, highly specific to *B. pertussis*, and lacks homologs. Our studies have found that TcfA is functionally competent, promoting host cell adhesion, yet exhibits no distinct structural features (via multiple methods of structural determination), pushing us to rethink rigid structure-function paradigms.

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Bacterial membrane vesicles promote horizontal gene transfer in *Helicobacter pylori*.

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Numerous pathogenic bacteria are naturally competent. *Helicobacter pylori* is a human gastric pathogen that is highly diverse and relies on natural transformation to acquire antimicrobial resistance genes, adapt to the human host and promote lifelong colonisation. However, we have limited understanding of factors that influence the efficiency of *H. pylori* to naturally acquire DNA encoding AMR and promote genetic diversity. Bacterial membrane vesicles (BMVs) have emerged as a novel mechanism used to package DNA, mediate horizontal gene transfer (HGT) and disseminate AMR within and between bacterial species. However, the contribution of BMVs to the development of AMR within *H. pylori* remains unknown and was examined as part of this study.

BMVs were isolated from *H. pylori* strains harbouring AMR genes within the genome. The size and morphology of BMVs isolated from each strain were examined by transmission electron microscopy and nanoparticle tracking analysis, and DNA associated with BMVs was quantified. Natural transformation of a range of *H. pylori* clinical isolates using BMVs isolated from *H. pylori* strains encoding AMR resulted in the generation of antimicrobial-resistant *H. pylori* transformants. Furthermore, BMVs showed varying strain-specific efficiency in transforming *H. pylori* compared to transformation of *H. pylori* with genomic DNA alone. In addition, the mechanisms whereby BMVs deliver AMR genes into recipient bacterial cells are currently being investigated using a range of molecular and imaging techniques.

Collectively, these findings reveal a novel mechanism whereby *H. pylori* can acquire AMR mediated by BMVs. Further understanding of the mechanisms whereby BMVs orchestrate horizontal gene transfer within and between bacterial species will advance knowledge regarding AMR transmission within and between microbial communities.

Identification of immunomodulatory proteins in *Helicobacter pylori*-derived extracellular vesicles.

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PUBLISH CONSENT WITHHELD.

Light-induced circadian rhythm disruption protects the host from *Clostridioides difficile* infection

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The brain, intestinal functions and normal gut microbiota communicate through bidirectional signalling pathways known as the gut-microbiota-brain axis to maintain health and homeostasis (1). The gut-microbiota-brain axis is also regulated by circadian rhythms which optimise almost all biological processes to the natural 24-hour light/dark cycle (2). Many gut components which oscillate according to a circadian rhythm such as the microbiota are also central to the pathogenesis of many gut bacteria (2). However, research is lacking about how circadian rhythms, gut infections and the gut-microbiota-brain axis interplay. One infectious gut pathogen is *Clostridioides difficile* which colonises the colon during gut dysbiosis (3). We have previously shown that *C. difficile* infection (CDI) can dysregulate gut circadian rhythms, suggesting that CDI may interact with brain-coordinated rhythms (López-Ureña, Mileto & Lyras, unpublished). To examine this, mice were housed in 24-hour constant light rather than a standard 12-hour light/12-hour dark cycle to disrupt their circadian rhythms before infection with *C. difficile*. In constant light, clinical severity scoring of CDI symptoms including diarrhoea and weight-loss were significantly 2.57-fold lower and delayed by ~12 hours compared to infected mice in a standard light/dark cycle. Furthermore, while all infected mice in standard light/dark conditions did not survive past 36-hours post-infection, constant light enabled 40% survival by 48-96-hours post-infection with significantly reduced colon and caecal damage in surviving mice. This accompanied a significant ~25% reduction in *C. difficile* numbers, ~40% reduction in *C. difficile* toxin in faeces and increase in beneficial short-chain fatty acid-producing commensal gut bacteria. This is the first study to construct a mouse model incorporating CDI and light-induced circadian rhythm disruption and to show that constant light reduces and delays symptoms, increases survival and protects mice from CDI. These results demonstrate that external lighting can impact host-*C. difficile* interactions which is particularly relevant to infection in modern environments where artificial lights replace natural light cycles. It also suggests that acute host circadian rhythm disruption may perturb rhythmic fluctuations in periods of vulnerability to infection and exploiting this via light therapy may be an innovative strategy for resolving CDI and other gut infections of healthcare concern.

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AIMing for Streptococcus pyogenes-specific memory T cells before and after experimental human challenge

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S. pyogenes, or Group A *Streptococcus* (GAS), is a ubiquitous human-restricted bacterium causing skin, throat, and invasive infections as well as autoimmune sequelae including rheumatic heart disease. Collectively, these diseases claim over half a million lives annually. There is an urgent need to develop an effective GAS vaccine, however a lack of knowledge of cellular and antibody-mediated immune memory against GAS continues to impede vaccine development. Here, we provide novel insight into CD4+ memory T cell (Tmem) responses following human experimental pharyngeal challenge of healthy adults with *emm75 S. pyogenes* (n=25). We developed a multiplexed activation-induced marker (AIM) assay and flow cytometry-based immunophenotyping panel to characterise the specificity of CD4+ Tmem specific for 4 leading candidate vaccine protein antigens (SLO, SpyCEP, ScpA, and SpyAD) before and after infection. We detected AIM+ Tmem specific for all antigens both before and after challenge. While baseline AIM+ frequencies did not differ between participants who did or did not develop pharyngitis, only participants with pharyngitis showed a transient increase in AIM+ Tmem in blood one week later. Acute increases in AIM+ frequency relative to baseline were correlated between antigens and were dominated by regulatory T cell, Th17, and Th17.1 subsets. Taken together, we found that pharyngitis elicited antigen-specific CD4+ Tmem responses, but that circulating pre-existing Tmem did not affect infection severity. Our results show these vaccine antigens are immunogenic, further supporting their inclusion in vaccine development. However, they suggest that immunisation, like infection, may recall a regulatory T cell-dominated response, which could impede vaccine efficacy.

Barbet: A Deep Learning Approach to Bacterial Taxonomic Classification

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The determination of the species of a bacterium from its genome sequence is an essential early step in bioinformatic workflows, both for quality control and for automatically deciding on proceeding species-specific analyses to perform. Many approaches to this problem either require very large databases (more than available RAM), have high computational requirements, or have long running time. We developed Barbet, a novel deep learning tool built on state-of-the-art protein language models and a hierarchical neural network architecture, to classify bacterial genomes with high accuracy and low resource usage. The latest model was trained on 732,475 labelled bacterial genomes in GTDB r226, leveraging large-scale datasets to capture taxonomic signals. We detail the design, training regimen, and validation approach used to ensure robust performance across diverse bacterial taxa. Barbet demonstrates the high performance for a deep learning-based method for bacterial taxonomic classification, while using significantly less computational resources. During model validation, Barbet consistently achieved higher accuracy (>99% genus, >97% species) on a much larger taxonomy than previous deep learning-based classifiers. The barbet model is 1.9GB which is around 99% smaller than the equivalent GTDB dataset, uses a fraction of the memory, and can be GPU accelerated. The Barbet model weights, architectures, and training process is open allowing users to train custom Barbet models on their own dataset. Our findings underscore the potential of advanced deep learning techniques in microbial genomics. Barbet delivers a powerful, open-source solution for bacterial taxonomic classification, facilitating accurate and large-scale analyses of new genomes.

ABSTRACTS
SESSION 5
Oral Presentations

Revealing dose and repeat effects of a novel Influenza vaccine on humoral immunity

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Targeted Depletion of CCR6$^+$ Cells Elicits Robust Therapeutic Effects Across Multiple Autoimmune Diseases

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Leukocyte migration to sites of inflammation is essential for immune defence and maintaining homeostasis. However, an excessive or chronic influx of immune cells into specific tissues can trigger immune-mediated inflammatory conditions, including autoimmune diseases. This process is regulated by chemokine receptors on immune cells, making them attractive therapeutic targets for inhibiting the migration of pathogenic immune cells and treating inflammation. Among these, the chemokine receptor CCR6 is predominantly expressed on IL-17-producing Th17 cells and B cells, directing their migration to inflamed tissues and contributing to the development of autoimmune diseases such as scleroderma, psoriasis, and rheumatoid arthritis. Consequently, CCR6 has emerged as a promising therapeutic target for treating Th17-mediated inflammatory and autoimmune disorders.

In this study, we performed an “immunological surgery” to selectively eliminate pathogenic CCR6$^+$ cells using a Fc-engineered anti-human CCR6 monoclonal antibody (anti-hCCR6 mAb) and evaluated the therapeutic potential of this targeted depletion strategy in preclinical mouse models of scleroderma, psoriasis and rheumatoid arthritis, utilising human CCR6 knock-in mice. Our results showed that selective depletion of CCR6$^+$ cells through anti-hCCR6 mAb treatment exhibited remarkable efficacy in reducing disease severity across all models. Specifically, in a bleomycin-induced scleroderma model, treatment with anti-hCCR6 mAb significantly alleviated sclerotic skin phenotypes, and lung inflammation and fibrosis associated with scleroderma, compared to mice treated with an isotype control mAb. In the imiquimod-induced psoriasis model, administration of anti-hCCR6 mAb markedly reduced skin thickening, epidermal hyperplasia, and dermal infiltration of inflammatory cells. In the collagen-induced arthritis (CIA) model, anti-hCCR6 mAb treatment significantly alleviated all signs of joint inflammation. Notably, our mAb treatment resulted in a substantial reduction in the infiltration of key pathogenic leukocyte subsets, including IL-17-producing Th17 cells and γδ T cells, within the affected tissues and draining lymph nodes.

Our findings suggest that CCR6-targeted depletion therapy is a promising and effective strategy for treating autoimmune disorders. This approach holds significant therapeutic potential not only for the conditions studied but also for other IL-17-mediated inflammatory pathologies, offering hope for patients with unmet medical needs.

Single-Cell Analysis Reveals the Precise Timing of T Lineage Commitment in the Thymus.

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Early thymic progenitors (ETPs) seed the thymus with multipotent potential but are directed toward the T cell lineage by the thymic microenvironment. ETPs are double negative (DN) for CD4 and CD8 and are defined as $Kit^{+}CD44^{+}CD25^{-}$, subsequently upregulating CD25 as they progress to the DN2a stage. Our current understanding suggests that T cell lineage commitment, during which progenitor-associated genes are silenced, occurs during the transition from DN2a to DN2b, marked by downregulation of Kit and upregulation of the transcription factor *Bcl11b*. However, it remains unclear which non-T lineages develop intrathymically and precisely when their potential is lost. This question is further complicated by the heterogeneity of haematopoietic progenitors that migrate to the thymus, which exhibit varying lineage potentials.

To define the timing of T cell lineage commitment in the thymus more precisely, we profiled the DN stages of T cell development at single-cell resolution. We identified a novel transcriptionally distinct DN2a subpopulation expressing high CD25 and Kit but low CD90, while maintaining multipotency-associated markers including *Spi1* (PU.1), *Irf8*, and the haematopoietic stem cell-associated marker CD34 at levels comparable to ETPs. Both ETPs and this novel subpopulation were transcriptionally more similar to multipotent bone marrow precursors than to later thymocyte stages. *In vitro* differentiation assays revealed that this novel subpopulation was more developmentally advanced than ETPs but less advanced than other DN2a thymocytes. We also found that ETPs can give rise to B, natural killer (NK), and myeloid cells both *in vitro* and *in vivo*, consistent with previous studies. In the novel DN2a subpopulation, B cell potential was lost, while NK and myeloid potentials were retained. In contrast, all other DN2a thymocytes were fully committed to the T cell lineage.

These findings challenge the prevailing view that T cell lineage commitment occurs at the DN2b stage. Instead, DN2a thymocytes are heterogeneous, comprising a multipotent subpopulation alongside another that has entirely silenced non-T cell lineage potential. Our study precisely defines when the T cell transcriptional program becomes irreversibly fixed and provides new insights into the timing of T cell lineage commitment.

Targeted Regulatory T Cells to Treat Autoimmune Small Vessel Vasculitis

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Antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV) is a rare autoimmune disease characterized by the inflammation of small vessels, mainly affecting the kidneys. Myeloperoxidase (MPO) is the most common ANCA autoantigen and MPO-AAV patients with the HLA type DRB1*04:05 have the worst disease prognosis, including higher rate of renal failure. Current treatment relies on a combination of glucocorticoids which causes global immunosuppression and is often insufficient to halt disease progression. Therefore, targeted regulatory T cell therapy may be a better therapeutic option because targeted Tregs are able to specifically suppress the autoimmune response driving disease. Here, we have identified and solved the protein crystal structures of two MPO T cell epitopes, $MPO_{453-467}$ and $MPO_{724-738}$ in complex with disease-associated HLA-DRB1*04:05. Then, using high-throughput scRNA-seq, we identified three T-cell receptors (TCRs) that are specific for these MPO epitopes. Subsequently, via lentiviral transduction, we successfully expressed these MPO-specific TCRs onto human T cell lines and primary human Tregs. In addition, we engineered the TCRs with a V5 and FLAG tags on the alpha and beta chains respectively to enable visualization of the surface expression after transduction. We show that we can express these three MPO-specific TCRs on the surface of human T cells and Tregs; and we also compared the suppressive capacity of these TCR-Tregs. One of the TCRs showed superior surface expression and suppressive capacity compared to polyclonal Treg; with 5-fold and 2-fold superiority seen in disease-associated HLA-DRB1*04:05 specific $MPO_{453-467}$ and $MPO_{724-738}$ respectively. In conclusion, we have successfully engineered MPO-specific Tregs that had been shown to establish sufficient in vitro MPO-specific suppression. We would proceed to test the TCR-Treg in in vivo humanised model of MPO-AAV.

ABSTRACTS
SESSION 6
Science Bites II

Immunomodulatory Lipid Nanoparticles to Treat Experimental Glomerulonephritis

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Introduction: Glomerulonephritis, which can occur as a manifestation of autoimmune disease, is a major cause of chronic kidney disease.[1] While standard treatments improve patient outcomes, they come with challenges such as adverse effects (infections, metabolic syndrome, osteoporosis, malignancies) and the development of drug resistance.[2] The immunomodulatory and tissue-repairing properties of our novel immunomodulatory-lipid nanoparticle (LNP) present a promising therapeutic approach. **Objective**: To investigate the therapeutic capacity and mechanism of action of our novel immunomodulatory-LNP in experimental anti-glomerular basement membrane glomerulonephritis (anti-GBM GN). **Methods and Results**: Anti-GBM GN was induced in C57BL/6 mice by immunisation with normal sheep globulin subcutaneously on day 0, followed by intravenous administration of sheep anti-mouse GBM globulin on day 4. Mice received an intraperitoneal injection of immunomodulatory-LNP or empty-LNP on day 8, and immune responses and kidney injury were assessed on day 21. *Ex vivo* imaging of DiR-labelled immunomodulatory-LNP demonstrated enhanced and sustained kidney accumulation in mice with anti-GBM GN compared to healthy controls (7.81×10^8 vs 2.19×10^8 p/sec/cm^2/sr at 24 hours; 8.56×10^8 vs 1.45×10^8 p/sec/cm^2/sr at 72 hours). Compared to empty-LNP controls, survival was significantly higher in immunomodulatory-LNP-treated mice (83% vs 33%). Immunofluorescent staining of kidney sections showed that immunomodulatory-LNP-treated mice preserved higher expression of podocyte marker, nephrin, than empty-LNP-treated mice (56.1±7 vs 38.7±5.7 % expression per glomerulus). *In vitro* treatment of human undifferentiated podocytes with immunomodulatory-LNP promoted podocyte maturation, as indicated by reduced Vimentin mRNA (log2RQ -0.9±0.6) and S1A100 mRNA (log2RQ -2.4±0.5). Additionally, immunomodulatory-LNP reduced IL-6 level in DMSO-injured human podocyte cultures (0.51±0.14 vs 0.80±0.14 OD_{450nm}). Furthermore, treatment of human PBMCs with immunomodulatory-LNP *in vitro* significantly increased the percentage of Treg cells by fourfold; also decreased Th1 percentage to one-fifth and Th17 percentage to half of the untreated group. Toxicity study revealed that our novel immunomodulatory-LNP had a good safety profile. **Conclusions**: Immunomodulatory-LNP is a promising disease-targeted therapy for glomerulonephritis that reduces kidney injury, modulates intrarenal cytokine responses, promotes podocyte maturation, and regulates immune cells with a good safety profile.

Keywords: *immunomodulation, lipid nanoparticle, glomerulonephritis*.

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Harnessing MR1 Antigen Presentation with a Ligand-Restricted Nanobody for Immune Manipulation

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Solid cancers are challenging immunotherapy targets due to high mutational burden and tumour antigen heterogeneity. Major histocompatibility complex (MHC) related protein 1 (MR1), unlike conventional MHC molecules, is non-polymorphic and thus represents an attractive candidate for off-the-shelf therapies. MR1 is known for presenting microbial-derived metabolites to mucosal-associated invariant T (MAIT) cells. We have generated a single domain antibody, a nanobody (clone C11), that binds MR1 only when presenting a conserved microbial ligand 5-OP-RU. To harness this specificity therapeutically, a bispecific engager linking C11 with anti-CD3 was generated and harnessed to target tumour cells for killing by human $CD3^+$ T lymphocytes.

To develop the C11 nanobody, a synthetic yeast library was used. Structural studies using X-ray crystallography were performed to provide a molecular basis for specificity to the MR1-5-OP-RU complex. Affinity measurements via surface plasmon resonance revealed a high-affinity binding interaction in the low nano- to picomolar range. To investigate the bioactivity of the nanobody, functional assays revealed robust and reproducible labelling of MR1-5-OP-RU on tumour cells of diverse origins, along with blocking of MAIT cell activation and proliferation *in vitro* and *in vivo.* Similar assays, *in vitro,* revealed the efficacy of the bispecific engager in redirecting primary T cells to selectively kill breast and B cell-derived tumour cells pulsed with 5-OP-RU. These findings demonstrate the feasibility of exploiting ligand-restricted MR1 to overcome antigen heterogeneity in cancer and ongoing work is investigating the therapeutic efficacy of this engager in preclinical cancer models.

Overall, we have developed the first nanobody with defined molecular specificity for MR1-5-OP-RU, with structural and functional validation. By leveraging the non-polymorphic nature of MR1, this approach can address a key limitation of conventional immunotherapy targets that are constrained by patient-to-patient variation. The selective induction of MR1-5-OP-RU on cancer cells or targeting of the tumour microbiome by bispecific engagers for killing by T cells presents a novel and broadly applicable immunotherapeutic strategy for cancer.

Suppressing the Suppressors – New Antibody Strategies in Cancer Immunotherapy

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Tumor immune evasion is a hallmark of cancer that contributes to therapeutic resistance and disease relapse. Among the key mediators of immune suppression in the tumor microenvironment (TME), tumor-infiltrating regulatory T cells (ti-Tregs) are recognized as major players due to their potent immunosuppressive properties. Consequently, selectively targeting ti-Tregs based on their chemokine receptor signature has emerged as a promising therapeutic strategy. In particular, CCR8 is highly expressed exclusively on ti-Tregs, making it an attractive target for intervention. We developed 2H8, a monoclonal antibody (mAb) that specifically targets CCR8 and possesses a unique binding epitope. Using CCR8-knockin cell lines and patient tumour biopsies, we identified several unique functional attributes of 2H8 in comparison to other anti-CCR8 mAbs that target the immunodominant N-terminal epitope. We then examined the therapeutic efficacy of 2H8 in the MC38 and CT26 syngeneic mice models of colorectal cancer (CRC). 2H8 exhibited comparable binding compared to other anti-CCR8 mAbs, but possessed unique cross-reactivity to mouse, human, and cynomolgus monkey CCR8. In CRC patient biopsies, 2H8 demonstrated a superior ability to identify ti-Tregs compared to other N-terminal mAbs. 2H8 also displayed similar antibody-dependent cellular cytotoxicity when compared to an N-terminal mAb, suggesting its potential advantage in clinical settings. Additionally, in both the MC38 and CT26 models, 2H8 treatment displayed a significant reduction in tumor volume and weight compared to the isotype control. Flow cytometric analysis of tumor-infiltrating immune cells revealed that 2H8 treatment led to a marked depletion of ti-Tregs, accompanied by increased infiltration of CD8+ T-cells and a higher CD8/T-reg ratio, indicating a shift toward a more antitumoral TME. Notably, these immune changes were tumor-specific, with no significant changes observed in the spleen. Collectively, our findings highlight the therapeutic potential of our novel anti-CCR8 mAb that targets a non-immunodominant epitope of CCR8, enabling effective and selective depletion of ti-Tregs and enhancing antitumor immunity.

Pre-existing humoral immunity and comorbidity-associated inflammatory signatures shape responses toward seasonal influenza vaccination in Australian First Nations people

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Indigenous populations globally have increased morbidity and mortality from influenza viruses, together with disproportionate rates of chronic diseases, including diabetes, respiratory and renal disorders. We recently revealed that comorbidities negatively impacted antibody responses to COVID-19 vaccination. The impact of chronic diseases on immunity towards influenza vaccination in Indigenous people is however unknown. We recruited Australian First Nations and non-Indigenous people vaccinated with seasonal quadrivalent inactivated influenza vaccines (IIVs) in 2022-2024. We assessed influenza Hemagglutination Inhibition (HAI) antibody titres, haemagglutinin (HA)-specific memory B cells (MBCs), antibody-secreting B cells, circulating T follicular helper 1 ($cT_{FH}1$) cells post-IIV, together with baseline inflammation, antibody glycosylation and cellular activation profiles. Our study highlights over-representation of multimorbidity in our Australian First Nations cohort, associated with elevated baseline proinflammatory cytokine levels (MCP-1, IL-6, IL-18), inflammation-associated agalactosylated IgG antibodies and activated NK cells, $\gamma\delta$, $CD4^+$ and $CD8^+$ T cells. Participants with multimorbidities, irrespectively of ethnicity, also had higher HAI titres at baseline, potentially reflecting health-seeking behaviour and repeated vaccination. Following IIV, all vaccinees, including participants with multimorbidities, had increased HAI antibody titres against all vaccine components and higher HA^+-specific MBC frequencies as compared to baseline levels. However, our data also demonstrated, for the first time, increased prevalence of pro-inflammatory atypical $CD21^-CD27^-$ B cells (atBCs) within influenza-specific HA-positive IgD^-CD19^+ B cells and lack of significant $cT_{FH}1$ cell activation in individuals with comorbidities, correlating with multimorbidity-associated baseline inflammatory features. Our findings thus reveal that although Australian First Nations and non-Indigenous participants with multiple chronic diseases can mount antibody responses following influenza vaccination, their HA-specific B cell and cTFH1 compartments display differential suboptimal features post-IIV, linked to multimorbidity-associated inflammatory signatures and IgG glycosylation patterns at baseline. Our study supports influenza vaccination for individuals with chronic diseases, especially relevant to Indigenous populations with high prevalence of multimorbidities.

Influenza A virus drives TLR-dependent inflammatory responses in non-small cell lung cancer

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Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer cases and remains a leading cause of cancer-related mortality worldwide. Beyond established carcinogenic drivers, such as smoking and oncogenic mutations, recent epidemiological and experimental evidence implicates recurrent influenza A Virus (IAV) infection as a potential modulator of lung cancer progression by reprogramming the tumour microenvironment through inflammatory pathways. Detection of the RNA genome by endosomal toll-like receptors (TLRs), including TLR3 and TLR7/8, triggers antiviral and inflammatory signalling essential for viral clearance. However, in NSCLC, sustained TLR activation has been associated with chronic inflammation, tumour cell survival, immune suppression and chemoresistance, suggesting that persistent TLR stimulation may promote disease progression. Despite this, the IAV-TLR interactions in shaping the NSCLC microenvironment remains poorly understood. In this study, we infected a panel of lung adenocarcinoma (A549, CALU-3), squamous cell carcinoma (H520, SK-MES-1) and non-malignant bronchial epithelial cells (BEAS-2B) with PR8/A (H1N1) strain of IAV and assessed inflammatory cytokine expression by RT-qPCR. A consistent interferon response was observed across all cell lines while pro-inflammatory responses were variable. Interestingly, TLR3, but not TLR7, expression was consistently suppressed following infection across all NSCLC lines. Basal TLR protein levels, determined by Western blot, varied between cell types and correlated with their inflammatory responses to stimulation with corresponding TLR agonists. Furthermore, pharmacological inhibition of TLR3 or TLR7 altered IAV-induced inflammatory responses in NSCLC cells. Collectively, these findings provide new insights into how IAV modulates inflammation in NSCLC, and highlights TLRs as potential therapeutic targets to mitigate IAV-driven inflammation in lung cancer.

Beta-blockade reshapes the tumour immune landscape to enhance chemotherapy control of triple-negative breast cancer progression

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Preclinical and clinical studies are exploring the utility of pharmacological beta-blockade as a novel strategy to enhance existing cancer treatments. Beta-blockers inhibit signalling from the sympathetic nervous system (SNS) and have been shown to improve the efficacy of anthracycline chemotherapy in controlling metastasis in mouse cancer models. Studies have shown that immune cells play a crucial role in determining the outcomes of anthracycline chemotherapy. However, whether immune cells mediate the beneficial effect of beta-blockade during anthracycline chemotherapy remains unknown.

To understand the impact of beta-blockade on immune cells during anthracycline chemotherapy, we profiled the immune landscape of murine triple-negative breast tumours. We found that doxorubicin treatment significantly increased neutrophil and decreased dendritic cell infiltration compared to placebo control, resulting in an immunosuppressive tumour microenvironment. However, blocking SNS signalling using propranolol, a non-selective beta-blocker, during chemotherapy reversed these changes in immune cell infiltration and significantly inhibited primary tumour growth. To determine if a reduction in neutrophils in the tumour microenvironment is sufficient to improve response to chemotherapy, we depleted neutrophils during chemotherapy and found significantly increased dendritic cell recruitment into tumours and improved tumour control by chemotherapy. In separate experiments, activation of the beta-2 adrenergic receptor signalling using salmeterol in tumour-bearing mice significantly reduced dendritic cell infiltration and increased neutrophil recruitment into the tumour, suggesting that beta-2 adrenergic receptor signalling is sufficient to modulate the innate immune landscape of tumours.

Altogether these findings show that blocking SNS signalling using beta-blockers during chemotherapy reprograms a favourable tumour microenvironment, thereby enhancing chemotherapy control of triple-negative breast cancer progression and suggesting a potential therapeutic strategy for clinical management of triple-negative breast cancer.

Selecting β-synuclein epitopes for a targeted regulatory T cell therapy for progressive multiple sclerosis

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Multiple sclerosis (MS) is an autoimmune neurodegenerative disease affecting over 30,000 Australians, and this prevalence is increasing[1]. Grey matter damage and progressive disease worsening affect many MS patients and increase disability burden but are poorly treated by available therapies. Engineered T cell receptor-regulatory T cell (TCR-Treg) therapies are a cutting-edge treatment strategy for autoimmune diseases, using an antigen-specific TCR to localise the anti-inflammatory, pro-healing effects of Tregs in response to antigens presented on HLA in the inflamed area[2]. One such antigen is β-synuclein, a neural protein that is immunogenic in progressive MS patients[3]. In contrast to conventional MS antigens, β-synuclein concentrations are higher in the grey matter than the white matter, making it a uniquely well-suited target to enable specific Tregs to address grey matter damage.

This project aims to select immunogenic β-synuclein peptides to begin development of a TCR-Treg therapy targeting β-synuclein, and to investigate the molecular basis of β-synuclein antigen presentation on the MS-associated HLA allele DRB1*15:01 (DR15).

In silico methods were used to identify β-synuclein peptides that may be presented on HLA-DR15. HLA-DR15 was produced in mammalian cells and peptide exchanged to present chosen peptides. X-ray crystallography was used to solve the structure of HLA-DR15 presenting β-synuclein peptides, indicating that this presentation is possible by antigen presenting cells. Several immunogenic peptides were identified by coculture of dendritic cells and T cells from healthy DR15+ individuals with flow cytometry-based cell proliferation tracing, suggesting that reactivity to these peptides may contribute to autoimmunity in carriers of the DR15 allele.

Future experiments will include single-cell RNA sequencing to find the TCRs used by β-synuclein-specific T cells, and viral transduction to produce Tregs bearing these TCRs, which can then be tested in vitro and in vivo for therapeutic effect. This research ultimately aims to both contribute to explaining how HLA-DR15 predisposes to autoimmune diseases, and to develop a novel TCR-Treg therapy that could improve quality and length of life for people with progressive MS.

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Designing Improved Diagnostic Assays for IBDV Detection in Australia

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Infectious bursal disease virus (IBDV) is a highly contagious virus common in chickens, causing significant risk for the worldwide poultry industry, while having a thus far unknown ecological effect on wild bird populations. There are two genetically distinct serotypes of IBDV: serotype 1 which includes isolates that are pathogenic in chickens and serotype 2 which is non-pathogenic. Serotype 1 viruses are further categorised into nine genogroups (A1-9). These genogroups range in virulence with some, such as A3, capable of mortality rates in poultry flocks of up to 70%. These A3 viruses have spread to every continent except Australia- which currently only has A7 and A8 IBDV that cause little to no clinical signs of disease. The possibility of an incursion of a virulent IBDV strain is a major cause for concern for the Australian poultry industry.

To monitor circulating IBDV strains in Australia, regular surveillance for IBDV is conducted using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). However, the assay currently in use lacks the ability to distinguish between genogroups or serotypes. Design of new RT-qPCR assays addressing these limitations requires analysis of the whole genomes of each IBDV genogroup. However, there currently is a distinct lack of whole genomes for Australian A7 and A8 viruses. We addressed this by performing whole genome sequencing on IBDV positive samples collected from Australian poultry farms between 1994-2024. Forty-eight Australian whole genomes were generated and phylogenetically analysed to determine their genogroup. These new genomes, along with 350 already available IBDV whole genomes, were analysed to determine targets for new RT-qPCR assays. Two new RT-qPCR assays were designed for IBDV surveillance in Australia. The first assay is designed to detect all strains of IBDV and allows distinguishing between serotype 1 and 2 viruses, broadening the scope of IBDV surveillance. While the second assay is also capable of detecting all strains of IBDV, it also distinguishes between strains of IBDV endemic or exotic to Australia, enabling faster detection and response to any incursions of virulent IBDV into Australia.

Development of a rapid point-of-care test for active syphilis

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Syphilis, caused by the bacteria *Treponema pallidum* (TP), is re-emerging at an alarming rate seeing to a three-fold increase in Australia over the past ten years and affecting an estimated 8 million adults globally in 2022 alone (World Health Organisation, WHO). Although treatable with antibiotics, syphilis poses a serious threat to public health, with vertical transmission resulting in an estimated 200,000-300,000 neonatal deaths and still births per year. A major barrier in effective disease management is the differentiation of an active infection from that of past-treated cases. Distinguishing these populations requires either centralised laboratory testing, which can be out of reach in resource-limited contexts and to those with limited healthcare access – or results in the overtreatment of false-positive past treated cases. As delays and complexity in diagnosis increase the risk of loss to follow up, there is an urgent need for a more efficacious and streamlined diagnostic strategy.

In approaching this clinical deficiency, we have developed a test that uses a carefully curated set of treponemal antigens to target a more transient set of antibodies that correlate with active infection. This assay has been optimised for use in the AtomoRapid^{TM} Pascal device, an entirely self-contained platform consisting of an integrated lancet, buffer delivery pack and 10 µL blood collection unit that provides results within 15 minutes. To validate our prototype, we have tested it against an array of clinically characterized patient samples with current infection, past-treated infection and never-infected donors. Our prototype demonstrated 93.9% sensitivity (95%CI:83.1-98.7) for active syphilis, and 99% (97.8-99.7) and 83.3% (67.2-93.6) specificity for never infected and past-treated patients, respectively. Our test has been refined to fulfill the WHOs preferred target product profile for an active syphilis POC test. We are now conducting studies to establish the correlation between venous and fingerprick blood to evaluate feasibility in a standard of care setting and demonstrate real world application.

Overall, our test represents a significant breakthrough in point-of-care testing for active syphilis and offers a rapid, cost-effective and user-friendly approach to address longstanding limitations in clinical diagnosis.

ABSTRACTS
SESSION 8
Science Bites III

Investigation of microbe associated antibody cross-reactivity in SARS-CoV-2 Nucleocapsid immune response

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While infection with SARS-CoV-2 is characterized by a sequalae of COVID-19 symptoms, an early adaptive immune response is associated with protection. Most of the current immunological research focuses on the spike protein owing to its incorporation in major vaccine formulations. In comparison, studies on the nucleocapsid protein (NP) have been largely limited to serological testing for COVID-19 positivity. However, a prior study from our lab detected anti-nucleocapsid antibodies in SARS-CoV-2 NAT (Nucleic Acid Test) negative health care workers. This suggests potential cross-reactivity and a possible contribution to natural pre-pandemic immunity. We hypothesize that this cross-reactivity could arise from not only genetically similar viruses but also through prior exposure to unrelated microbes, such as environmental bacteria. To investigate this, novel NP B-cell epitopes were predicted and run through NCBI BLAST to identify microbial peptides with similar (conserved amino acid residues) sequences. This resulted in a total of 10 potential microbial cross-reactive (CR) peptides against 4 NP peptides. Homologous bacterial CR peptides obtained were from diverse sources belonging to environmental microbes, human microbiome and even pathogenic species. Indirect ELISA was subsequently employed to evaluate IgG reactivity to the NP and CR peptides, in plasma or sera collected from cohorts of COVID-19 negative and convalescent volunteers, from Victoria and Tasmania (n=290). Simultaneous NP and CR peptide recognition was preferentially observed in COVID-19 exposed individuals, indicating a probable boosting of prior immunity to homologous peptides. Donors with dual NP and CR peptide reactivities are being further evaluated for overlapping antibody binding (cross-reactivity), between the NP and CR peptides, via competition ELISA. Initial results suggest the existence of such cross-reactivity and that the regions of similarity or identity within these peptides could be responsible. Thus, the prediction of novel NP and corresponding CR epitopes, their validation and delineation of potential cross-reactivity could not only shed light on the breadth of anti-SARS-CoV-2 immunity but also have direct implications for leveraging cross-reactive epitopes to induce protective and sustained immunity via vaccine formulations.

Bispecific IgG4 display epitope-dependent ability to bind and mediate Fc effector functions against SARS-CoV-2

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Repeated COVID-19 mRNA vaccination (>3 doses) increases anti-spike IgG4, often associated with reduced antibody Fc-mediated functions. As Fc-mediated responses help maintain protection against SARS-CoV-2, this has raised concerns regarding reduced vaccine effectiveness. Moreover, IgG4 possesses the ability to form half-molecules which randomly recombine with other half-molecules, a process known as Fab arm exchange, so that the majority of IgG4 *in vivo* is thought to be bispecific. Currently, studies examining the properties and function of SARS-CoV-2 bispecific IgG4 are limited.

To identify potential epitopes targeted by bispecific IgG4 *in vivo*, we first mapped epitopes bound by SARS-CoV-2-specific IgG4. In a cohort that had received four mRNA vaccinations, the majority (>60%) of SARS-CoV-2-specific IgG4 targeted the ancestral receptor binding domain (RBD) of the spike trimer. This suggests a large proportion of SARS-CoV-2-specific bispecific IgG4 *in vivo* may consist of at least one RBD-targeting arm.

To characterise the functional properties of SARS-CoV-2 bispecific IgG4, we utilised controlled Fab arm exchange to generate a panel of IgG4 bispecific antibodies (bsAb) targeting RBD in combination with a non-overlapping RBD epitope, spike 2 (S2) or against an irrelevant antigen (Influenza hemagglutinin). Bispecificity was confirmed by multiplex assays and biolayer interferometry. We examined the ability of the bsAbs to mediate spike-specific FcγR binding against a range of SARS-CoV-2 variants and mediate antibody-dependent cellular phagocytosis (ADCP).

IgG4 bsAbs containing one binding RBD-binding arm displayed reduced binding to spike-specific FcγRIIaH and FcγRIIaR binding compared to their parent monospecific RBD mAb (median 1.6- and 4.3-fold decrease respectively). Consequently, these bsAbs exhibited reduced ability to trigger spike-specific ADCP (median 3.3-fold decrease). In contrast, RBD-RBD bsAbs improved spike-specific FcγRIIaH/R binding compared to the parent mAbs (median 2.8- and 2-fold increase respectively), and this was confirmed in cell-based ADCP assays.

Collectively, our study demonstrates that ability of bispecific IgG4 to mediate antibody Fc effector functions is dependent on the epitopes targeted. Ongoing studies will further characterise the role of bispecific IgG4 on mediating other antibody functions including neutralisation. Insights on the properties of bispecific IgG4 may provide better understanding on the role of vaccine-induced IgG4 on antibody-mediated responses and protection.

First molecular insight into HLA-C contribution to COVID-19 outcome

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T cells are paramount to the adaptive immune system in controlling infection upon activation. The central interaction of cell-mediated adaptive immunity is between the αβ T cell receptor (αβTCR) and the presentation of an antigen bound by Human Leukocyte Antigen (HLA). Understanding the TCR interaction with peptide-HLA (pHLA) with structural biology, as crucial key event of the immune response, unravels the parameters that drive this interaction and its impact on the disease outcomes.

Our project aims to understand the link between effective antiviral T cell response in COVID-19 and the expression of HLA-C*12:02, as the role of HLA-C is highly understudied in controlling viral infections unlike HLA-A and -B allomorphs. We have discovered a novel Nucleocapsid-derived peptide, able to elicit T cell responses in individuals expressing HLA-C*12:02.

We assessed the stability of each HLA in complex with the N-derived peptide by performing thermal stability assays. We then revealed the first crystal structure of HLA-C molecule presenting a SARS-CoV-2 peptide to a T cell receptor, giving the first insight into how T cells might engage with these HLA molecules. Furthermore, we also performed TCR repertoire analysis and revealed that clonotypic diversity underpins the response to this epitope and performed affinity measurement against the HLA-C molecules using surface plasmon resonance.

Our results represent the first study at the molecular level of HLA-C molecules associated with potent antiviral T cell responses, and how T cells engage with those detrimental HLAs. This work will provide new avenues on how to better activate T cells.

Defining molecular gene profiles that underpin superior antiviral CD8$^+$ T cell immunity in nonagenarians and centenarians

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Background: Nonagenarians and centenarians exemplify healthy aging and have survived decades of seasonal viral outbreaks and pandemics. Given the importance of CD8$^+$ T cells to antiviral immunity, we hypothesized that nonagenarians and centenarians have superior CD8$^+$ T cell immunity with unique transcriptomic features.

Methods: We performed single cell RNA sequencing and measured the cell-surface proteome of CD8$^+$ T cells specific for influenza A and B, SARS-CoV-2, Epstein-Barr virus and cytomegalovirus to define epitope-specific CD8$^+$ T cells from children (5-15y), adults (24-39y), older adults (68-75y) and long-lived individuals (93-102y) to establish their key transcriptomic and protein signatures.

Results: Our analyses of bulk CD8$^+$ T cells across age groups revealed more differentiated memory CD8$^+$ T cell subsets in centenarians and nonagenarians that had unique transcriptomic signatures. Strikingly, 90+ year olds displayed differential clustering of terminally differentiated CD8$^+$ T cells. Unique age-specific signatures were also observed for epitope-specific CD8$^+$ T cell populations in both chronic and acute viruses. The extent of CD8$^+$ T cell differentiation in nonagenarians and centenarians also varied between virus-specific populations being less differentiated in influenza A-specific CD8$^+$ T cells and more differentiated in SARS-CoV-2-specific CD8$^+$ T cells.

Conclusion: We identified immune profiles for virus-specific CD8$^+$ T cells across the human lifespan and provide key insights into biomarkers associated with healthy immune aging that may identify potential therapeutic targets to reduce disease severity.

A High-Dimensional Flow Cytometry Profiling on Peripheral Blood Mononuclear Cells of Transitioning Transgender Individuals

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There are well-known sex differences in immune function, with implications for infection response and autoimmune disease. The contribution of sex hormones to these differences is not completely understood, and it is often difficult to separate the effects of hormones from the underlying genetic differences between males and females. Estrogen has been shown to increases immune cell reactivity, whereas testosterone has an immunosuppressive effect. The effects of gender affirming hormone therapy (GAHT) on the immune system of transgender individuals is not well understood, which is a significant gap in health knowledge.

We isolated peripheral blood mononuclear cells from whole blood of transgender women before GAHT (baseline), and at 3- and 6- months after feminizing GAHT. This study included two antiandrogen groups, cyproterone acetate (CPA) (n=12) and spironolactone (SPI) (n=12). Using a 30-marker high-dimensional flow cytometry approach, we profiled 40 distinct immune cell subpopulations and determined differentially abundant subsets using linear mixed effects model.

Our analysis has shown that changes in circulating immune cells is CPA-specific, with not many changes in the SPI group. Notably, we found an increase in Th17 CCR4- (log2FC= 0.6, p = 0.008) and activated regulatory T cells (log2FC = 0.16, p = 0.018), alongside a decrease in transitional (log2FC = - 0.44, p = 0.05) and classical Memory B cells (log2FC = -0.36, p = 0.037) after 6 months of GAHT. In addition, to estrogen and testosterone, levels of prolactin were associated with the modulation of the investigated cell subsets. Overall, our data suggest that feminizing GAHT with CPA changes immune cell proportions in the circulation, influencing cells related to autoimmune disease risk, such as Th17 CCR4- cells.

This is the first longitudinal immunophenotyping study conducted in transgender women receiving feminizing GAHT. While several cross-sectional studies have previously characterized immune profiles in this population before, the longitudinal aspect of our study gives us more resolution to investigate the dynamic changes in immune cell reactivity for these individuals overtime during GAHT. Our study has implications for the long-term health of transgender women and highlights that only 6-months of GAHT can significantly remodel specific immune cells in the blood.

Decoding chronic urinary tract infections: A predictive multi-omic trio of bladder transcriptomics, urinary metabolomics, and the gut microbiome

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Urinary tract infections (UTIs) are among the most common infections worldwide, with over 400 million cases annually, disproportionately affecting women. Chronic UTIs—a severe and under-recognised subset—are characterised by persistent or recurrent bladder infections that evade immune clearance and resist standard antibiotic treatment, leaving patients symptomatic for years. Diagnosis is challenging because uropathogenic bacteria can embed within the urothelium, escaping detection by conventional midstream urine cultures. Without reliable diagnostics or treatments, patients endure repeated—and often unnecessary—antibiotic courses and misdiagnoses, compounding their physical, psychological, and financial burdens. This unmet clinical need underscores the urgency for mechanistic insights and predictive biomarkers of chronic UTIs. To address this, we aimed to identify multi-omic signatures predictive of chronic UTI using a translational murine model.

Female C3H/HeNRj mice (6-8 weeks old) were inoculated with the clinical uropathogenic *Escherichia coli* strain UTI89 or sham-inoculated with sterile PBS. At 28 days post-infection (dpi), urinary bladder bacterial load was assessed to retrospectively stratify mice as having persistent (chronic) or resolved infections. Bulk RNA sequencing was also performed on bladder tissue collected at 28 dpi. Longitudinal urine and faecal samples were collected weekly from 7 days before infection to 28 dpi and analysed using untargeted metabolomics and shotgun metagenomic sequencing, respectively.

Mice with chronic infection exhibited markedly altered bladder transcriptomic profiles, with signatures of persistent immune activation and chronic inflammation. In contrast, transcriptomes from mice that had resolved the infection closely resembled those of sham-inoculated controls. The urinary metabolome of chronically infected mice was enriched over time for metabolites and lipids linked to immune dysregulation and cell membrane damage, while mice that had resolved the infection displayed increased levels of antioxidant-associated metabolites, suggesting a more effective immune response. Distinct faecal microbiome profiles were detectable as early as 7 dpi in mice that later developed chronic infection, with several bacterial species consistently differentially abundant across the time course.

Together, these findings reveal unique bladder transcriptomic, urinary metabolomic, and faecal microbiome signatures predictive of chronic UTI. This integrated multi-omic approach lays the foundation for early biomarker-driven diagnostics and novel therapeutic strategies to tackle treatment-refractory chronic UTIs.

Altered gastrointestinal permeability and immune profiles in the *Shank3B*$^{-/-}$ mouse model of autism and modulation by the microbial metabolite-sequestering drug, AB-2004

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Introduction: Phelan-McDermid syndrome (PMS) is a syndromic form of autism caused by variants in the SHANK3 gene. People with PMS commonly experience inflammation and altered gastrointestinal (GI) permeability, but the role of the autism-associated *Shank3B*$^{-/-}$ gene remains unknown. Clinical trials suggests that the microbial metabolite-adsorbing drug AB-2004 modulates autism-associated irritability, but the effects on GI physiology and inflammatory responses are unclear.

Methods: C57Bl/6 mice (minimum 8 mice/group) were fed standard or 5% (w/v) AB-2004-containing chow from age 5-to-12 weeks. Ex vivo permeability was assessed in the duodenum, jejunum, ileum, and colon via passage of 1mg/ml 4-kDa Fluorescein isothiocyanate across the mucosal barrier. The colitis-inducing agent Dextran sodium sulphate (DSS, 1.5-2%) was given from age 9-to-10 weeks, with daily disease activity index (DAI) scores, representing colitis severity, recorded for one week of treatment and one week of recovery. Serum cytokine levels (IL-1β, IL-6, IL-10, IL17A, IL-22, IFN-γ, and TNF-α) were analysed using a Bio-Plex 200, with q-values accounting for false discovery.

Results: We identified altered permeability in specific gut regions for both genotype and AB-2004 drug treatment. Interestingly, *Shank3B*$^{-/-}$ mice showed decreased jejunal permeability compared to wild-types. AB-2004 decreased permeability in WT jejunum and colon and serum IL-22 levels compared to standard-chow-fed wild-types. AB-2004 also reduced colon permeability in *Shank3B*$^{-/-}$ mice. Compared to WT, *Shank3B*$^{-/-}$ mice showed lower levels of serum IL-1β, IL-6, and IFN-γ cytokines and a statistical trend towards decreased TNF-α (q=0.059). In response to DSS-induced inflammation, *Shank3B*$^{-/-}$ mice showed increased DAI scores from day 5 of DSS treatment persisting until day 14 alongside decreased survival compared to WT.

Conclusion: Unexpectedly, we identified decreased gut permeability and serum cytokine levels in *Shank3B*$^{-/-}$ mice, indicating that a gene variant impacting neuronal synapses modifies gut physiology and potentially affects nutrient absorption. AB-2004 also decreased gut permeability in both WT (jejunum and colon) and colon of *Shank3B*$^{-/-}$ mice. These findings indicate gut and immune dysfunction in *Shank3B*$^{-/-}$ mice. In addition, AB-2004 may potentially benefit gut health by decreasing gastrointestinal permeability.

Macrophage heterogeneity influences cellular response to HIV infection and latency modulation

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PUBLISH CONSENT WITHHELD

Selective viral modulation of IFN-regulated genes to supress antiviral genes while permitting pro-viral gene expression

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Rabies virus (RABV) infection triggers a type I interferon (IFN)-mediated innate immune response, the primary host defence against viruses. IFN activates STAT1/2, which regulate expression of hundreds of interferon-regulated genes (IRGs), many with antiviral functions. This antiviral gene induction is traditionally considered IFN's main role. To replicate successfully, viruses' express IFN-antagonist proteins that disrupt IFN induction and signalling. The main IFN antagonist of RABV is P protein, which targets/inhibits IFN-activated (phospho-) STAT1/2 as a critical factor in pathogenicity. However, while the IFN system is viewed as antiviral, significant numbers of IRGs have 'pro-viral' roles, including genes required for RABV replication, some of which are upregulated during RABV infection. Notably, IFN signalling uses multiple signalling pathways (other than the classical phospho-STAT1/2), which are not known to be inhibited by P. Thus, it is possible that, rather than inducing global shutdown of IFN signalling, IFN-antagonists such as P protein can selectively modulate IRGs to prevent antiviral outcomes while exploiting proviral functions.

Using RNA sequencing analysis together with transcription factor prediction, and ChIP-seq data, we have investigated the global effect of P on the IFN-regulated transcriptome. While IFN responses are typically considered in terms of IRG induction, we confirmed that IFN treatment affects several hundred IRGs and found that similar proportions of IRGs are stimulated or repressed. P inhibited the expression of certain IFN-stimulated genes, while enhancing the expression of IFN-repressed genes, indicative of important roles for both types of genes (and their modulation by IFN antagonists) in infection. Notably, P did not impact IRGs globally, but rather selectively affected subsets, including inhibiting known antiviral IFN-stimulated genes while promoting expression of other IFN-stimulated genes, including genes with known pro-RABV function. Transcription factor analysis indicated P targets STAT1/2-regulated genes but not IRGs regulated by MAPKs, consistent with highly selective targeting of phospho-STATs. This supports the idea that selective modulation rather than global inhibition of IFN signalling is important to viral infection

ABSTRACTS
SESSION 9
Oral Presentations

Macrophages play critical roles in MR1 antigen presentation and regulation of MAIT cell immunity against bacteria

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Antigen presentation plays a critical bridging role between the innate and adaptive immunity. Among the non-classical antigen-presenting molecules, major histocompatibility complex class I-related protein 1 (MR1) is one of the most evolutionarily conserved but least understood. MR1 antigen presentation activates Mucosal-Associated Invariant T (MAIT) and other MR1-restricted T cells. This function is carried out by MR1 Antigen Presenting Cells (APC). It remains unclear whether specialized MR1 APCs exist and how their activity is regulated during immune responses. Here, we identify macrophages as critical MR1 APCs both at steady-state and during bacterial infection, and we uncover mechanisms controlling the extent and duration of MR1-mediated MAIT cell activation.

Using an MR1-reporter mouse model, we show that in the steady-state, MR1 is predominantly expressed by tissue-resident macrophages. Further studies demonstrate that elimination of MR1 expression in macrophages alters the microbiota and impairs MAIT cell immunity against bacteria. We also show the dynamics of MR1 expression and antigen presentation in a murine model of *Staphylococcus aureus* peritonitis. MR1-dependent MAIT cell activation peaks at 3h and returns to baseline at 1 day post-infection. Early activation is mediated by tissue-resident macrophages, but these are mostly replaced by monocyte-derived inflammatory macrophages that express little MR1, thus preventing further MAIT cell activation. *Mr1* transcription and protein expression are not fully restored until 21 days post-infection. To identify factors that regulate *Mr1*, we used ATAC-seq to describe *Mr1* chromatin regulatory elements and conducted a genome-wide CRISPR screen of transcriptional regulators in a macrophage cell line. We found a repressor of *Mr1* transcription and protein expression in macrophages that shows elevated expression in inflammatory (MR1-low) compared to tissue-resident (MR1-high) macrophages.

Our findings establish tissue-resident macrophages as fundamental participants in MR1-mediated MAIT cell immunity. Their replacement during infection by inflammatory macrophages may prevent excessive MAIT cell activation and/or exhaustion of activated MAIT cells. Our description of APC specialized in MR1 presentation, and of *Mr1* regulators, open exciting opportunities to harness the function of MAIT and other MR1-restricted T cells in immunity against self-antigens, infection and cancer.

Memory B cells and plasmablasts expand following experimental infection with *Streptococcus pyogenes* in humans

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There is an urgent need for a vaccine against *Streptococcus pyogenes* (Group A *Streptococcus*), which causes over 600 million pharyngitis cases and 160,000 deaths from invasive infectious disease annually as well as life-threatening post-infectious autoimmune sequelae. It remains unclear whether protective and durable immune memory can be generated in humans through natural infection with *S. pyogenes*, a knowledge gap which continues to hinder vaccine development. To address this, we have used blood samples collected during the recently developed human challenge model of *emm*75 *S. pyogenes* pharyngitis in healthy adults (CHIVAS-M75). Focused on 6 leading vaccine candidate antigens, we analysed antigen-specific B cells by flow cytometry to uncover how B cell phenotype and frequency differed between those who did and did not develop symptomatic pharyngitis. Overall, we observed pre-existing memory B cell responses to most antigens in most adults. Post-challenge, only those participants that developed symptomatic pharyngitis had significant but transient increases in memory B cells post challenge. For some antigens, like SpyCEP, memory B cells remained elevated up to 3 months post challenge. Infection also induced a transient burst of antibody-secreting cells specific for some antigens, which mirrored increases in serum IgG for those antigens. There was an inverse correlation between the breadth of the peak memory B cell response and the peak bacterial load in the throat of participants. This suggests that the rate of bacterial clearance is linked to memory B cell abundance post challenge. Our work provides foundational insights into how a single infection shapes immunity and suggests that pre-existing B cell memory in adults can influence the outcome of *S. pyogenes* infection.

Dynamic Single-Cell Microfluidics Uncovers Heterogeneous Mechanisms of Response to Anti-PD-1 Therapy

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Abstract

Cancer remains a leading cause of death worldwide. While chemotherapy lacks specificity and causes systemic toxicity, immunotherapies are limited by variable patients. A major obstacle is T cell exhaustion, a dysfunctional state arising from prolonged exposure to tumour antigens, marked by impaired cytotoxicity and upregulation of immune checkpoint receptors such as PD-1 and TIM-3.

Although PD-1 blockade has shown clinical benefits, its efficacy remains inconsistent across patients. Restoring T cell function requires T cell receptor (TCR) stimulation and subsequent Ca^{2+} flux, and monitoring these dynamic signalling events is crucial for assessing PD-1 inhibition in exhausted populations. However, conventional techniques such as flow cytometry and microscopy cannot capture the spatiotemporal complexity of T cell signalling in real time, nor can they maintain single-cell resolution under defined stimulation conditions.

Here, we present a novel microfluidic platform that enables real-time, long-term observation of individual T cells under precisely controlled stimulation to investigate the mechanistic basis of immune exhaustion and rejuvenation. The device incorporates a single-cell trapping array that allows simultaneous tracking of 100 immune T cells at the single-cell level, supporting fluid exchange without cell loss, enabling the observation of both rapid TCR-Ca^{2+} flux and static PD-1 expression on the same cell.

Results of either long-term Ca^{2+} dynamics or static PD-1 expression from our platform align with conventional assays, but with improved spatial and temporal control. We demonstrated that PD-1/PD-L1 signalling not only dampened Ca^{2+} intensity but also delayed response onset in a PD-1-dependent manner. Furthermore, the administration timing of PD-L1, PD-1 blockade can be precisely controlled in our platform. Results suggest that pre-binding to PD-1 blockade was crucial to rejuvenation, and there was a strong correlation between low PD-1 expression and favourable PD-1 therapy. Leveraging the on-chip staining techniques, we identified a TIM-3^{hi}PD-1^{low} subset that resists PD-1 therapy, suggesting that TIM-3 may be upregulated in response to PD-1 therapy.

This platform overcomes the limitations of traditional approaches by enabling dynamic, high-throughput, single-cell analysis under physiologically relevant conditions. It provides a powerful tool to dissect immune exhaustion, model early activation events, and better predict immunotherapy responses. (350 words)

References: (Zhu, 2023) (Makuku, 2021) (Meng, 2020) (Ngiow, 2015)(Graves, 2019)(Newton, 2020)

Pro-apoptotic Agents Reduce Chronic HIV Infection in Vivo

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Introduction: Human Immunodeficiency Virus (HIV) is a significant global health threat. Although antiretroviral therapy (ART) suppresses HIV viremia and has improved health of people living with HIV, it doesn't lead to a cure. The existence of a latent HIV reservoir (cells harbor replication-competent HIV provirus in their genome) leads to HIV rebound following ART interruption. This turns HIV infection into a chronic, life-long disease.

Aims: Recent *ex vivo* studies suggest that resistance to cell death is an important feature of the HIV reservoir, which serves as a therapeutic target. In this project, we used pro-apoptotic agents such as SMAC Mimetics and BH3 Mimetics to target the pro-survival proteins (cIAPs, BCL2) in HIV infected cells in a Humanized Immune System (HIS) mouse model.

Methods: Newborn NOD/SCID/IL2rγ^{null} mice were irradiated and engrafted with human CD34^{+} cord blood cells. After a reconstitution period of 16 weeks, they were infected with HIV-JRCSF. 3 weeks after infection, mice began ART treatment until week 16. During the ART treatment, HIV-suppressed mice were given either vehicle or pro-apoptotic treatments to reduce HIV reservoir. After treatment, the efficacy of treatments was assessed using either a 4-weeks Analytical Treatment Interruption (ATI) or the Intact Proviral DNA Assay (IPDA).

Results: HIS mice recapitulate key features of human HIV infection. 6 weeks of Xevinapant (a SMAC Mimetic) treatment induced a significant delay in HIV rebound comparing with vehicle group. Xevinapant treatment also reduced HIV intact proviral DNA level in the spleen and lymph nodes. Combining Xevinapant with Venetoclax (a BH3 Mimetic) did not show a synergistic effect in ATI or IPDA assays.

Conclusion: Our 6-week Xevinapant treatment showed a significant delay in HIV rebound, which suggests a reduction of HIV reservoir size. This finding is also supported by our IPDA data. This is among the first *in vivo* studies to characterize SMAC Mimetics to reduce the HIV reservoir in a latent model. We are currently studying the impact of these treatments on HIV latency, HIV-specific immune response, and cytokine responses. Our findings suggest that exploiting cell death pathways offers a potential new direction towards an HIV cure.

ABSTRACTS
POSTER SESSION
I

Mechanism-based modelling of antibiotic effects on Pseudomonas strains with different resistance mechanisms

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Aim: To evaluate dosing regimens of meropenem (MER) and ciprofloxacin (CIP), alone and combined, against isogenic P. aeruginosa strains with different resistance mechanisms in a dynamic hollow fibre infection model (HFIM) and develop a mechanism-based mathematical model (MBM) accounting for the resistance mechanisms.

Background: Pseudomonas aeruginosa has a large armamentarium of mutational resistance mechanisms enabling resistance emergence during therapy against almost all antibiotics in monotherapy. Pharmacokinetic/pharmacodynamic (PK/PD) indices are based on minimum inhibitory concentrations (MICs) and link bacterial response to antibiotic exposure. However, it has been shown that these indices have limitations, including not accounting for combination therapies. Therefore, an alternative way to optimise dosing regimens is needed.

Methods: Four isogenic P. aeruginosa strains were used: PAOD1 (spontaneous oprD mutation/loss of porin OprD), PAΔADmexR (ampD knock-out/AmpC overexpression and mexR knockout/MexAB-OprM overexpression), PAOD1ΔmexR and PAOD1ΔAD (other arrangement of combinations of the resistance mechanisms). Dosing regimens were: MER continuous infusion (CI, 6g daily dose, 12g daily dose additionally against MER-resistant strains), CIP intermittent infusions (400mg, 8-hourly [Q8] as 1-h infusions), and both combinations. MICs were determined in triplicate. The HFIM was run for 240h. MBM was performed.

Results: All monotherapies resulted in regrowth with amplification of MER- and CIP-resistant subpopulations. The combination regimens suppressed total and resistant counts of PAOD1, PAΔADmexR and PAOD1ΔAD. Against PAOD1ΔmexR, the MER 6g + CIP regimen performed synergistically from 24h to 120h, while MER 12g + CIP was synergistic from 24h to 192h. MER-resistant counts emerged from 72h and 168h for the respective combination regimens; CIP-resistant colonies emerged from 216h with the low-dose combination only. An MBM with subpopulation synergy was developed that described the bacterial response to antibiotic based on the resistance mechanisms. The model could describe bacterial response to mono- and combination therapies of MER and CIP.

Conclusions: Combination regimens of MER and CIP enhanced bacterial killing and suppressed regrowth and resistance in three of four strains. Even against PAOD1ΔmexR substantial synergy occurred up to 120 or 192h. An MBM, accounting for different resistance mechanisms could describe the impact of double mutations and combination therapies on bacterial response to CIP and MER.

Exploring the hepatoprotective role of glucagon

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Although the peptide hormone glucagon is best known for regulating metabolism, studies have also implicated potential roles of glucagon in hepatocellular infection resistance and survival [1-2]. Given the non-canonical roles of glucagon, we aimed to investigate whether glucagon could influence infection-induced cell death in the liver. We administered the long-lasting, highly specific glucagon-receptor agonist IUB288 to a mouse model of infection-induced liver injury (i.e. the D-galactosamine/ lipopolysaccharide (D-GalN/LPS)). We also used the agonistic anti-FS-7-associated surface antigen (Fas) ligand-induced apoptosis-triggered liver model as a second model of liver injury. We then evaluated protection by histological analysis, cleaved caspase-3 immunostaining, and liver damage markers such as serum transaminases (AST, ALT). Parallel *in vitro* studies performed used human SNU398 hepatocarcinoma cells overexpressing the glucagon receptor and staurosporine challenge, with viability assessed at 6 hours and 20 hours post-treatment, by bright-field microscopy and trypan blue exclusion. *In vivo*, glucagon treatment significantly reduced hepatic necrosis, loss of tissue structure, caspase-3 activation, as well as liver damage markers in both injury models. *In vitro*, glucagon markedly improved SNU398 cell survival 20 hours post-staurosporine treatment. These findings establish glucagon as a potent hepatoprotective agent against multiple liver injury agents (infection-triggered or apoptosis-triggered) in preclinical models, both *in vivo* and *in vitro*. Studies are ongoing, assessing the broader therapeutic potential of glucagon as well as the mechanisms involved.

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Exploring a possible role for immune-responsive gene 1 (IRG1) in modulating influenza A virus replication

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Influenza A virus (IAV) causes outbreaks of seasonal influenza disease with individuals experiencing a spectrum of mild to severe symptoms. Interferon stimulated genes (ISGs) play a key role in host antiviral defence during IAV infection. One ISG of interest is immune-responsive gene 1 (IRG1) protein. IRG1 is a mitochondrial enzyme involved in regulating the production of reactive oxygen species. It has been well characterized for its role during bacterial infection, where the IRG1 derived metabolite itaconate inhibits the growth of certain bacteria. While itaconate has been described to be antiviral, a direct role for IRG1 in modulating viral infection, including IAV, has not been defined. Herein, a constitutive overexpression system was used to investigate whether IRG1 was antiviral towards seasonal strains of IAV (both H1N1 and H3N2 subtypes). We identified that IRG1 did not impact the early stages of IAV infection, as the IRG1 overexpressing 293T cells were as equally susceptible to IAV infection as control cells. However, lower viral titres were detected in supernatants from IAV-infected IRG1 overexpressing 293T cells compared to control cells. While this suggested that IRG1 has potential to inhibit the late stages of IAV infection, significant restriction of IAV growth was not observed in A549 and THP-1 cells engineered with inducible overexpression of IRG1. To conclusively determine if IRG1 was antiviral towards IAV, we generated THP-1 cells deficient in endogenous IRG1 expression by CRISPR editing to look for enhanced IAV growth. There was no significant difference in IAV growth (H1N1 and H3N2 subtypes) in the presence or absence of IRG1, suggesting that IRG1 itself is not directly antiviral. Metabolomics analysis of itaconate levels in IRG1 overexpressing and knockout cell lines showed that the highest levels of itaconate were present in 293T cells with constitutive overexpression of IRG1. Interestingly, in the IRG1 deficient cells, while mesaconate (metabolite of itaconate) levels were downregulated, itaconate was not. Overall, these findings suggest that any antiviral activity of IRG1 towards IAV replication is likely to be indirect and mediated through the elevated levels of itaconate as observed in 293T cells with constitutive overexpression of IRG1.

Evaluation of Neglected Tropical Diseases Exposure in an Urban Population Cohort in Quito, Ecuador

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Introduction:
Neglected Tropical diseases (NTDs) represent a group of more than 20 diseases, that are most prevalent in tropical and subtropical regions. These diseases predominately affect low-income countries where limited access to healthcare and ongoing exposure to vectors and livestock contribute to their persistence. Elimination of NTDs has been a key public health priority across South America and Ecuador, for instance, has successfully eliminated onchocerciases. However, there remains a critical need for continued surveillance and access to data that facilitate monitoring of the status and transmission dynamics of other NTDs that continue to affect the country. In our current study, we aimed to evaluate exposure to NTDs and seroprotection against of vaccine-preventable diseases (VPDs) in an urban Ecuadorian population.

Methods:
A bead-based multi-pathogen serology assay was performed on plasma samples from an Ecuadorian cohort. Samples were incubated with a 34-antigen panel, including antigens from 11 NTDs (bacterial, parasitic disease, parasitic diseases transmitted by vectors, arbovirus) and 7 VPDs. The procedure followed a CDC standardized antibody detection protocol using multiplex Luminex technology. The mean fluorescence intensity (MFI) values were obtained as a measure of antigen-specific IgG antibody binding and analysed using a in house R-script to determine the cutoff values of each pathogen tested.

Results:
A total of 148 plasma samples were analysed. Antibody responses to at least one of the NTDs were detected in 98 % of the participants, with the highest prevalence observed for parasitic diseases Cryptosporidiosis (82%; n=121), Toxocariasis (33%; n= 49), and Amebiasis (29 %; n=43). Antibodies to bacterial antigens were also detected where seropositivity to Trachoma was the most prevalent (46%; n= 63). For vaccine-preventable diseases, most participants exhibited seroprotection (98%, 81.8%), against Rubella and Measles whereas a substantial proportion of individuals lacked protective immunity against *Bordetella pertussis* (59.5%), underscoring potential gaps in immunisation coverage.

Conclusions:
Our findings highlight the benefit of measuring IgG antibody responses to NTDs to establish patterns of exposure and immunity within the population. Regular serological monitoring can facilitate detection of circulating pathogens, identify gaps in vaccine coverage, and inform strategies for disease control and prevention.

Cross-serotypically conserved epitope recommendations for a universal T cell-based dengue vaccine

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Dengue virus (DENV)-associated disease is a growing threat to public health across the globe. In recent year, the geographical footprint of DENV is increasing and becoming endemic in more countries. Co-circulating as four different serotypes, DENV poses a unique challenge for vaccine design as immunity to one serotype predisposes a person to severe and potentially lethal disease upon infection from other serotypes. Emerging evidence suggests that an effective vaccine should elicit a strong T cell response against all serotypes of DENV., which could be achieved by directing T cell responses toward cross-serotypically conserved epitopes while avoiding serotype-specific ones. Here, we used experimentally determined DENV T cell epitopes and patient-derived DENV sequences to assess the cross-serotypic variability of the epitopes. We define the conservation profile of these epitopes and identify a set of 55 epitopes that are highly conserved in at least 3 serotypes. Most of the highly conserved epitopes lie in functionally important regions of DENV non-structural proteins. By considering the global distribution of human leukocyte antigen (HLA) alleles associated with these DENV epitopes, we identify a potentially robust subset of HLA class I and class II restricted epitopes that can serve as targets for a universal T cell-based vaccine against DENV while covering ~99% of the global population. In addition, we assess the temporal conservation profile of all known DENV T cell epitopes and assess their potential towards T cell escape. We also present an easy-to-use dashboard accompanying our analysis to facilitate the research community in understanding the emerging landscape of DENV T cell epitopes.

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Exploring the natural resistance of juvenile abalone to haliotid herpesvirus (HaHV-1) infection: The role of algae

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HaHV-1 is a re-emerging viral pathogen characterised by mass mortality events in native wild and farmed Australian abalone, with no current treatment options. HaHV-1 and its associated disease (Abalone Viral Ganglioneuritis) are a major threat to the Australia's abalone industry, currently worth upwards of AUD$150 million. Most Australian HaHV-1 studies have been conducted on adult abalone of one species, so this study sought to expand the current understanding of the dynamics of infection in abalone of differing ages and species.

Naive abalone of different ages and species were exposed to HaHV-1 via immersion in infectious water and real-time PCR detected HaHV-1 in nerves. In collaboration with three Victorian abalone farms, juvenile abalone (6-12 months old) of differing species (greenlip, blacklip and hybrid) were challenged with HaHV-1. Abalone <1 year old were significantly less susceptible to HaHV-1 infection compared to older abalone which contrasts with oyster herpesvirus (OsHV-1) infection.

The main lifestyle change between juvenile and adult abalone on farm is their diet, with juveniles eating algae and adults fed formulated pellets. To investigate if diet components influence HaHV-1 susceptibility we conducted an on farm feeding trial which demonstrated that 6-month old abalone fed an algae only diet were significantly less susceptible to HaHV-1 challenge in comparison to a genetically matched cohort that were fed a mixed pellet and algae diet. To assess the major bioactive compound within the algal diet we extracted algal polysaccharides and assessed their anti-viral capacity against the tissue culture optimised herpesvirus, herpes simplex virus (HSV-1). The algal derived compounds from the mixed algal cultures fed to juvenile abalone on farm, were shown to be highly anti-viral against HSV-1 in astrocytes. HSV-1 mRNA was reduced by 98% and 50%, respectively when administered during and after viral adsorption.

This work provides an insight into the differential susceptibility of abalone of differing ages and species and the anti-viral capability of algal derived polysaccharides from the diet of juvenile abalone on farm. This work informs future investigations into the use of bioactive compounds of natural origin against viruses of concern in clinical, agricultural and aquacultural settings.

Cedar virus and Salt Gully virus are capable of viral mRNA editing during henipavirus infection

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Henipaviruses are a genus of single-stranded, negative sense RNA viruses, the most notable of which are Hendra (HeV) and Nipah (NiV) virus that can cause severe illness with a 40-70% fatality rate in humans. These viruses are handled at the highest level of biocontainment – Physical Containment Level 4 (PC4). Not all viruses in this genus are equal in pathogenicity, Cedar Virus (CedV) was detected in Australian bats in 2009 and animals experimentally infected with this virus failed to show disease. Previous studies determined that CedV lacks a mRNA editing site within its phosphoprotein (P), present in pathogenic henipaviruses. This editing site gives rise to non-structural proteins V and W known to play key roles in immune evasion and inhibition of host interferon responses, contributing to pathogenesis. Salt Gully virus (SGV) is a recently discovered Australian henipavirus isolated from bat urine collected in 2011 and isolated in 2022. The pathogenicity of this virus is unknown. This study aimed to assess viral non-structural protein expression of SGV to gain information on the pathogenic potential of this novel henipavirus as well as whether CedV can edit its mRNA and express a V and W. Here, we have determined that SGV edits and expresses a V and W protein, and that during *in vitro* infection of human cells, the virus displays similar inhibition of the interferon response to HeV, which could correlate to potential pathogenic outcomes. Contrary to previous studies, we found that CedV edits its viral mRNA and expresses a V protein. Illumina amplicon sequencing of the editing site in a variety of CedV and SGV infected host cell lines (human, bat, hamster, non-human primates) and proteomic analysis of infected cell lysates detected the expression of V and W proteins. Additionally, western blots of infected cell lysates using antibodies against the common region of P for CedV detected P and V proteins. These comprehensive analyses elucidate that the editing of CedV utilises a non-conventional sequence to yield a V protein, while SGV edits to express both a V and W protein using the classical format known to henipaviruses such as HeV and NiV.

Exploring SAMHD1 as a cross-species restriction factor against Herpesvirus in cattle and humans

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Bovine Respiratory Disease (BRD) is a major global health concern and the leading cause of illness and mortality in feedlot cattle, with bovine herpesvirus 1 (BHV-1) being the most prevalent viral contributor. Furthermore, an outbreak of highly pathogenic avian influenza (HPAI) (H5N1) in dairy cows was reported in early 2024. Despite the critical role of cross-species virus transmission and zoonotic potential, research on viral pathogens in cattle and other species remains limited. In this study, we explore the role of bovine restriction factors, focusing on SAM and HD domain-containing deoxynucleoside triphosphate triphosphohydrolase protein 1 (SAMHD1), a potent restriction factor known to inhibit DNA viruses and retroviruses in human. Bovine SAMHD1 has recently been suggested as a restriction factor against different primate lentiviruses. Here, we demonstrated that human epithelial cells, bovine epithelial cells and bovine macrophages overexpressing bovine or human SAMHD1 inhibit both human and bovine herpesvirus replication. This indicates that the antiviral activity of SAMHD1 is conserved across different cell types and species. These findings suggest that SAMHD1 may play a critical cross-species role in restricting herpesvirus replication, highlighting its potential as a therapeutic candidate for controlling viral infections in cattle and other species.

Beyond Storage: Lipid droplets as novel extracellular communicators in flavivirus infection

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Objectives
Lipid droplets (LDs) were traditionally regarded as fuel sources and flavivirus replication sites. They play a vital role in the production of an effective host immune response against early ZIKV and DENV replication and our recent work uncovers a novel role for LDs as extracellular communicators during flavivirus infection.

Methods
Plin2-mCherry/GFP cell lines were created in neurons and primary immortalised astrocytes for direct and indirect co-culture exploration. LD isolations were performed from either mock, ZIKV infected or dsRNA stimulated astrocytes cells. Using LC-MS we performed proteomic and lipidomic screens alongside confocal, super-resolution microscopy and molecular techniques to determine LD antiviral effects.

Results
LD secretion and movement between cells was observed using both co-culture systems, with increased transfer of LDs occurring following flavivirus infection at early time points. To understand if LDs could influence surrounding cells following secretion, we isolated LDs from cells following activation of early innate immunity (dsRNA viral mimic) and placed them on naïve cells prior to ZIKV infection. The treatment of LDs increased type I/III interferon responses, resulting in decreased ZIKV RNA. To identify key players involved in mediating LD movement we performed a side by side proteomic/lipidomic screen on LDs and EVs (extracellular vesicles, known small particle movers between cells). We identified a large protein overlap between LDs and EVs, and using western blot and super resolution microscopy we demonstrated ESCRT pathway members to be co-localised to both LDs and EVs.

Conclusion
These findings highlight a previously unknown role for LDs as novel extracellular communicators. Our findings point to early flavivirus infection triggering the release of LDs to act as extracellular communicators between cells, with this release potentially being facilitated via ESCRT pathway proteins. A better understanding of this process will enhance our knowledge of how cells communicate to combat flavivirus infection.

Transport of Japanese Encephalitis Virus neutralising monoclonal antibodies across the blood-brain barrier

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Japanese Encephalitis Virus (JEV) is increasingly prevalent in Australia, with a recent outbreak in 2022 infecting 45 people and causing 7 deaths[1]. While only 0.1-1% of JEV infected people develop encephalitis[2], the fatality rate amongst this group is ~30%, and 30-50% of the survivors are left with permanent neurological damage[3]. JEV vaccines prevent disease by inducing neutralising antibodies, however, no licensed therapeutics exist[2]. Given the importance of neutralising antibodies in vaccine-induced protection, we reasoned that therapeutic antibodies may provide an effective JEV-specific treatment.

To this end, we recruited a cohort of JEV vaccinated donors and collected peripheral blood. In addition, we expressed recombinant E protein (the protein responsible for viral entry and the main target for neutralisation). This E protein was fluorescently tagged for flow cytometric sorting of E-specific memory B cells from PBMCs. B cell receptor sequencing identified E-specific immunoglobulins, which we then expressed as monoclonal antibodies (mAbs). These mAbs were screened for binding to JEV virus-like particles (VLPs) and for viral neutralisation activity against the vaccine strain (IMOJEV).

Our strongest neutralising mAb (MJE011) bound to JEV VLPs strongly (EC_{50} of 6.2ng/ml) and potently neutralised IMOJEV (IC_{50} of 9.2ng/ml). While this mAb was not cross-reactive with the closely related Murray Valley Encephalitis Virus, MJE011 represents a potential therapeutic for people with recent JEV exposure or early-stage infection.

However, poor penetration across the blood-brain-barrier (BBB) may limit the effectiveness of therapeutic mAbs during the later encephalitic stages of infection. To overcome this, we developed bispecific 'BBB shuttle' versions of MJE011 to facilitate receptor-mediated transcytosis across the BBB. The BBB shuttle consists of a single chain variable fragment (scFv) that binds to endogenous proteins (e.g. TfR1) expressed by BBB endothelial cells[4] . MJE011 attached to BBB shuttles: i) bound to both their brain endothelial target and to JEV, ii) retained its neutralisation capacity and iii) accumulated in the brain at much higher levels than their unmodified counterparts in mice.

This study, to our knowledge, is the first application of BBB shuttle technology to a viral infection scenario and provides promising therapeutic options for both early and late stages of JEV infection.

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Characterisation of novel bat pararubulavirus identified in Australian Pteropus bat urine

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Emerging zoonotic viruses from wildlife reservoirs pose an ongoing global health challenge, with Pteropus bats recognised as key hosts for a diverse range of viral families. Within the Paramyxoviridae family, highly pathogenic henipaviruses such as Hendra and Nipah are well characterised, yet pararubulaviruses belonging to the rubulavirinae subfamily, remain poorly understood despite their frequent detection in bats. Although some rubulaviruses, including Menangle (MenV) and Sosuga viruses, have been linked to human and animal disease, the diversity, ecology and spillover potential of pararubulaviruses are largely unexplored. Addressing this gap is critical for improving early detection of emerging pathogens and strengthening preparedness for future zoonotic threats. This study aimed to characterise three novel pararubulavirus isolates obtained from pooled urine samples collected beneath a *Pteropus* bat roost in Boonah, Queensland. Viral isolates were propagated in *Pteropus alecto* (PaKi) and African green monkey kidney (Vero) cells, generating six samples that were assessed separately to compare potential differences. Reverse transcription PCR confirmed their classification within the pararubulavirus genus using paramyxovirus and rubulavirus specific primers. MinION sequencing generated nucleotide reads that enabled identification of viral genomes for each sample. To further validate these findings, antisera raised against MenV, Teviot (TeV), and Tioman (TioV) viruses were tested in immunofluorescence assays using Vero cells infected with the six virus samples . TeV antisera reacted with Samples 1 and 2, MenV antisera reacted with samples 3 and 4, while both MenV and TeV sera reacted with samples 5 and 6. These results indicate conserved antigenic sites among pararubulaviruses that may influence host range and interspecies transmission. Growth kinetics were assessed across multiple human and animal cell lines to identify differences in replication dynamics, including viral growth rate and cell type susceptibility. Together, these findings expand current knowledge of pararubulavirus diversity in Australian Pteropus bats, reveal antigenic relationships with recognised zoonotic paramyxoviruses and highlight the need for continued surveillance to prevent future bat-borne disease outbreaks.

Cancer-related gene expression in bat retrovirus infection

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Bats are critical reservoirs for zoonotic viruses, including coronaviruses, lyssaviruses, filoviruses and paramyxoviruses. Zoonotic retroviruses are one the most impactful viral families to jump from animals to humans. Human immunodeficiency virus (HIV) and human T lymphotropic virus (HTLV) are zoonotic retroviruses that emerged from non-human primates and cause a large human disease burden worldwide.

Retroviral infections in humans and animals are often oncogenic. Retroviral replication involves the insertion of proviral DNA into the host genome, leading to oncogenesis when insertion disrupts the expression of cellular oncogenes. Recently, the first confirmed infectious bat retrovirus, Hervey pteropid gammaretrovirus (HPG), was discovered in the Australian black flying fox (*Pteropus alecto*) (1). HPG is closely related to the gibbon ape leukemia virus (GALV) and koala retrovirus (KoRV), both of which cause blood cancers in their hosts. Notably, HPG has been reported in a bat with leukemia, suggesting that HPG may also be oncogenic (2). Whether HPG can cause cancer in bats or other mammalian hosts is not presently known. *In vitro* studies show that HPG can establish persistent infection in human cells by utilising the PiT-1 receptor, which is highly conserved and widely expressed across mammalian tissues.

In this study we investigate if HPG infection in human and bat cell lines will alter the expression of cancer-related genes. Human and bat cell lines will be infected with HPG and repeatedly passaged. RNA will be extracted and compared to uninfected cells through RNA-seq. Differential gene expression and oncological pathway enrichment analyses will be used to reveal the impact of HPG infection in bat and human cell lines. This will be supported by RT-qPCR expression analysis of genes previously reported to be regulated by KoRV infection. These data will represent the first step toward revealing the potential cancer-related impact of HPG infection in bats and humans, and advance our understanding of this novel bat retrovirus.

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Development of an *ex vivo* airway epithelium model to study bat innate immune responses

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Bats are natural reservoir hosts for a variety of viruses that are highly pathogenic in other susceptible species, including humans, yet rarely cause clinical disease in bats. The Australian black flying fox (*Pteropus alecto*) is a native Australian bat species that has been identified as a natural host for Hendra, Menangle, and ABLV. Of these, Hendra causes fatal disease in humans and horses. Therefore, decoding bats' ability to control virus infection has the potential to inform the development of interventions to prevent spillover of viruses to other susceptible hosts and identify new therapeutic targets. Previous studies to characterise the host-pathogen responses of bats have predominantly used 2D cell culture systems consisting of a monolayer of cells. In recent years, more complex cell culture systems, including organoids and transwell models, have become standard for studies of humans and other species, providing more physiologically relevant *in vitro* systems. A few physiologically relevant 3D culture models have been established for other bat species to understand their antiviral capabilities, but no *ex vivo* models have been reported for *P. alecto*. In this study, we isolated airway epithelial cells from *P. alecto* tracheobronchial tissues and then differentiated them at the air-liquid interface to generate pseudostratified 3D airway epithelium cultures. The bat epithelial cell model exhibited a mucociliary phenotype, characterised by mucus secretion and beating cilia on the surface of ciliated cells. Histological- and immune-staining confirmed that the bat tracheobronchial cells formed a pseudostratified epithelium consisting of basal, goblet, club and ciliated cells. Stimulation of this differentiated *P. alecto* airway epithelium with the Poly I:C, a pattern-recognition receptor agonist resulted in upregulation of the innate immune response. Here, we describe the characteristics of this model, including the innate immune response and demonstrate that bat epithelial cells provide a physiologically relevant model for studying the host-pathogen response of *P. alecto*.

Breaking the Barrier: The IL-33–ILC2 axis drives severe urinary tract infections

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Structural basis of biofilm formation by the oral pathogen *Treponema denticola*

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Biofilms are structured microbial communities that drive chronic infections by conferring resilience to antibiotics and host defences, yet the structural adaptations underpinning their assembly remain poorly defined. The periodontal pathogen *Treponema denticola* forms complex oral biofilms linked to periodontal disease. Using an integrated, cross-scale approach combining confocal laser scanning microscopy with fluorescence in situ hybridization (CLSM-FISH), cryo-electron microscopy, cryo-electron tomography (cryo-ET) and phylogenomics, we reveal the structural basis of biofilms formation by *T. denticola*. Our cryo-ET analysis revealed that during planktonic-to-biofilm transition, *T. dentic*ola undergoes striking morphological transitions from spiral-shaped planktonic cells to densely packed, intertwined architectures. We demonstrate that periplasmic flagella undergo dramatic structural remodelling during biofilm development and play a crucial role in mediating cell–cell contact within biofilms. Sub-3 Å structures of flagellar filaments in two distinct conformations revealed planktonic flagella is made of 7 different proteins (including 4 previously uncharacterized) forming a complex asymmetric lattice whereas within biofilms, flagellar filaments are made of a single protein. These findings reveal structural and functional adaptations linking motility machinery to stable biofilm architecture with broad implications across diverse bacterial pathogens.

Structural and functional characteristics of legionella effectors targeting mitochondria

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Legionella pneumophila is an intracellular pathogen that delivers over 330 effector proteins into host cells through its Dot/Icm Type IV secretion system (Qiu and Luo, 2017, Lockwood *et al.*, 2022). There are several effectors targetting mitochondria, but the molecular basis and functional significance of these effectors remain poorly defined (Escoll *et al.*, 2017). It is unclear whether these effectors primarily facilitate organelle localisation of host proteins or actively modulate key mitochondrial biochemical pathways to promote bacterial replication and survival (Escoll *et al.*, 2016). Given the rising incidence of Legionnaires' disease in Australia, elucidating the bacterial strategies that manipulate host mitochondrial functions during infection is crucial.

This study focuses on two possible *Legionella* effector–host enzyme pairs: *L*pPIP–PPP1CA and LpTA1–glycerol kinase. PPP1CA was identified as an *L*pPIP interactor through proteomic analysis by our collaborator (Yek *et al.*, 2025). AlphaFold modelling predicts intrinsic disorder within *L*pPIP; therefore, co-expression with human phosphatase PPP1CA in a pETDuet-1 vector to stabilise the complex was designed. In parallel, LpTA1 and glycerol kinase were expressed separately to evaluate their potential functional interaction.

Progress toward characterising the enzymatic activities of PPP1CA and glycerol kinase—with and without their respective effectors—will be described using colorimetric enzyme assays. Additionally, structural studies by X-ray crystallography are underway to resolve the effector–host complexes at atomic resolution. These studies aim to identify key binding residues, conformational changes, and molecular mechanisms by which *Legionella* effectors modulate host enzymes. Ultimately, this work seeks to advance our understanding of *Legionella's* manipulation of host mitochondrial pathways to support its intracellular survival.

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Subcellular localisation of RNA-binding effectors from *Legionella pneumophila*

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Background

Legionella pneumophila is an intracellular, bacterial pathogen and the causative agent of Legionnaires' disease, a severe form of pneumonia. A key virulence mechanism is the secretion of >300 bacterial 'effector' proteins into host cells by the Dot/Icm type IVB secretion system. Recently, our laboratory performed an RNA-interactome capture screen to isolate *Legionella* effectors bound to host messenger RNA (mRNA) during infection. This included the Dot/Icm effectors Lpg1666, LegLC8 and LepB, which had no previously known function in *Legionella* pathogenesis or the modulation of host transcriptional responses to infection.

Aim

The aim of this study was to characterise these novel RNA-binding effectors and their impact on host mRNA processing within the cell.

Key Methods

To characterise these effectors, human cell lines were transfected with fluorescently-tagged effector constructs, and key cellular organelles and RNA processing structures were subsequently detected by indirect immunofluorescence for analysis using confocal microscopy. Furthermore, mRNA localisation was examined using fluorescent *in situ* hybridisation (FISH).

Results

We found that *Legionella* RNA-binding effectors localised to different parts of the cell. While LegLC8 localised exclusively to the cytoplasm, Lpg1666 localised to the nucleus, and LepB displayed an asymmetrical expression pattern, suggesting potential association with the secretory pathway. Using quantitative cell profiling, we showed that Lpg1666 distorted the nuclear envelope through the disruption of the localisation of lamin B1, a key structural component of the nuclear envelope. We also observed that each of the RNA-binding effectors altered the formation of stress granules within the cytoplasm of transfected cells. Stress granules are cytoplasmic structures formed to sequester stalled or damaged mRNAs during cellular stress.

Conclusion

Overall, each of the RNA-binding effectors characterised had unique localisation within the cell, suggesting that *Legionella* may target host mRNA processing at various stages. Future investigation of these effectors during *Legionella* infection will aid in elucidating their impact on host mRNA processing and its significance for bacterial pathogenesis.

Prior *S. aureus* colonisation impacts future immune responses to invasive *S. aureus* infections

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Staphylococcus aureus is a ubiquitous component of the normal human microbiota and among the first commensal bacteria to seed the nasal mucosa, with initial exposure occurring at birth. This bacterium is also an opportunistic pathogen and can disseminate from the nasal tissue to cause invasive disease. It is unclear whether the presence of *S. aureus* within the nasal microbiome from neonatal life has formative effects on the adaptive immune response, or whether this perturbs the development of an effective immune response against this bacterium later in life. We established a mouse neonatal colonisation model of *S. aureus* to examine the adaptive immune response to colonising *S. aureus* and examined whether this immunity had any protective effect during an invasive *S. aureus* infection. Mice remained colonised with *S. aureus* in both the nasal and intestinal compartments for at least 6-7 weeks after birth. Colonisation by *S. aureus* induced the development of *S. aureus*-reactive $CD4^+$ T cells as well as anti-*S. aureus* antibodies. These $CD4^+$ T cells subsequently conferred site-specific protection against an invasive systemic *S. aureus* infection later in life. Adaptive immunity generated towards commensal *S. aureus* provides a protective advantage against invasive *S. aureus* infection. This neonatal colonisation model will enable further investigation into how early-life exposure to *S. aureus* influences vaccine efficacy and immune responses to infection across the lifespan.

Detection and Characterization of Antimicrobial Resistance Genes in Mothers and Infants During the First Year of Life

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Background: Antimicrobial resistance (AMR) is a critical global health concern, yet the establishment and progression of the infant gut resistome during the first year of life remains underexplored.

Methods: We analysed faecal samples from mothers and their children at birth, 1 month (O), and 12 months (T) within the Barwon Infant Study, alongside data on delivery mode (vaginal (VD) or caesarean (CS)) and antibiotic exposure during pregnancy (mothers (M)) and infancy (children).

Results: We detected 151 unique AMR genes spanning 15 antibiotic classes. Mothers harboured more abundant and diverse resistome than infants, who had the lowest detection at 1 month but showed a marked increase by 12 months. Both maternal and infant resistome included genes conferring resistance to the three most prescribed antibiotics in Australia: tetracyclines, β-lactams, and macrolide–lincosamide–streptogramin B (MLSB). Tetracycline was the most prevalent class across delivery modes (VD: M=104, O=102, T=108; CS: M=76, O=75, T=69), while β-lactams exhibited the highest gene diversity (34 genes). MLSB genes were also prominent (VD: 246 detections; CS: 183 detections). Among minor classes, trimethoprim resistance was most frequent (VD: 75; CS: 63), followed by glycopeptides (VD) and phenicol (CS).

Richness and Shannon diversity increased from birth to 12 months in both groups ($p<0.001$). VD infants showed a 73.1% increase in richness (7.6 → 13.2 genes) and a 28.2% increase in Shannon diversity (1.73 → 2.22), while CS infants showed a 51.2% increase in richness (9.6 → 14.5 genes) and a 20.2% increase in Shannon diversity (2.15 → 2.58).

Conclusions: Significant AMR carriage was detected in both mothers and infants, with the infant resistome increasing over the first year of life. Tetracycline and β-lactam resistance genes were dominant across cohorts.

Implications: These findings highlight the acquisition of AMR from maternal and environmental sources and underscore the importance of early-life antimicrobial stewardship.

Disruption of *mecR* impairs MecA-mediated cephamycin resistance in *Clostridioides difficile*

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PUBLISH CONSENT WITHHELD

Simple sequence repeats power *Staphylococcus aureus* adaptation

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Background
Staphylococcus aureus is a major human pathogen with a remarkable capacity for rapid adaptation. Its ability to undergo phenotypic switching enables evasion of antimicrobial therapies and contributes to treatment failure and resistance emergence. However, the specific genetic mechanisms and molecular pathways that enable this phenotypic plasticity remain poorly defined.

Methods
To uncover recurrent adaptive events, we developed the Convergent Mutation Adaptive Score (CMAS), a scalable metric for detecting convergent adaptive mutations across bacterial genomes. Applying CMAS to 7,099 *S. aureus* genomes revealed a significant enrichment of loss-of-function mutations (n = 500, $p \leq 0.001$), predominantly arising from frameshifts mediated by simple sequence repeats (SSRs). Among these, an SSR frameshift in the DNA mismatch repair gene *mutL* (*mutL*-N343fs) emerged as a top candidate for functional validation. We reconstructed *mutL*-N343fs in a wild-type background and first quantified its impact on genome-wide mutation rate via spontaneous rifampicin resistance. Next, we subjected mutant and wild-type strains to five serial passages in vancomycin and daptomycin broth microdilution to monitor resistance emergence. Finally, we deployed a chromosomal *ermC* reporter bearing synthetic SSR tracts of 5–9 nt to measure how loss of MutL alters SSR expansion and contraction frequencies.

Results
Relative to wild type, the *mutL*-N343fs mutant exhibited a > 36-fold increase in mutation frequency ($p \leq 0.0001$) and accelerated emergence of non-susceptibility to daptomycin and vancomycin over five serial passages. In the *ermC* reporter system, *mutL*-N343fs drove a significant rise in SSR expansion and contraction rates ($p \leq 0.0001$). Presence of *mutL*-N343fs also correlated with an elevated genome-wide SSR frameshift burden ($p < 0.001$).

Conclusions
Our study establishes CMAS as a powerful tool for mapping adaptive convergence and identifies SSRs as central, reversible switches of genetic variation in *S. aureus*. The *mutL*-N343fs frameshift functions as a master switch, transiently elevating mutation rates and unlocking downstream SSR-mediated adaptations. This mechanism provides a dynamic, reversible pathway for accelerated evolution under selective pressure.

Aztreonam plus ciprofloxacin synergistically kills resistant *Pseudomonas aeruginosa* strains.

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Investigating the immunomodulatory and clinical effects of fermentable fibre intervention to reduce symptoms of Long COVID

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Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or COVID-19 is a type of respiratory infection that has had a tremendous role with regards to contribution to global disease burden, morbidity and economic instability. Although thought of as an acute infection, more cases of chronic symptoms of COVID-19 have been noted, with and the clinical term being post-acute COVID-19 syndrome (PACS) or Long COVID. Symptoms experienced by individuals are heterogenous in nature and include fatigue, cognitive functioning problems, muscle pain, immune dysregulation and significant changes to the gut microbiota. Therefore, this study aims to investigate whether dietary intervention with fermentable fibre (inulin and resistant starch) over the course of 21 days can lead to symptom alleviation in people affected by PACS, and whether this is modulated by changes to the immune system.

Methodology: A 3-week randomised dietary intervention study with a parallel design is currently being undertaken comparing the effects of fermentable fibre (18 g/day) versus a low-fibre control. Blood samples are collected pre- and post-intervention for immune-phenotyping and serology. Long COVID symptoms, gastrointestinal symptoms and dietary intake are also being assessed at baseline and during intervention.

Results: Long COVID patients have been found to have different dietary intake and gastrointestinal symptoms to healthy people. No significant differences to absolute numbers of leukocytes, monocytes and lymphocytes (B and T cells) have been observed to date. However, ongoing work is being conducted to examine the T-cell activation through flow cytometry, as well as quantification of SARS-CoV-2 specific antibodies.

Conclusion: People with Long COVID may have significantly different dietary habits and gastrointestinal symptoms compared to healthy people. This study is ongoing to assess if dietary fibre intake may have benefit for symptoms and if these are associated with changes to the immune system and gut microbiota.

Investigating the impact of diet on invasive fungal infection outcomes

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Diets modulate whole-body metabolism in humans. The role of diets in controlling metabolic health is characterised, but less is understood about the role of dietary components in modulating outcomes in infectious diseases. We are focusing on fungal invasive candidiasis, a high-mortality disease caused by fungal pathogen *Candida albicans*. As prior preclinical study indicated an effect of diet quality on viral infection outcomes (1), this project aimed to compare the roles of acute feeding of macronutrient matched ultra-processed diet (UPD) and an isocaloric grain-based (GBD) control diet in enhancing protective metabolic responses in systemic candidiasis.

Male and female C57Bl/6J mice, aged 7 weeks, were fed one of the two diets for 7 days followed by intravenous infection with 3*10^5 CFU of *C.albicans*. Of note, there was no differences in between the diets with total food caloric intake prior to infection, nor caloric intake or body weight reduction after *C. albicans* infection. After 48 hours, the groups were compared for fungal burden, systemic damage markers (ALT and AST), tissue cytokine levels, and serum metabolites. UPD-fed male mice had a slightly higher fungal burden, however this was not seen in the female mice, and no other parameters including survival were affected. Furthermore, serum metabolomic analysis revealed both diet- and infection-induced alterations in serum metabolites, indicating clear effects of both infection and individual diets.

Although results so far indicate slightly higher fungal burden with UPD feeding, the mechanism remains unclear. Our ongoing efforts aims to generate a comprehensive knowledge about how diets that differ in sources, and ratio of macronutrients can affect host metabolism to modulate infection outcomes. This knowledge can be translated to develop diets as a therapeutic tool to support the recovery of patients with systemic candidiasis.

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Genetic dissection of *Toxoplasma* differentiation

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Toxoplasmosis is an infectious disease caused by the intracellular, obligate Apicomplexan parasite *Toxoplasma gondii*. Approximately one-third of the global human population is chronically infected with *Toxoplasma,* and no treatment exists to clear the latent reservoir, putting immunosuppressed patients at risk of reactivation and severe disease. Only a handful of genes have been identified to be important for *Toxoplasma* differentiation from the acute 'tachyzoite' form into the slow-growing latent 'bradyzoite' form, including a master transcription factor – *BFD1* which is necessary and sufficient for this process. To expand our understanding of the underlying molecular pathway controlling *Toxoplasma* differentiation, our lab recently performed a series of *in vitro* and *in vivo* CRISPR screens, identifying over 30 new genes, including a series of nucleic acid-binding proteins (NBPs) and an E3 ubiquitin ligase complex (GID). Focussing on three NBPs, we show that two are localised in the parasite's cytoplasm, whilst the other is found in the nucleus. Using quantitative imaging and sophisticated genetics we show that two NBPs likely operate early in the differentiation program upstream of *BFD1*, whilst the other likely more constitutively required. We show that the level of these proteins is regulated by the E3 ubiquitin ligase. We are currently determining how these NBPs act to control translation repression during bradyzoite development. This study therefore shows the importance of RNA regulation in controlling fate determination in this single celled eukaryote and their role in pathogenesis.

Ecological Surveillance of *Mycobacterium ulcerans* (MU) in Urban Possums and Development of a CRISPR-SHERLOCK MU Assay

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PUBLISH CONSENT WITHHELD

Dissecting Host Manipulation by *Cryptosporidium*.

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The apicomplexan parasite *Cryptosporidium* causes the severe diarrheal disease, cryptosporidiosis, a leading cause of childhood mortality globally. There is no vaccine and only one FDA approved drug – nitazoxanide – which is ineffective in young children and immunocompromised individuals. *Cryptosporidium* infects host enterocytes and like its cousins, *Plasmodium* and *Toxoplasma*, secretes proteins into its host cell to establish infection. It has long been understood that *Cryptosporidium* drastically remodels the host actin cytoskeleton, including the formation of an actin patch at the host-parasite interface. Another hallmark of *Cryptosporidium* infection in the enterocytes include actin-dependent elongated microvilli, a morphological feature shared between enteric pathogens. Recently, a highly disordered exported effector, MVP1, was identified to facilitate microvilli elongation through the activation of actin polymerisation pathways. Despite new findings, mechanistic involvement of secreted effectors in parasite invasion remains poorly understood.

In this study, we used TurboID and APEX- expressing host cell lines to identify effector proteins of *Cryptosporidium*. Using newly developed CRISPR-Cas12 technology and mouse models, we have generated transgenic HA-tagged parasites and show that two of these effectors are exported into the host upon invasion, localising with host actin. Further, we implicate an aspartyl protease, CpASP4, in the proteolytic processing and maturation of these effectors and show that this process is essential for parasite survival. This study greatly expands our molecular understanding of host manipulation in *Cryptosporidium* infection and highlights a new therapeutic pathway for the development of an efficacious anti-cryptosporidial agent.

Investigating the role of Kelch-13 during sexual development in *P. Falciparum*

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Malaria remains one of the most widespread and significant infectious diseases worldwide, causing substantial morbidity and mortality despite ongoing prevention and treatment efforts. *Plasmodium falciparum*, a causative agent of malaria, has a complex life cycle with stages in both human and *Anopheles* mosquito hosts. Currently, primary treatment for malaria depends on artemisinin-based combination therapies, however, the emergence of artemisinin resistance independently in both south-east Asia and now Africa threatens the control of this disease. The most prevalent mutations associated with artemisinin resistance are in the parasites' *Kelch13* (K13) gene. During asexual development, the K13 protein plays an important role in the formation, stability and function of a double-membraned invagination called the cytostome, whose role is to facilitate uptake of haemoglobin from the host red blood cell into the parasite. This provides the parasite with essential amino acids required for growth and simultaneously releases haem-iron as a bioproduct which is crucial for artemisinin activation. Whilst K13 and it's role in haemoglobin uptake is well studied during asexual development, it's role during sexual blood, mosquito and liver stages of development are yet to be investigated.

Using fluorescence and super resolution microscopy, we show that K13-GFP displays a dynamic localisation pattern throughout gametocytogenesis, with several K13-GFP puncta often converging to form a unique rod-shaped structure in late-stage gametocytes. We are using lattice light-sheet microscopy to track the fate of K13-GFP and cytostomes during gamete formation and further investigating the localisation and function of K13 in mosquito stages. Using a knock-sideways approach, we show that mislocalisation of K13 during early-stages of gametocyte development leads to parasite death. This has implications for the role of K13 in mediating haemoglobin uptake and artemisinin resistance during transmission.

Immune profiling of severe and fatal influenza infections in First Nations patients with multimorbidities

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Defining protective, long-lasting immune mechanisms that underpin rapid recovery from life-threatening respiratory viral infections in hospitalised patient cohorts, including influenza disease and COVID-19, is essential to understand why some people develop severe disease, leading to hospitalisation or death, while others develop mild infection. The presence of underlying comorbidities can impact the patient's ability to respond to respiratory viral infections.

We determined how innate and adaptive immune cells work together to resolve respiratory viral disease in hospitalised-infected patients with comorbidities. Since 2014, we have been actively recruiting hospitalised patients across multiple sites within Australia. We measure cytokine and chemokine profiles within the blood and perform direct whole blood cytometry staining to measure activation of immune cellular subsets.

We found high comorbidity rates (80%) in our hospitalised influenza patients, ranging from 0 up to 7 comorbidities per patient. First Nations patients with influenza disease had lower median age by 12 years and higher rates of multimorbidities such as diabetes and renal disease. Inflammatory cytokines and chemokines, particularly IL-8 and IL-18, were higher in First Nations patients, associated with multiple comorbidities. First Nations patients also had reduced circulating ICOS+PD-1+ activated T follicular helper (Tfh) cells, in comparison to non-Indigenous patients.

We provide a detailed analyses of immune response networks that promote recovery for hospitalised patients with respiratory virus infections, particularly in the context of people living with comorbidities. Potentially, patients presenting to hospital with high comorbidities may need different treatment strategies given their higher inflammation levels.

Investigating Antibody Responses to Endothelial Protein C Receptor Binding *Plasmodium falciparum* Erythrocyte Membrane Protein 1 Associated with Severe Malaria in Children

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Introduction: Malaria remains a major global health problem, with 263 million cases and 597,000 deaths reported in 2023 (1). Variant surface antigens expressed on infected erythrocytes (IEs), mainly the PfEMP1s play role in malaria pathogenesis and immunity (1-2). Antibodies targeting the CIDRα1 domains of EPCR-binding PfEMP1 variants have been associated with reduced disease severity (2–4). However, the acquisition of these antibodies particularly when assessed using IEs and their functional roles in mediating protection are not well understood. **Methods:** Plasma sample from sixty PNG children with severe malaria (SM) and thirty-eight with uncomplicated malaria (UM) was collected at enrolment and 8 weeks later and used to measure IgG binding to EPCR binding IT4-*Var19* parasites and antibody dependent phagocytosis by THP-1 cells and neutrophils using flow cytometry. Moreover, the level of IgG afucosylation to the CIDRα1.1 recombinant protein was measured using the Fucose-sensitive Enzyme-linked Immunosorbent Assay (FEASI) assay. The Wilcoxon signed-rank sum test was used to compare IgG levels and functional responses between two paired groups whereas, Mann-Whitney U test was used for two unpaired groups using GraphPad prism. **Results:** At presentation, IgG response to EPCR binding IT4-*Var19* parasites, ADCP by THP-1 cells and ADNP were higher in children with UM. The ADCP increased from presentation to convalescence in SM children. At presentation, afucosylated IgG was higher in UM and it was increased from presentation to convalescence in children with SM. **Conclusion:** Higher IgG to EPCR binding PfEMP1 observed in children with UM, may indicates prior exposure and development of protective immunity. Moreover, the increased IgG in convalescence in SM children could suggest that *Var19* parasites have role in SM. Higher levels of ADCP and ADNP at presentation in children with UM suggests phagocytosis of IEs are protective. The increase in ADCP from presentation to convalescence in children with SM, despite stable IgG levels, highlights the importance of measuring antibody function not just antibody levels.

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The Effects of N^6-methyladenosine on the Proteome of the Malaria Parasite *Plasmodium falciparum*

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Plasmodium falciparum accounts for 95% of the nearly 600,000 estimated deaths from malaria annually. Multiple rounds of asexual replication during the intra-erythrocytic developmental cycle (IDC) stage of *P. falciparum* results in the pathology associated with malaria. Each cycle of the IDC is approximately 48 hours and begins with the parasite invading a new erythrocyte. Completion of the IDC requires precise regulation of gene expression. mRNA modifications — such as N^6-methyladenosine (m6A) — are known to regulate mRNA export, abundance and translational efficiency in yeast and mammalian cells.

Like other eukaryotes m6A is the most prevalent modification in *P. falciparum* mRNA. Adenosine is converted to m6A by the nuclear protein complex known as the m6A writer. Orthologs of the human writer components such as METTL3 (PF3D7_0729500; annotated as *Pf*MT-A70 in *P. falciparum* genomes) have been detected in *P. falciparum*. In our lab, we use the rapamycin inducible knock sideways system to mislocalise *Pf*MT-A70 away from the nucleus to the parasite plasma membrane to study the effects of m6A.

Using nanopore direct RNA sequencing we have observed that knock sideways of *Pf*MT-A70 depletes m6A in mRNA and changes in the abundance of some mRNA. Other studies have reported changes to the translational efficiency of some mRNA based on polysome profiling. The consequence of this altered mRNA biology on protein synthesis has not been studied. The aim of this study was to study the effects of *Pf*MT-A70 knock sideways on protein abundance using label-free quantitative proteomics.

Parasites that were 28±2 (28HPI) and 36±2 (36HPI) hours post invasion of a new erythrocyte were used for the analysis. Knock sideways was induced for 4 and 8 hours for each time-point along with uninduced controls. Peptides for 1,548 and 2,117 were detected for the 28HPI and 36HPI time-points respectively, however small proportion of these (6.9% and 11.8% respectively) had statistically significant changes to their abundance following 8 hours of knock sideways.

Although these results seem to indicate that m6A in mRNA has some impact on protein abundance, these results are not conclusive. Further optimisation of the technique is being carried out to yield more conclusive results.

Mechanistic insights into repurposed compounds as potential antimalarials

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Malaria remains a leading cause of morbidity and mortality, with 608,000 deaths reported in 2022, predominantly among children under five in sub-Saharan Africa. The disease, predominantly caused by Plasmodium falciparum, faces escalating challenges due to the emergence of strains resistant to all frontline antimalarials, including artemisinins. This highlights an urgent need for new therapeutic strategies. While de novo drug discovery is slow and costly, repurposing existing compounds offers a rapid and cost-effective alternative.

We screened the Structural Genomics Consortium's Donated Chemical Probes Library, which consists of compounds originally developed for human diseases, to identify candidates with antimalarial potential. From 200 compounds, 11 demonstrated sub-micromolar potency against the P. falciparum Pf3D7 reference strain and retained activity across five resistant lines. Their equipotent profiles suggest novel mechanisms of action distinct from existing drug classes. In vitro pulse activity assays further characterized compound speed-of-action and asexual stage-specific antimalarial activity.

Future work will employ unbiased multi-omics approaches, including as untargeted metabolomics, solvent proteome profiling and limited-proteolysis mass spectrometry, to elucidate molecular targets and validate these candidates as starting points for drug development. These findings highlight the potential of compound repurposing to accelerate antimalarial discovery and identify new druggable pathways to combat resistance.

Predicting the impact of antimalarial resistance in West African parasites

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Background

Africa bares the highest malaria burden, with 569,000 deaths in 2023. Artemisinin combination therapies (ACT) remain the frontline treatment but are becoming less efficacious due to delayed parasite clearance linked to mutations in the *Plasmodium falciparum* Kelch 13 (K13) gene. While well documented in East Africa, similar resistance is predicted to emerge in West Africa.

Aims and Methods

This study investigates artemisinin phenotypes in contemporary West African parasites. A total of 380 isolates from Ghana and The Gambia will be genotyped, with 15 multi-drug resistant isolates selected for whole-genome sequencing. Known resistance markers, including *pfcrt*, *pfmdr1*, and *plasmepsin 2/3*, will be assessed and correlated with phenotype across adapted parasite lines. Three genetically diverse culture-adapted Ghanian strains will be transfected with key K13 mutations (R561H, 622I, A675V, C469Y) to analyse their resistance phenotypes. Multi-omics approaches will identify biochemical hallmarks linked to artemisinin resistance.

Results

Preliminary genotyping has revealed pyrimethamine resistance markers typically associated with mild resistance. However, phenotypic assays suggest strong resistance, indicating additional mechanisms may be involved. Initial work will correlate genotypic markers with phenotypic profiles across the same parasite populations.

Conclusion

These findings will inform ACT resistance surveillance and treatment strategies in West Africa.

Impacts of apicoplast-targetting antibiotics on dihydroartemisinin activation in *Plasmodium falciparum*

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Malaria is a disease of significant and ongoing global burden, caused by parasites of the *Plasmodium* genus and carried by *Anopheles* mosquitoes. *Plasmodium falciparum* is responsible for 90% of global malaria mortality. Effective drug treatment is instrumental to control of this disease, and the World Health Organisation (WHO) recommends artemisinin (ART) combinations for treatment of uncomplicated *Plasmodium falciparum* malaria. In the event that these frontline treatments fail, the WHO guidelines recommend second line treatments that combine artesunate with doxycycline, clindamycin, or tetracycline. Further, these common antibiotics may be used in treatment of other infectious diseases, which risks inadvertent combinations. Previous work in the Ralph laboratory used the Chou and Talalay isobologram method to assess the nature of interaction between these apicoplast-targeting antibiotics and ART derivatives, revealing an antagonistic relationship between dihydroartemisinin (DHA) and antibiotics including doxycycline and clindamycin. The key to this antagonism lies in a relict plastid organelle in *Plasmodium* parasites known as an apicoplast. This endosymbiotic organelle is the target of a number of antibiotic therapies due to its bacterial-type translation machinery. Both doxycycline and clindamycin are classified as 'delayed death' inhibitors, as the initial parasites are apparently unaffected, yet the apicoplasts of their daughter parasites are defective. These defects result in reduced isoprenoid synthesis, protein prenylation, and ultimately in the parasites' inability to traffic haemoglobin into their digestive vacuole, where oligopeptides and toxic free haem would otherwise be released. Crucially, the iron in this free haem activates artemisinin derivatives to produce free radicals that impair protein function and ultimately leads to parasite death. We posit that this reduction in haemoglobin degradation may contribute to the observed antagonist effect between apicoplast-targeting antibiotics and ART derivatives. To test this, we are performing novel trophozoite stability assays established by the Creek Laboratory at the Monash Institute of Pharmaceutical Science to directly measure artemisinin activation within *Plasmodium* parasites pre-treated with apicoplast-targeting antibiotics. This assay, in combination with further haemoglobin fractionation, will shed light on the nature of the observed antagonism between doxycycline and clindamycin and DHA and may inform how these drugs should be used in the future.

Investigating primary and recall humoral immune responses in malaria and the role of type I interferon signalling

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Protective immunity to malaria relies primarily on high level of circulating antibodies. However, individuals in high-endemic areas often fail to mount a protective antibody level efficiently following vaccination, with antibody levels declining after each subsequent booster. The mechanism to establish and maintain the humoral and memory responses against the parasite is unclear. This study aims to investigate the qualitative and quantitative differences between primary and recall anti-malarial humoral immune responses, and to determine how malaria-induced immunoregulatory network via type I interferon, influences these responses.

We will use plasma and PBMCs samples collected from healthy volunteers participating in controlled human malaria infection (CHMI) study treated with Ruxolitinib, a licensed orally bioavailable small molecule inhibitor of type I IFNs signalling pathway, and anti-malaria drugs artemether/lumefantrine. We will investigate the specific epitope and breadth of the primary and recall antibody responses against blood-stage plasmodium antigen merozoite surface protein 2 (MSP2) using peptide array. The phenotype of plasmodium-specific peripheral B cells will be detected and analysed with high-dimensional spectral flow cytometry and scRNAseq with the aid of B cell tetramers. Collectively, this study will give important insights into the development of next-generation malaria vaccines.

Keywords: B cell, antibody, malaria infection

Investigating the role of Kelch 13 protein in *Plasmodium falciparum* gametocytes

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Malaria is one of the deadliest infectious diseases globally, responsible for over 200 million infections each year. *Plasmodium falciparum* causes the most severe form of the disease and the most deaths. *P. falciparum* has a complex life cycle between human and mosquito hosts: the intraerythrocytic asexual cycle is responsible for the symptomatic infection in humans. However, for transmission from human to mosquito, a portion of the parasites gradually differentiate into mature gametocytes, which can be taken up by *Anopheles* mosquitoes. Current frontline treatment against malaria relies largely on artemisinin combination therapies. Emergence of resistance against artemisinin therefore threatens global malaria control. Resistance maps to mutations in the parasites' *k13* gene coding for the Kelch 13 (K13) protein. During the intraerythrocytic cycle, K13 stabilises the parasites' cytostome, a double membrane invagination responsible for haemoglobin uptake from the host red blood cell, by forming a ring around the cytostomal neck. Activation of artemisinin requires reaction with haem released from digested haemoglobin. The K13 mutation is thought to cause drug resistance by decreasing cytostomal uptake of haemoglobin. However, how K13 modulates this process is undetermined. K13's role in gametocyte stages is unknown, and the role of K13 and K13 mutations on parasite transmission is also unresolved. Using an endogenously tagged version of K13, we imaged K13 throughout gametocyte development, using standard fixes as well as ultrastructure expansion microscopy. In early gametocytes, K13 was also resolved to form a ring structure, similar to its asexual counterpart. However, in late-stage gametocytes, which are nearing maturity for mosquito transmission, K13 formed a hollow tubular structure, likely serving a function distinct from nutrient uptake. We will investigate the essentiality of K13 in gametocyte stages and investigate its potential role in differential transmission of artemisinin-resistant parasites.

Investigating the activation of new permeability pathways in *Plasmodium falciparum* infected erythrocytes

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Malaria, caused by parasites of the *Plasmodium* genus, remains a global disease burden accounting for over half a million deaths annually. With resistance reported across all currently available antimalarials, there is an urgent need to identify new drug targets and one approach is through gaining a better understanding of *Plasmodium's* complex biology. *Plasmodium falciparum* is the most lethal of the five species that infect humans where its life cycle begins in the liver and subsequently in the blood where it undergoes asexual reproduction by invading host erythrocytes. About 12-15 hours post invasion, the parasites modify the host cell membrane to form 'new permeability pathways' (NPPs) to facilitate enhanced nutrient uptake and accommodate the parasite's increased metabolic demands for subsequent growth and proliferation. Whilst a lot is understood about the transport properties of NPPs, the identity of the proteins that form NPPs remain poorly understood. The RhopH complex, a trimeric protein consisting of CLAG3, RhopH2, and RhopH3, has been implicated to play a role in the formation of NPPs though the mechanism as to how the complex does this remains unclear. This research focuses on the proposed mechanism in which the RhopH complex interacts with integral erythrocyte membrane transporters to enhance nutrient uptake, following a recent crosslinking study which consistently identified an interaction between RhopH2 and Glut1. By applying metabolomics and structural proteomics, we aim to explore the functional consequences of the interaction between RhopH2 and Glut1 and elucidate the mechanism of NPP activation in *P. falciparum* infected erythrocytes.

Antibody-dependent activation of NK cells as a potential correlate of protection from placental malaria: Insights from Malawi and PNG cohorts with varying infection status at enrolment

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Abstract

Background: In malaria-exposed pregnant women, antibodies to VAR2CSA induce NK cell activation; whether the role of antibody-mediated activation contributes to protection from placental malaria remains unclear.

Method: This study explored activation of NK92-CD16A and primary NK cells by IEs opsonised with plasma samples collected mid-pregnancy from 1) Malawian pregnant women who were infected at enrolment with (n=72) and without (n=84) evidence of past placental malaria at delivery or 2)73 PNG pregnant women (most whom were uninfected at enrolment), 46 with placental malaria at delivery and 27 with peripheral infection but not placental malaria.

Results: NK cell responses from IE opsonised with plasma from Malawian women were not associated with gravidity and were higher with plasma from women with placental malaria than without, with differences between the two groups observed for CD107a in NK92-CD16A ($p = 0.011$) and IFNγ in primary NK cells ($p=0.012$). Using PNG women's plasma, responses of NK92-CD16A cells (CD107a) and NK cells (CD107a, TNF and IFNγ) were lower for plasma from women with than without placental malaria ($P<0.039$).

Conclusion: Antibody dependent activation of NK cell responses show potential as an immune correlate of protection from placental malaria, but associations vary with the timing of infection.

Identification of B cell epitopes in serological exposure markers for improved *Plasmodium vivax* surveillance

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PUBLISH CONSENT WITHHELD.

Unfolding malaria parasite biology: targeting protein disulphide isomerases to block *Plasmodium* invasion and transmission

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Antimalarial resistance is a looming barrier to malaria control, necessitating new therapeutics with novel targets. *Plasmodium* parasite egress from human erythrocytes and subsequent invasion of new host cells is responsible for clinical manifestations of malaria, while transmission to the mosquito vector drives ongoing disease burden.

All essential biological processes within the parasite critically rely on the correct manufacturing of protein machinery to complete the lifecycle across both hosts. Egress, invasion, and transmission are underpinned by a repertoire of highly folded and disulphide-bonded proteins.

Protein disulphide isomerases (PDIs) are established eukaryotic protein folding chaperones and disulphide bonding mediators. The primary malaria parasite, *P. falciparum*, possesses four highly conserved PDIs. Previous work has demonstrated essentiality of PDI-Trans in transmission and its vulnerability to chemical inhibition.

We now show that PDI-Trans knockdown also perturbs folding of essential egress and invasion proteins, and treatment with repurposed PDI inhibitors recapitulates this effect. We have generated compounds that prevent *Plasmodium* invasion, egress, transmission, and growth across multiple species with single digit nanomolar potency and irresistibility in asexual stages. We have also generated tagged transgenic *P. falciparum* lines to characterise the remaining three PDIs, enabling further interrogation of the biology underlying the activity of the inhibitors.

With an established relationship between highly conserved PDIs and integral processes of egress, invasion, and transmission in *Plasmodium*, repurposing existing PDI inhibitors may offer an expedited and novel means of eliminating malaria parasites through dual-stage pan-species activity.

γδ T cell receptor recognition of CD1d in a lipid-independent manner

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T cells comprise the cellular arm of the adaptive immune system and can be categorised into either αβ or γδ T cells. Extensive cellular and biochemical characterisation of αβ T cells has unearthed the principles governing their activation and subsequent recruitment into the immune response. Comparatively there persists a poor understanding on the functions of γδ T cells that has been hampered by an incomplete ligand repertoire. This is despite γδ T cells playing a vital role in the immune response against viral, bacterial and *Plasmodium* infections. Therefore, we investigated how γδ recognised a known antigen, CD1d, to shed light on this enigmatic T cell population (1). Both CD1d and γδ T cells are enriched within mucosal tissues suggesting CD1d-reactive γδ T cells may maintain homeostasis within this compartment.

The monomorphic antigen-presenting molecule CD1d presents lipid antigens to both αβ and γδ T cells. Type I Natural Killer T cells (NKT) display exquisite specificity for CD1d presenting α-Galactosylceramide (α-GalCer), while the extent of lipid specificity exhibited by CD1d-restricted γδ T cells remains unclear. Here we demonstrate that human γδ T cell receptors (TCRs) can recognise CD1d in either a lipid-reactive or auto-reactive manner with weak to moderate affinity. Using small-angle X-Ray scattering we identify conserved γδ TCR-CD1d binding modalities across unique CD1d-restricted TCRs that is distinct from αβ NKT TCR-CD1d binding modes. Contrasting our affinity measurement analysis, in functional assays CD1d presenting endogenous lipids was sufficient to stimulate γδ T cell lines. Moreover, CD1d alone induced γδ TCR-CD3 clustering and phosphorylation in a dose-dependent manner while type I NKT TCR-CD3 clustering required α-GalCer. We then determined the crystal structure of a Vδ1 $\gamma\delta^+$ TCR-CD1d complex, that showed the γδ TCR sat atop of the CD1d antigen-binding cleft but made no contacts to the presented lipid antigen. This was in stark contrast to type I NKT CD1d recognition that co-recognised CD1d and the presented lipid antigen.

Ultimately, through combined biochemical, cellular, and structural based approaches, we provide a molecular basis for lipid-independent CD1d recognition by γδ TCRs and demonstrate ligand availability is a regulator of their activation.

(1) Rice, M.T. *et al* γδ T cell receptor recognition of CD1d in a lipid-independent manner, 2025. *Under Review*, *available on Research Square*, doi.org/10.21203/rs.3.rs-7182106/v1

Runx3 acts as a rheostat for CD8$^+$ tissue-resident memory T cell formation in the liver

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After resolution of an infection, both circulating (T_{CIRC}) and tissue-resident memory T (T_{RM}) cells emerge to combat recurrent infections. While T_{CIRC} cells traffic through the blood and secondary lymphoid tissues, T_{RM} cells do not recirculate. Instead, T_{RM} cells inhabit tissues such as the gut and liver, enabling rapid local immune protection. Despite T_{RM} cells across tissues sharing a core gene module that inhibits recirculation, they adopt tissue-specific transcriptional programs shaped by local environmental cues. This suggests that unique combinations of intrinsic and extrinsic signals collaborate to establish residency across tissues. For example, TGFβ is essential for T_{RM} cell formation in epithelial tissues like the skin and gut, where high Runx3 expression permits TGFβ-responsiveness to enforce residency and drive CD103 expression. Conversely, liver T_{RM} cells lack CD103 and develop independently of TGFβ, raising the question of whether elevated Runx3 expression is also required for these cells. Here, we show that liver T_{RM} cells are uniquely sensitive to enforced Runx3 expression, which suppresses key regulators of their development, including T-bet, LFA-1 and the β-subunit of the IL-2 and IL-15 receptor, CD122. This defect was not rescued by T-bet overexpression and occurred independently of TGFβ-signalling. Enforced Runx3 expression also induced a subset of epithelial-like CD103$^+$ T_{RM} cells in the livers of mice. Given that both CD103$^-$ and CD103$^+$ liver T_{RM} cells exist in humans, with the latter linked to fibrotic diseases, our findings provide insight into how distinct liver T_{RM} populations arise and how they could contribute to pathology. Together, our findings reveal that fine-tuning Runx3 expression is critical for establishing tissue-specific residency programs, underscoring the distinct transcriptional requirements of T_{RM} cells across non-epithelial and epithelial sites.

Exploration of small intestine and colon plasma cell survival heterogeneity

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Background and Aims: As a frequent site of antigen exposure, intestinal immunity is critical to modulate the homeostasis of the microbiota and the protection against pathogens. In turn, it is highly populated with plasma cells (PC) and especially the IgA isotype. The large and small intestine are two anatomically distinct regions, differing in structure, cell composition and local cytokine production. As microenvironment is one of the key regulators for PC survival, we hypothesise that PC survival in these two regions may be differentially regulated. We aim to characterise the PC in large and small intestine and determine the relative sensitivity of PC in these two regions in response to the depletion of survival factors.

Methods and Results: The kinetic of PC turnover was studied by the blocking the major PC survival factors, A proliferation-inducing ligand (APRIL) and B-cell activating factor (BAFF), in a previously established PC-timestamping mouse model by our lab. The mouse strain, BLTcre.Mcl1fl/+, allows time-sensitive marking of PC from tamoxifen. This led to detection of newly generated and previously established IgA PC population in both large and small intestine Swiss roll through immunohistochemistry. It revealed that lack of APRIL and/or BAFF affects both persistence and generation of PC in intestines. Overall, PC in small intestine showed higher sensitivity to APRIL and BAFF blocking.

Conclusions and Future Perspectives: These results reflect the importance of APRIL and/or BAFF as survival factor of PC even in the mucosal site as well as lymphoid tissue. The higher susceptibility of the PC in small intestine suggests the possibility of inherent phenotypic difference in particular anatomical localisation. Identification of such phenotypical distribution may deepen the understanding of intestinal IgA which has integral role in protection of food allergy.

Investigating T cell responses to a nested peptide

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One important component of the adaptive immune response is the T cells, either "helper" $CD4^+$ T cells and the "killer" $CD8^+$ T cells. Activation of these T cells involves their T cell receptor (TCR) recognising peptides presented by Human Leukocyte Antigens (HLAs). During infection, pathogen derived proteins are degraded into peptides that can be presented by HLA molecules to T cells. Typically, $CD4^+$ T cells recognise long peptides (>11 residues) presented by HLA-II, while $CD8^+$ T cells recognise shorter peptides (8-10 residues) presented by HLA-I molecules.

The concept of nested peptides, where a shorter $CD8^+$ T cell peptide could be nested within a longer $CD4^+$ T cell peptide, allows a single (long) peptide to activate both $CD8^+$ and $CD4^+$ T cells, and has been relatively understudied.

This project investigated this phenomenon using an influenza-derived longer 13-residue T cell peptide ($PB1_{411\text{-}423}$) containing a shorter 9-residue known $CD8^+$ T cell epitope ($PB1_{413\text{-}421}$).

Using T cell activation assays, we confirmed that the long $PB1_{411\text{-}423}$ peptide is a novel immunogenic peptide which could be presented by multiple HLA-II allomorphs. Interestingly, $CD8^+$ T cells specific to the shorter $PB1_{411\text{-}423}$ peptide were expanded when the peptide was in the nested conformation in some donors. However, a greater proportion of $CD8^+$ T cell activation was observed when using the shorter peptide alone. We are currently working on determining the TCR repertoire of responding $CD4^+$ and $CD8^+$ T cells, and the structure of both peptides presented by both HLA to understand the similarity shared by the nested and unnested peptides.

This suggests a potential influence of peptide processing on the outcome of the T cell response, highlighting an area for future investigation to enable the optimal design of nested peptides to be used in vaccines.

Immune Cell Robbery: Tracking Dendritic Cell-to-B Cell Material Transfer

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Trogocytosis is a process by which cells extract membrane fragments from other cells during direct contact, potentially gaining new functions. This phenomenon plays critical roles in diverse biological contexts, including embryonic development, tumour progression, and immunity. Most immune cells engage in trogocytosis; however, the underlying molecular mechanisms remain poorly understood. To address this, we used the interaction between dendritic cells (DC) and B cells as a model system. In this context, B cells recognise the MHC II-C3 complex on the DC surface, initiating the transfer of DC fragments to B cells. This study aimed to determine the dynamics and molecular mechanisms driving trogocytosis using a combination of confocal, live-cell, and super-resolution microscopy, alongside inhibitors targeting various molecular pathways.

Splenic DC from mice expressing cytoplasmic Venus fluorescent protein (VFP) were co-cultured with B cells from VFP- mice and imaged hourly for seven hours. Initially, DC–B cell contacts were frequent. Over time, the number of contacts declined, while the proportion of B cells associated with VFP+ vesicle-like structures steadily increased. Super-resolution imaging revealed VFP localisation in discrete structures near the B cell surface. By seven hours, most B cells had acquired VFP signal, indicating transfer of DC-derived cytoplasmic material.

To investigate the fate of transferred membrane material, we performed co-cultures using DC with GFP+ membrane and B cells with tdTomato+ membrane. Samples were analysed by confocal microscopy with Z-stack acquisition and three-dimensional reconstruction. This analysis revealed that DC-derived membrane fragments partially intermix with the recipient B cell membrane over time.
To dissect the molecular mechanisms driving trogocytosis, we employed inhibitors targeting membrane trafficking, actin and tubulin dynamics, integrin-mediated adhesion, and calcium signalling. Disruption of actin polymerisation reduced cytoplasmic transfer acquisition by 40%, revealing a central role of cytoskeletal remodelling.

Together, these findings support a model in which B cells actively extract DC-derived fragments through an actin-dependent pinching mechanism, consistent with models proposed in other trogocytic systems. These observations raise questions about how trogocytosis influences B cell functios. Elucidating the molecular basis of trogocytosis is crucial for understanding immune interactions and may uncover novel therapeutic strategies in cancer, infection, and autoimmunity.

ABSTRACTS
POSTER SESSION
II

Evaluating cell:cell antiviral protection by *Wolbachia* in *Aedes aegypti* cells

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The intracellular bacterium *Wolbachia* is a biocontrol tool used to limit the transmission of arboviruses such as dengue virus (DENV) by *Aedes aegypti* mosquitoes. While its antiviral properties in *Ae. aegypti* are well-established, the exact mechanisms by which *Wolbachia* inhibits viral replication remain unclear. Previous studies have used surrogate systems including *Drosophila melanogaster*-derived cells, and the alphavirus Sindbis virus to show that *Wolbachia'*s antiviral effects are confined to *Wolbachia*-infected cells and do not extend to neighbouring *Wolbachia*-free cells. Since the antiviral effects of *Wolbachia* can be host-specific, here we replicate these experiments using biologically-relevant *Ae. aegypti*-derived cells and DENV. By co-culturing cells with or without *Wolbachia* in a transwell system, we assessed whether *Wolbachia*-infected cells can restrict DENV replication in *Wolbachia*-free cells separated by a porous membrane. Our findings demonstrate that *Wolbachia*-free cells co-cultured with *Wolbachia*-infected cells had no reduction in viral replication compared to those co-cultured with other *Wolbachia*-free cells. This indicates that *Ae. aegypti* cells infected with *Wolbachia* do not release factors that can confer antiviral protection to surrounding *Wolbachia*-free cells and provides further insight into the mechanism of viral restriction by *Wolbachia*.

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Investigating the mechanism of viral control in HIV-infected individuals

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Background:
Human Immunodeficiency Virus (HIV) remains a major global health burden, affecting millions worldwide. While antiretroviral therapies (ARTs) can suppress viral replication to undetectable and untransmittable levels, they do not eliminate the virus due to persistent latent reservoirs. As a result, lifelong treatment and monitoring is required.
A rare subset of individuals, known as HIV controllers, maintain low to undetectable viral loads (<50–2,000 RNA copies/mL) without ART. Though they make up less than 1% of people living with HIV, controllers provide valuable insight into natural viral suppression.
In this study, we aim to characterise the $CD4^+$ and $CD8^+$ T cell profiles and responses in an understudied cohort of HIV-positive individuals from Nepal, where HIV still remains a major public health issue.
Methods:
Peripheral blood mononuclear cells (PBMC) were isolated from venous blood collected from a cohort of 190 HIV-positive individuals in Nepal. In this cohort, ~5% (n=10) are controllers and the rest are typical progressors or non-controllers. Absolute counting of T cell subsets ($CD4^+$ and $CD8^+$) was conducted on the PBMCs using BD Trucount tubes. Ex vivo intracellular cytokine staining (ICS) assay was used to measure the responses of $CD4^+$ and $CD8^+$ T cells to a HIV Gag peptide pool.
Results:
$CD4^+$ T cell count in controllers was found to be trending higher (222-1124 cells/μL) compared to non-controllers (11-2658 cells/μL), $CD8^+$ T cell count was trending lower in controllers (203-3596 cells/μL) compared to non-controllers (19-4839 cells/μL), and the CD4/CD8 ratio was significantly higher ($p<0.05$) in controllers (0.1894-2.191) compared to non-controllers (0.03720-3.117). Preliminary results from ex vivo ICS of HIV-positive PBMCs show positive IFNγ, TNF, and CD107 response to the HIV Gag peptide pool.
Conclusions:
The difference between CD4/CD8 ratio between controller and non-controllers were found to be statistically significant, aligning with known characteristics of controllers from previous literature. Further investigation into the immune response and phenotype profiles of the T cells will provide a better insight into the link between T cells and protection to HIV.

Understanding viral and host determinants that influence infection outcomes for influenza A virus in human macrophages

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Airway epithelial cells (AECs) and airway macrophages (AMs) represent primary cellular targets of infection by seasonal influenza A virus (IAV). IAV infection of AECs results in productive replication, defined as the release of newly synthesised infectious virus. In contrast, infection of mouse macrophages is blocked at a late stage of replication where infectious virus is not released (abortive replication). IAV infection outcomes are not as well defined for human macrophages. Herein, we infected two sources of primary human macrophages isolated from matched donors (AMs from bronchoalveolar lavage and monocyte-derived macrophages (MDMs) from peripheral blood). Despite similar levels of IAV infection in AMs and MDMs at 8 hours post-infection, infectious virus was released from MDMs, but not AMs, at 24 and 48 hours post-infection. These data suggest that while IAV infection of AMs is abortive, productive replication can occur in MDMs. Indeed, this pattern was consistent across a wide range of H1N1 and H3N2 IAV strains. Interestingly, only a small subset of recent H1N1 pdm IAV strains (2009 to 2016) underwent abortive replication in MDMs. Comparison of sequences of all eight genes from closely related strains that underwent abortive vs. productive replication in MDMs identified differences in the haemagglutinin (HA), neuraminidase (NA), matrix protein (MP) and non-structural (NS) genes, implicating these segments as potential viral determinants influencing replication outcomes. Additionally, disruption of specific innate sensing molecules (RIG-I, MDA5/RIG-I and MAVS) enhanced the replication of a representative pre-2009 H1N1 strain in the human monocytic cell line, THP-1, but did not impact the replication of representative abortive (A/Auckland/1/2009) or productive (A/Newcastle/65/2015) H1N1 pdm strains. Furthermore, blockade of downstream JAK/STAT signalling enhanced the replication of both the representative pre-2009 H1N1 strain and the H1N1 pdm strains (A/Auckland/1/2009 and A/Newcastle/65/2015) in THP-1s, although the increases observed for the H1N1 pdm strains were relatively modest when compared to the pre-2009 H1N1 strain. Together, these findings provide novel insights into potential viral and host determinants, including IFN-dependent responses, that can influence IAV infection outcomes in human macrophages.

Differential temporal immune responses to influenza A virus and PRR stimulation in lung epithelial versus macrophage cells

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Influenza A virus (IAV) is a highly transmissible pathogen responsible for significant global morbidity and mortality worldwide. Host detection of IAV in cells is mediated by pattern recognition receptors (PRRs) such as toll-like receptors-3 (TLR3) and -7 (TLR7), and retinoic acid-inducible gene I (RIG-I), which sense viral RNA and trigger antiviral and inflammatory signalling. These pathways promote viral clearance, although their dysregulation can contribute to lung tissue damage and immunopathology. Importantly, PRR expression can vary between cell types, yet the cell-specific contributions of these receptors to IAV induced responses remain poorly defined. To address this, we investigated the temporal dynamics of cytokine and interferon responses in murine alveolar macrophages (MHS) and alveolar type II epithelial cells (MLE12) following IAV infection (HK-X31 and PR8 strains) or stimulation with PRR agonists. Preliminary findings indicate that epithelial cells initiate inflammatory gene expression earlier than macrophages in response to TLR3 (poly(I:C)) stimulation, although with a lower overall magnitude. In contrast, TLR7 and RIG-I activation (via imiquimod or 3pRNA, respectively) elicited a stronger cytokine response in macrophages. Following IAV infection, induction of IL-1β, TNF-α, IFN-λ3, and IFN-β in epithelial cells was transient, while inflammatory markers were significantly higher in macrophages and remained elevated for longer. These transcriptional patterns also correlate with Western blot data, which confirmed distinct PRR expression between the two cell types. CRISPR/Cas9-mediated deletion of TLR3, TLR7, or RIG-I in each cell type further delineated their distinct roles in cytokine and interferon induction. Moreover, propidium iodide uptake assays indicated that epithelial cells were more susceptible to IAV-induced cell death than macrophages. This study provides a cell-type–specific map of PRR-driven immune signalling kinetics in the lung. Defining these pathways will support the development of targeted strategies to limit virus-induced immunopathology while preserving protective antiviral immunity.

Identifying novel antibody biomarkers of Asian-Pacific *Anopheles* vector bite exposure for malaria transmission surveillance

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Malaria is a parasitic disease, transmitted by the bite of female *Anopheles* mosquitoes. To progress towards malaria elimination, sensitive and scalable surveillance tools are required to monitor human exposure to *Anopheles* vectors. However, the current gold-standard method of entomological surveillance, whereby an entomologist exposes a limb and catches mosquitoes that land on it, is crude, logistically challenging, and only provides population-level estimates of bite exposure. This is further complicated in the Asia-Pacific region, where malaria transmission is low and geographically heterogeneous. Here, the diverse range of dominant vector species exhibit unique behaviours, preferring different biting times and locations, making the capture of a representative sample of mosquitoes unlikely. A potential alternative is serosurveillance of antibodies generated against *Anopheles* salivary proteins injected during a mosquito bite, which could provide a proxy measure of individual-level bite exposure. The current leading candidate antigen, gSG6, from the African vector *An. gambiae*, which has been shown to be a reliable biomarker of bite exposure in Africa. However, reliable anti-salivary antibody biomarkers of Asian-Pacific *Anopheles* species bites are yet to be identified and characterised.

To address this, we screened antibody responses against salivary antigens belonging to Asian-Pacific *Anopheles* species to identify novel biomarker candidates. We identified several region-specific biomarker candidates that showed higher responses in individuals from the Asia-Pacific compared to African and naïve Australian donors ($p<0.05$). Furthermore, individuals with malaria infection had 23%-30% higher odds of IgG seropositivity to certain salivary antigens evaluated. These findings suggest that the identified biomarker candidates may be indicators of exposure to Asian-Pacific *Anopheles* species bites and could be further validated to improve vector serosurveillance to aid malaria elimination efforts in the Asia-Pacific region.

Defining conserved sites of vulnerability on the Influenza B HA Using Human Monoclonal Antibodies

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Introduction: Influenza B viruses (IBV) circulate primarily in humans, causing periodic outbreaks and a public health burden. Although annual vaccination remains the most effective preventative measure, antigenic drift in viral haemagglutinin (HA) can compromise vaccine efficacy. A deeper understanding of the IBV antigenic landscape, including the breadth of recognition and extent of cross-neutralization by IBV HA-specific antibodies could guide improved IBV vaccine design.

Methods: A panel of 115 IBV-specific human monoclonal antibodies (mAbs) was established by single-cell sorting and BCR sequencing of IBV HA specific memory B cells from seasonal IIV4 recipients, expressed in mammalian cells. Cross-strain recognition and neutralization was assessed via ELISA, HIA, and micro-neutralization assays against IBV isolates spanning 1940-2021. Epitope mapping of the mAbs was conducted by competition ELISA and escape mutant generation. Protective capacity was assessed *in vivo* in a C57BL/6 mouse model of IBV infection.

Results: 77 IBV mAbs exhibited broad cross-lineage recognition, with multiple epitopes conserved within the head, vestigial esterase (VE) domain and stem. Neutralisation activity was identified among cross-reactive antibodies targeting the head and VE domain but not the stem. Different mAbs targeting the head, VE domain or stem showed comparable protective efficacy in vivo, measured by bodyweight loss, survival and lung viral load.

Conclusion: Multiple sites of the IBV HA are highly conserved despite over 80 years of viral evolution and display a degree of protective potential. Notably, the relative commonality of cross-lineage neutralizing mAbs targeting the VE domain may indicate an underappreciated site exploitable by targeted vaccine design. Our comprehensive mapping of the IBV HA antigenic space provides valuable insight for the rational design of next-generation, broadly protective IBV vaccines.

Modelling a microphysiological system to mimic pig lymph nodes

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Abstract

Secondary lymphoid organs, such as lymph nodes, exhibit a highly organized and complex cellular architecture that is fundamental to their immunological function. In pigs, lymph nodes not only act as key immune regulators but also serve as entry points and reservoirs for a wide range of infectious agents. Their strategic anatomical location makes them among the earliest sites of infection and valuable indicators of disease progression. Developing an *in vitro* model of porcine lymph nodes would represent a significant advancement in the study of porcine infectious diseases.

To address this need, we present a novel hydrogel-based organ-on-a-chip platform designed to replicate the structural and functional intricacies of lymphoid tissues. The system features a tri-channel configuration, comprising two medium-based channels flanking a central extracellular matrix (ECM) channel, and supports a streamlined workflow with flexible cell integration. The modular design allows for the development and interconnection of multiple organ models within a single platform.

Primary cells isolated from porcine lymph nodes were seeded into the chip using various strategies, including heterogeneous cell populations and compartmentalized cell groupings. Cell viability and longevity will be assessed through viability assays and live-dead staining. The system will be further characterized for cellular diversity and tissue architecture via immunostaining, and its functional capacity will be evaluated using multiplex ELISA. To assess immune responsiveness, the platform will be challenged with mock antigens and infectious pathogens.

Once fully validated, this organ-on-a-chip model offers strong potential as a versatile tool for investigating porcine immune responses and advancing infectious disease research. Moreover, its adaptability across species positions it as a promising candidate for One Health applications.

Lymph node entrapment of CD8$^+$ T cells underlies lymphopenia in severe influenza disease

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Influenza viruses impose a significant global health burden, but there is extensive heterogeneity in how the disease manifests. While some individuals develop only mild to moderate illness, others develop more severe disease and require hospitalisation. A prominent feature of severe influenza is substantially reduced lymphocyte counts in the blood, known as lymphopenia. Although associated with poor prognosis in influenza disease, the underlying mechanisms driving lymphopenia are poorly characterised. Here, we tracked the expansion and tissue localisation of the virus-specific CD8$^+$ T cell response in a C57BL/6 mouse model of moderate and severe influenza virus infection. Using flow cytometry, molecular biology, and mass-spectrometry based proteomics, we profiled the immunological perturbations in severe influenza and examined how viral antigen is differentially distributed amongst professional antigen presenting cells (APCs) in mild and severe influenza disease. We found that CD8$^+$ T cells failed to exit the lung-draining lymph node in severe influenza disease, causing a significant reduction in CD8$^+$ T cells in the blood and lung. This defect in lymph node egress was partially driven by impaired re-expression of the transcription factor KLF2 and its downstream target gene, the sphingosine-1-phosphate receptor, S1PR1, and was accompanied by the rapid and selective apoptosis of antigen-specific CD8$^+$ T cells in the lungs in severe influenza infection. Lastly, we showed that the introduction of effector CD8$^+$ T cells into the lung led to worsened survival outcomes and resulted in increased lung pathology, suggesting that lymphopenia may reflect a protective adaptation to mitigate excessive immunopathology in severe infections. Collectively, these findings show that CD8$^+$ T cell lymphopenia in severe influenza disease is driven by a two-part mechanism consisting of (1) sequestration in the lung-draining lymph node, and (2), the apoptosis of antigen-specific CD8$^+$ T cells in the lung.

Further insights into asymptomatic outcome after SARS-CoV-2 in HLA-B*15:01+ individuals

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T lymphocyte activation is driven by the recognition of Human Leukocyte Antigens (HLA) on the surface of infected cells. HLAs present viral peptides to T cells, which then recognize them as antigens via their T cell receptor (TCR). Our previous work showed that a specific HLA-B15:01 can present both a SARS-CoV-2 peptide (NQK-Q8) and a Seasonal Coronavirus peptide (NQK-A8) in the same conformation, with the same thermal stability. We also demonstrated that these peptides elicit a similar T-cell response, driven by high-affinity binding of identical TCRs found in different HLA-B15:01+ individuals.

To further investigate these results, I used X-ray crystallography to show that these public TCRs contact two residues on NQK-Q8 and NQK-A8, with half of the TCR interaction involving the HLA alone. To prove how vital these peptide residues are to TCR recognition, I solved the crystal structures, thermal stability, and binding affinity of HLA-B*15:01 presenting homologous NQK peptides containing different mutations. I found that even a single residue mutation can cause substantial changes in peptide conformation. This can reduce overall complex stability, limit binding affinity, and decrease CD8+ T cell stimulation.

My work shows that CD8+ T cell activation in HLA-B*15:01+ individuals after SARS-CoV-2 infection is driven by unique TCR interactions. It also highlights the important role that NQK peptide side chains play in this process. Additionally, I show that single self-derived peptides similar in sequence to NQK can induce T cell activation through high-affinity binding of these same TCRs, highlighting the potential for T cell auto-reactivity within these same individuals.

[1]Augusto, D.G., **Murdolo, L.D.**, Chatzileontiadou, D.S.M. *et al.* A common allele of *HLA* is associated with asymptomatic SARS-CoV-2 infection. ***Nature* 620**, 128–136 (2023). https://doi.org/10.1038/s41586-023-06331-x

Selective Endocytosis-mediated Omicron S1-RBD Internalization Revealed by Reconstitution of ACE2-S1-RBD Interaction on Micropatterned Membrane Substrates

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Keywords: ACE2; SARS-CoV-2; S1-RBD; Supported Lipid Bilayer; Endocytosis; Cytoskeleton.

Abstract

The SARS-CoV-2 spike protein, through its receptor binding domain (S1-RBD), binds to the angiotensin-converting enzyme 2 (ACE2) receptor on the host cell membrane leading to viral infection. Mutations in S1-RBD in SARS-CoV-2 variants are known to enhance infection through an increased affinity for ACE2. While many reports are available describing the SARS-CoV-2 infection mechanism, there is a dearth of studies towards understanding the initial interaction of the S1-RBD with ACE2 on living host cells, and the role of endocytosis and cytoskeleton in the process. Here, we reconstituted the interaction between S1-RBD and ACE2 expressing host cells in a hybrid live cell-supported lipid bilayer (SLB) platform and show that cells depleted Omicron S1-RBD from SLB corrals, likely, through endocytosis. Interaction of living host cells resulted in the enrichment of SLB-substrate bound S1-RBD and host cell membrane-localized ACE2. Restriction of S1-RBD on micropatterned SLB corrals revealed a depletion of Omicron S1-RBD from many corrals, which was generally not observed with the WT S1-RBD and was reduced with the Omicron Revertant S1-RBD. The pattern S1-RBD depletion was coincident with the recruitment of the early endosomal marker EEA1. Importantly, treatment of cells with the clathrin inhibitor, pitstop 2, but not the myosin II inhibitor, blebbistatin, significantly reduced Omicron S1-RBD depletion. Collectively, these observations suggest that the SARS-CoV-2 Omicron variant has evolved, through mutations in its S1-RBD, to take advantage of the cellular endocytic pathway for enhanced infection, which is not observed with the parental SARS-CoV-2 and appears to be lost in the Omicron Revertant variant likely due to the reversion of Q493R mutation. Additionally, these results underscore the significance of the hybrid live cell-SLB platform in studying SARS-CoV-2 S1-RBD-ACE2 interaction and the potential impact of mutations in the S1-RBD on adapting to a specific cellular entry mechanism.

Preclinical Evaluation of Cytokine-Adjuvanted MVA Vaccines Encoding SARS-CoV-2 Antigens

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Modified Vaccinia Ankara (MVA) is a highly attenuated versatile viral vector with an excellent safety profile and strong immunogenicity, making it an attractive platform for universal vaccine development. MVA does not replicate in mouse or human cells. Previous clinical trials presented no adverse events under immunodeficient conditions, thus making it a safe and effective vector in millions of vaccinations. MVA is also highly thermostable and genetically flexible, capable of accommodating large gene insertions—as demonstrated by the licensed Ebola Vaccine.

As a live viral vector, MVA assesses intracellular antigen-processing pathways, thereby stimulating robust T cell responses. Given the continues antigenic drift and recombination events in SARS-CoV-2, the Spike protein frequently evades neutralising antibodies, promoting the need to rethink long-term vaccination strategies. Targeting conserved viral proteins that elicit durable T cell responses may offer broader and more sustained protection. In this study, we evaluate cytokine-adjuvanted MVA vaccines encoding conserved SARS-CoV-2 antigens with integration of immune-stimulating cytokines, to enhance both humoral and cellular immune response, thus generating a highly immunogenic vaccine that can be used to 'immunise the immune' in the post COVID-19 pandemic era.

A combination of SARS-CoV-2 genes selected from both surface and internal viral proteins including Spike (S), Nucleocapsid (N), Membrane (M), and Open Reading Frame (ORF) 3a, known to elicit robust antibody and T cell responses in convalescent individuals. As an initial step, we focus on MVA constructs expressing SARS-CoV-2 S protein, either alone or integrated with immunomodulatory cytokines IL-15, GM-CSF, and Flt3L, which have been shown to augment dendritic cell activation and T cell expansion. Mice are vaccinated in a prime-boost schedule, and vaccine induced responses are assessed for serum antibody titres and T cell responses. The impact of cytokine integration as a self-adjuvating approach will be compared for immunogenicity by quantifying vaccine-induced memory responses in both the short and long term, as well as by evaluating recall capacity following viral challenge to determine protective efficacy.

This preclinical study aims to define optimal antigen-cytokine vaccine formulations to enhance T cell immunity for cross-reactive protection, supporting the development of MVA-based multivalent vaccines against SARS-CoV-2 and other respiratory pathogens.

Leveraging RNA-based approaches to develop new therapies and discovery tools to overcome current treatment limitations for HTLV-1

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Human T cell Lymphotropic Virus 1 (HTLV-1) infects an estimated 10-20 Million people worldwide and primarily targets CD4 T cells within the immune system to establish a lifelong infection. Clinical outcomes range from adult T-cell leukemia/lymphoma (ATL), which typically arises decades after infection, to chronic inflammation of the spinal cord (HAM/TSP), systemic inflammation, immune dysregulation, and increased susceptibility to opportunistic infections. The HTLV-1c subtype is highly endemic in Indigenous communities in central Australia, reaching infection rates of up to 35% - the highest prevalence globally. Many aspects of host-pathogen interactions during infection remain unclear, and as a result there are currently no curative treatments or vaccines available.

To address this, the Doerflinger lab developed humanized mouse models for HTLV-1 infection, reflecting key aspects of human disease. Previous work in the lab showed that extrinsic as well as intrinsic apoptosis are dysregulated in HTLV-1c infected cells, and that induction of apoptosis with small molecule drugs reduces infection and disease severity. However, current inhibitors cause substantial toxic side effects, limiting their clinical translation.

In my PhD project, I will employ T cell targeting lipid nanoparticles (LNPs) to deliver siRNA to selectively induce apoptosis via downregulation of MCL-1 (intrinsic) and cIAP1/2 (extrinsic) to kill infected cells in vitro and in vivo and mitigate disease in humanised mice. In parallel I will investigate how viral proteins drive infection by developing intrabodies that can be tagged for discovery biology to uncover new host interaction partners and cellular localization patterns. Importantly, I aim to identify nanobodies with inhibitory function that can disable the pathogenic function of these HTLV-1 virulence factors and be delivered as therapeutic by mRNA into infected T cells via LNPs. Ultimately, I aim to multiplex siRNA-based and intrabody-based strategies to simultaneously target host and viral factors essential for HTLV-1 persistence and provide first pre-clinical proof for these novel therapeutic approaches.

The investigation of the T cell receptor diversity of skin-resident and circulating CD8$^+$ T cells in psoriasis patients

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Psoriasis is a T cell-mediated skin autoimmune disease. The human leukocyte antigen class I molecule HLA-C*06:02 presents peptide antigens to CD8$^+$ T cells and is a major predisposing genetic risk factor for psoriasis. CD8$^+$ skin-resident memory T cells (T_{RM}) are predominantly located in the epidermis and contribute to psoriasis by producing proinflammatory cytokines. This response is potentially driven by HLA-C*06:02-restricted antigens presented in lesional epidermis.

To address the T cell receptor (TCR) diversity of CD8$^+$ skin T_{RM} in psoriasis, I performed single-cell TCR sequencing of CD8$^+$ T_{RM} from the epidermis and dermis of lesional and non-lesional skin of four HLA-C*06:02$^+$ and three HLA-C*06:02$^-$ patients. TCR clonotypes in lesional epidermis showed minimal overlap with other skin compartments and exhibited a high level of clonal expansion, regardless of HLA-C*06:02 status. Across three patients who shared similar HLA allotypes, 13 clonotypes from lesional epidermis shared highly similar TCRα CDR3 sequences paired with same TCRBV chain usage, suggesting potential similar antigen specificity.

To assess the correlation of TCR clonotypes between CD8$^+$ skin T_{RM} and circulating CD8$^+$ T cells, I performed matched single-cell transcriptomics and TCR sequencing on six HLA-C*06:02$^+$, three HLA-C*06:02$^-$ patients and three healthy HLA-C*06:02$^+$ individuals. I focussed on CLA$^+$ CD8$^+$ T cells known to recirculate through skin and KIR$^+$ CD8$^+$ T cells that have been implicated as antigen-specific regulatory T cells in several autoimmune diseases. In three HLA-C*06:02$^+$ patients, independent of KIR expression, some expanded clonotypes from lesional epidermis were detected in circulating CLA$^+$ CD8$^+$ T cells with strong transcriptional signatures of IL-17-related mediators and skin-homing genes. Compared to healthy donors, patient circulating cell populations showed higher TCR engagement and cytotoxicity.

Altogether, this study bridges tissue-resident and circulating T cell repertoires in psoriasis in the context of the main risk gene HLA-C*06:02. Identification of prevalent clonotypes in lesional tissue provides a platform for further antigen discovery in psoriasis.

Persistent low antigen in chronic infections sustain a potent CD8$^+$ effector T cell response which leads to severe immunopathology

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CD8+ T cell responses to chronic infections often become functionally impaired, a state known as 'exhaustion', which facilitates disease progression and prevents the formation of durable memory. However, the individual contributions of continuous high antigen levels and chronic inflammation on the impaired CD8+ T cell memory development remain incompletely understood. To dissect these factors, we utilised a previously developed a chronic Lymphocytic Choriomeningitis Virus (LCMV) infection system that allowed us to selectively modulate antigen presentation without altering the overall inflammatory milieu or viral persistence. Using this model, we show that CD8+ T cells exposed to low antigen levels amidst ongoing inflammation do not acquire hallmark features of exhaustion, including high expression of PD-1 and TOX while retaining robust cytokine production. Single-cell RNA sequencing further revealed that these cells acquire an effector memory T cell phenotype in lymphoid organs and give rise to cytotoxic tissue-resident memory T cells in peripheral tissues. Strikingly, the emergence of these T cell subsets coincides with increased host mortality, which could be rescued by CD8$^+$ T cell depletion. Together, our findings demonstrate that chronic inflammation can sustain functional effector T cells in the absence of high antigen, leading to severe immunopathology. Further, our model offers a powerful platform to disentangle the effects of antigen and inflammation on CD8$^+$ T cell differentiation and highlights the role of exhaustion as a protective adaptation to limit immunopathology.

A Novel Targeted Regulatory T Cell Therapy for HLA-DR3-associated Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is a multi-organ autoimmune disease with no known cure. T-cells play a central role in SLE pathogenesis, wherein aberrant T-regulatory (Treg) cell function and decreased Treg numbers are strongly implicated. HLA-DR3 is a known dominant SLE risk allele,[1] and anti-Smith (Sm) seropositivity is associated with more severe disease and with lupus nephritis.[2] Data from the Australian Lupus Registry and Biobank (ALRB) has shown a strong link between Sm autoreactivity and HLA-DR3, with 30% of anti-Sm$^+$ SLE patients being HLA-DR3$^+$.[3]

We have developed a Sm-specific Treg therapy targeting HLA-DR3-associated, anti-Sm$^+$ SLE.

We have previously identified two HLA-DR3-specific immunogenic regions of the Sm antigens (SmD1$_{78\text{-}92}$ and SmB/B'$_{7\text{-}21}$). To investigate the structural basis of antigen-presentation, we successfully expressed soluble recombinant HLA-DR3 in complex with both SmD1$_{78\text{-}92}$ and SmB/B'$_{7\text{-}21}$ using the Expi293 mammalian expression system, followed by purification via immobilised metal affinity chromatography (IMAC) and size exclusion chromatography (SEC). Protein crystallography confirmed stable peptide presentation and revealed key peptide residues involved in immune recognition by autoreactive TCRs.

To explore the T-cell response to these epitopes, we employed single-cell RNASeq (10X Genomics). CD4$^+$ T-cells from a HLA-DR3$^+$ healthy donor were labelled with a proliferative dye (CellTrace^{TM} Violet; CTV) and co-cultured *in vitro* with autologous monocyte-derived DCs which have been pulsed with SmD1$_{78\text{-}92}$ and SmB/B'$_{7\text{-}21}$. Proliferated (CTV^{lo}) CD4$^+$ T-cells were sorted for and subjected to single gene expression and pair TCR V(D)J profiling. This approach identified a dominant TCR clonotype (SmTCR) specific to both Sm peptides and is notably of Treg origin (FoxP3^{hi}).

To assess functional potential, Sm-specific, DR3-restricted Tregs (SmTCR-Tregs) were engineered by lentiviral transduction of CD4$^+$ Tregs isolated from HLA-DR3$^+$ donors. SmTCR Treg suppressive function was evaluated using an *in vitro* co-culture with Sm peptide-activated CD4$^+$ conventional T-cells. Preliminary results demonstrated that engineered SmTCR-Tregs exhibited up to a 2-fold increase in suppressive capacity, compared to non-engineered polyclonal Tregs.

We next aim to engineer the lead SmTCR into Tregs derived from HLA-DR3$^+$ anti-Sm$^+$ SLE patients. We will assess regulatory phenotype stability and suppressive function *in vitro*. These SmTCR-Tregs will then be evaluated *in vivo* using an already-established humanised SLE mouse model.

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Understanding the Development of Terminally Exhausted T cells

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Robust activation of T cells is crucial to protect the host against harmful pathogens. However, prolonged T cell activation, such as in chronic viral infection, can lead to life-threatening autoimmunity or immunopathology. T cell exhaustion is an important adaptation that prevents excessive immune-mediated damage by dampening the function of chronically-stimulated cytotoxic T cells. However, it is also a common mechanism that inhibits antitumour immunity in many cancers. Importantly, exhausted T cells are characterised by their irreversible loss of effector function, partly enforced by a specific epigenetic program, such that their dysfunction persists even T cells are removed from antigens.

Exhausted T cells are transcriptionally and epigenetically distinct from conventional effector T cells that are typically derived from an acute infection. The exhausted T cell pool can be largely divided into precursor of exhausted T cells (Tpex) and effector exhausted T cells (Tex). Tpex cells possess stem-like capacity that can give rise to Tex cells, which lack stem-like potential, but possess cytotoxic capacity that can mediate viral control. Tpex cells thus maintain the overall immune response by continuously replenishing the pool of Tex cells. Tex cells are transcriptionally and functionally heterogenous, which can be largely divided into two distinct subsets. Specifically, Tex cells that express the chemokine receptor CX3CR1 are known to possess greater cytotoxic capacity compared with their CX3CR1$^-$ counterparts. Moreover, CX3CR1$^+$ and CX3CR1$^-$ Tex cells exhibit differential localisation to anatomical sites in both systematic chronic viral infection and cancer. Such characteristics are also observed in exhausted CAR-T cells in treated cancer patients. Given their difference in cytotoxicity and residence capacities, manipulating the fate decision of Tex cells may allow specific tailoring of both systemic and localised immunity. However, it is currently not known what cell-intrinsic mechanisms control the development of different Tex cells. In this project, we set out to elucidate the underlying molecular mechanism that control fate decision in Tex cells development.

γδ T cell-derived IL-4 initiates CD8 T cell immunity

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Resident-memory T (T_{RM}) cells are poised to rapidly protect against repeat tissue-specific insults. Immunisation with radiation-attenuated sporozoites (RAS) generates liver resident-memory CD8 T cells that confer protection against *Plasmodium* infection. These protective CD8 T cells are primed in the spleen by type 1 conventional dendritic cells (cDC1) in a manner that is critically dependent upon CD4 T cell help. An additional layer to this reaction is observed in which γδ T cells are crucial for effective RAS priming. Loss of γδ T cells abrogates clonal expansion of antigen-specific CD8 T cells in the spleen, resulting in insufficient precursors for the formation of protective CD8 liver T_{RM} cells. Here, we reveal that Vγ1+ γδ T cell-derived IL-4 is pivotal for effective expansion of these antigen-specific CD8 T cells. IL-4 elicits two distinct effects: it acts directly on CD8 T cells to promote expansion, and it synergises with IFN-γ to drive bioactive IL-12 production by cDC1, further enhancing this response. Collectively, this project reveals an indispensable role for γδ T cells in bridging DC activation and effective T cell priming in the context of the weak stimulus of RAS vaccination.

Ly108 Preserves CD8$^+$ T Cell Fitness Under Chronic Antigen Stimulation

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CD8$^+$ T cells are essential for controlling chronic infections and tumours, yet their persistence is often compromised by exhaustion. Ly108 (encoded by *Slamf6*) has been widely used as a marker of progenitor-exhausted CD8$^+$ T cells (Tpex), yet whether it actively regulates their fate remains unresolved. Here, we demonstrate that Ly108 is a critical regulator of CD8$^+$ T cell longevity and function in chronic antigen settings. Using lymphocytic choriomeningitis virus (LCMV) infection, we found that Ly108 expression was indispensable for CD8$^+$ T cell persistence. Loss of Ly108 led to attrition of virus-specific T cells, while enforced Ly108 expression selectively promoted survival following chronic but not acute infection. This survival advantage required interactions with other Ly108-expressing lymphocytes and distinct spatial localisation within lymphoid tissue. Mechanistically, Ly108 promoted both proliferative and survival advantages without major global changes in transcriptional or surface phenotypes. Ly108 engagement also enhanced effector cytokine production, and consistent with this, Ly108-overexpressing CD8$^+$ T cells provided superior protection across multiple tumour models. Together, these findings establish Ly108 as a key regulator of CD8$^+$ T cell longevity and function in chronic antigen settings and highlight this molecule as a potential immunotherapy target with translational potential.

miRNA expression of bovine CD4+, CD8+ and γδ T cells

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Maintaining livestock health is essential for animal welfare, upholding consumer confidence in animal products and the financial viability of individual producers and broader industry. One key aspect of livestock health is the ability for producers to reliably diagnose disease in their herds. The accuracy, cost and practicality of diagnostics for cattle and other livestock species are among the most limiting factors that influence how animal disease is managed on farm. Gaps in our understanding of the bovine immune system further hinders the development of diagnostic and therapeutic assays.

An expanding area of research with potential to improve diagnostic capabilities is microRNAs (miRNAs). This emerging class of nucleic acid molecules, while only around 20 nucleotides in length are now understood to regulate most biological process in most species. miRNA post-transcriptional regulate gene expression, acting on host cells or being excreted to facilitate cell-to-cell communication. Differentially expressed miRNA have been correlated with disease states in human studies and may have translational value as biomarkers. Particularly, for pathologies which have limited or ineffective existing assays.

We conducted a study to determine the miRNA species expressed in T-cell populations and identify differentially expressed miRNA. CD4+, CD8+ and γδ T cells were isolated from whole bovine PBMCs using Magnetic Activated Cell Sorting (MACS). Following which, miRNA was extracted. The resulting RNA sequencing data is being analysed at the time of this abstract's submission. We are expecting to identify the miRNA species present within each subset of T cell and, to determine if CD4+, CD8+ and γδ T cells have district miRNA profiles. This information will help support biomarker discovery for cattle and possibly have application across a range of other species. Furthermore, identifying the miRNA species present in bovine immune cells contributes to our understanding of the regulation of bovine immune functions.

Mechanisms of Antigen Presentation and CD8 T cell Priming by mRNA Vaccines

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RNA vaccines have demonstrated their capacity to generate robust CD8 T cell immunity. However, how naïve T cell response is primed following mRNA vaccination remains poorly defined. The first goal of this study was to investigate the distribution and kinetics of T cell priming, as well as the immune cells involved in mRNA vaccine uptake, translation, and antigen presentation to T cells after intramuscular mRNA-Lipid Nanoparticle (LNP) vaccination in mice. We also evaluated how inflammation-induced 'immune paralysis' affects the capacity of these vaccines to induce immune responses. Our findings offer new perspectives for the development of more efficient and targeted mRNA vaccines to fight complex intracellular infections and tumours.

A Cre-expressing mRNA vaccine was used in the mT/mG reporter mice to detect vaccine uptake and expression in different immune cells in the muscle and lymph nodes (LN) following vaccination by flow cytometry. An Ovalbumin (OVA)-coding mRNA vaccine was administered to C57/BL6 mice to characterise the OVA-specific CD8 T cells responses. The immunoparalysis mouse model was established during the recovery phase following intravenous injection of inflammatory agent (CpG) and T cell priming and lymph node dendritic cell (DC) response to the vaccine was analysed.

We identified the specific subsets of immune cells involved in uptake, expression, and antigen presentation of mRNA vaccine, along with the spatiotemporal dynamics of each event. In the immunoparalysed mice, we showed CD8 T cell priming was severely impaired with mRNA vaccination, for up to 21 days after CPG administration. We then fully characterised LN DC alterations in this model, and demonstrated that the impaired T cell priming is linked to the DC failure to uptake, translate, transfer and present mRNA-LNP vaccine.

Our findings can lead to the development of more efficacious vaccines specifically targeted to the antigen presenting cells involved. We also found that DC functional paralysis impairs mRNA vaccine uptake and antigen presentation, suggesting the mechanism of capture and processing of such antigens does not differ from the antigens in other vaccine formulations. The poor mRNA vaccination outcome calls for careful considerations when administrating mRNA vaccines to these immunocompromised individuals.

Crohn's associated invariant T cells recognise small benzofuran-sulfonate molecules with self-lipids on CD1d

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Natural killer T (NKT) cells are a family of T-lymphocytes that recognise via their T-cell receptor (TCR) lipid antigens presented by the non-polymorphic MHC-I-like molecule CD1d. An unusual NKT cell population has been described which recognises small benzofuran-sulfonate molecules in association with specific self-lipids presented by CD1d.[1] Strikingly, these small molecules resemble sulfa-based compounds that trigger hypersensitivity in humans. A large subset of this population expresses a semi-conserved TRAV12-TRAJ6 TCR gene rearrangement.

Recently, the same semi-invariant TRAV12-TRAJ6 TCR-motif was found to be highly expanded in Crohn's disease (CD) patients, termed Crohn's associated invariant T cells (CAITs),[2,3] suggesting an unexplored link between these cells and CD. CD is a multifactorial chronic inflammatory bowel disease affecting around 300 in every 100,000 Australians. In those studies, CD1d-dependent reactivity to small benzofuran-sulfonate molecules was only clearly demonstrated for one of two CAIT clones tested, and direct CD1d-recognition by the TCR remained to be investigated.

Here, we generated transiently transfected and stably transduced TCR reporter cell lines over-expressing CAIT TCRs from five distinct T cell clones reported to be accumulated amongst different CD patients. All five transiently transfected CAIT TCR reporter cell lines bound CD1d-tetramers treated with small benzofuran-sulfonate molecules. Three of five CAIT TCR reporter lines responded to small benzofuran-sulfonate molecules in a CD1d-dependent manner (via CD69 upregulation). Co-loading these tetramers with the long-chain self-lipid sphingomyelin SM34:1 improved TCR binding, whereas the very-long-chain self-lipid SM42:2 prevented it.

Using optimised CD1d-tetramers co-loaded with small benzofuran-sulfonate molecules and permissive SM34:1 lipids, we isolated T cells from healthy donor blood and phenotyped them using flow cytometry. Upon mitogenic stimulation, cultured T cells produced pro-inflammatory cytokines TNF and IFNγ, which are associated with CD.

Overall, our results further support a role in CD pathology for small benzofuran-sulfonate molecule-reactive NKT cells, which are normally found at very low levels in healthy individuals. This also highlights an unexplored relationship between CD1d antigen presentation and small molecules, including from drugs, diet or microbes, together with a dysregulated lipidome. We aim to further investigate this complex mechanism and the NKT/CD1d axis in CD and other aberrant immune responses.

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Harnessing the immunotherapeutic potential of MAIT cells with a novel MAIT TCR nanobody

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Bispecific antibodies capable of targeting T cell effector functions toward tumour targets have revolutionised cancer immunotherapy. However, the broad targeting of all T cell subsets can result in toxicity from cytokine-release syndrome, or the activation of pro-tumour T cell subsets such as Tregs. Mucosal associated invariant T (MAIT) cells are ‘innate-like’ T cells characterised by an evolutionarily conserved semi-invariant T cell receptor (TCR). The unique biology of MAIT cells – rapid effector responses, enrichment in solid tissues, and donor-unrestricted recognition – positions them as promising targets for novel immunotherapeutics. However, the limited diversity of tools capable of specifically engaging MAIT cells has limited their translational use.

Here, we developed a nanobody (Nb-MT) using an *in vitro* yeast display system, where Nb-MT binds the MAIT TCR with high specificity in both human and mouse models, outperforming traditional surrogate identification markers via flow cytometry. We demonstrate that Nb-MT can activate human and mouse TCR cell lines, can induce robust proliferation of primary MAIT cells from both species *in vitro,* and can activate mouse MAIT cells *in vivo.* Furthermore, bispecific constructs incorporating Nb-MT and an antibody specific for the model tumour antigen HER2 induced MAIT cell-specific killing of HER2+ tumour cell lines.

By enabling the precise engagement of MAIT cells across species, Nb-MT allows both for the investigation of their unique non-polymorphic biology, and for the translation of these insights into novel immunotherapeutic strategies.

Title: Identification of the skin cells specialised in MR1 antigen presentation to MAIT cells

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Introduction: Mucosal-Associated Invariant T (MAIT) cells play a crucial role in the immune system, particularly at mucosal surfaces like the skin. These cells recognize metabolites from vitamin B2 (riboflavin) synthesis produced by various bacteria, presented by the MHC class I-related molecule MR1. MAIT cells contribute to wound healing by producing cytokines and growth factors that facilitate tissue repair and respond rapidly to injury. Additionally, they are responsive to bacterial infections, producing pro-inflammatory cytokines and directly killing infected cells. Despite the well-documented role of MAIT cells in skin repair, the identity of skin cells that express and present MR1 to activate MAIT cells remains unclear.

Objective: This study aims to identify MR1-expressing skin cells and assess MR1 antigen presentation via topical application.

Methods: We used a novel reporter mouse line, the *Mr1*-Tomato (Mr1Tom) model, to assess *Mr1* expression in skin cells via tdTomato fluorescence measured with flow cytometry. We administered MR1 ligand,5-OP-RU, via topical application in the ears of K14-cKO mice, where MR1 is knocked out specifically in keratinocytes, the highest MR1-expressing cells. We assessed MAIT cell numbers and activation status in response to this treatment compared to controls.

Results: tdTomato fluorescence was primarily observed in keratinocytes and Langerhans cells, with a novel subpopulation of Langerhans cells identified as high MR1 expressers. Following topical application, MAIT cells rapidly increased in both number and CD69 expression, while non-MAIT cells remained inactive. Notably, K14-cKO mice could not activate MAIT cells after topical application, whereas wild-type mice recruited MAIT cells more effectively.

Conclusion: Our findings indicate that keratinocytes and Langerhans cells are key MR1-presenting cells in the skin. The specific presentation of MR1 ligands highlights the role of keratinocytes in activating MAIT cells. Future studies will explore the implications of these interactions in maintaining skin immunity against bacterial infections.

Identification of checkpoint markers, cancer pathways and cancer genes in a mouse model of chronic colitis: Implications for new cancer biomarkers

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Chronic inflammation is a key driver of oncogenesis, and inflammatory bowel disease is strongly associated with the development of cancer. In this study, the Winnie mouse model of inflammatory bowel disease is used to show that the severity of inflammation leads to the expression of a wide range of cancer genes. This study provides important insights into the genetic basis for malignancy in inflammatory bowel disease, as well as identifying markers that could be used to screen for the development of cancer in patients. The presence of checkpoint markers in cancer cells aids in immune escape. The identification of checkpoint markers and early cancer markers is of utmost importance to gain clarity regarding the relationship between colitis and progressive inflammation leading to cancer. Herein, the gene expression levels of checkpoint makers, cancer-related pathways, and cancer genes in colon tissues of mouse models of chronic colitis (Winnie and Winnie-Prolapse mice) using next-generation sequencing are determined. Winnie mice are a result of a Muc2 missense mutation. The identification of such genes and their subsequent expression and role at the protein level would enable novel markers for the early diagnosis of cancer in IBD patients. The differentially expressed genes in the colonic transcriptome were analysed based on the Kyoto Encyclopedia of Genes and Genomes pathway. The expression of several oncogenes is associated with the severity of IBD, with Winnie-Prolapse mice expressing a large number of key genes associated with development of cancer. This research presents a number of new targets to evaluate for the development of biomarkers and therapeutics.

Bispecific antibody approach to harness dendritic cells in cancer immunotherapy

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Without immune intervention, cancer rapidly grows and can lead to deleterious patient outcomes. In 2024 in Australia, there were over 53,000 cancer-related deaths. While patient survival improves when combining traditional chemotherapy with immune checkpoint inhibition (ICI) only about 20% of patients respond to this immunotherapy. Cancer develops in the body because of several immune evasion mechanisms. One consists of an indirect decrease of local levels of the XCL1 chemokine resulting in poor infiltration of type 1 conventional dendritic cells (cDC1s), a cell type that responds to XCL1-XCR1 interactions to recruit cytotoxic T cells ($CD8^+$). Another mechanism involves upregulating inhibitory proteins, e.g. PDL-1, to forcibly trigger a reversible immune evasion mechanism via interaction with immune checkpoints on T cells, e.g. PD-1. These allow cancer to form a complex biological environment known as the tumour microenvironment (TME) from which immune cells can be excluded, forming what is known as a “cold” tumour. The presence of cDC1s in the TME has also been shown to correlate with successful ICI therapy, emphasizing the importance of crosstalk between cDC1s and $CD8^+$ cells. In this study, we explore the possibility of using bispecific cell engagers (BiCE) to increase the crosstalk between these cell types. Five different bispecific antibodies have been generated by adding single chain variable fragments (scFvs) with antigen recognizing capability to an IgG2a structure to allow the molecule to bind to both cDC1s and $CD8^+$ cells. An SDS-PAGE was performed as quality control; this indicated the formation of some protein aggregates. To assess binding to respective receptors we conducted ELISAs to test each arm individually and flow cytometry to test binding to cells *in vitro*. While all BiCEs bind to the desired receptors, two have superior affinity. Additionally, the scFv region of the antibody does not bind as efficiently as the IgG2a. Further *in vitro* flow cytometry testing is needed to confirm these results and to test the different fractions obtained after size exclusion chromatography to remove aggregates. This will allow for the BiCEs to be tested in CT26 models in mice to understand their potential as immunotherapy.

Loss of expression of the tumour surveillance protein NLRC5 in chief cells undergoing transdifferentiation in *Helicobacter pylori*-associated carcinogenesis

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Background: NOD-like receptor family CARD domain-containing protein 5 (NLRC5) plays a key role in immune surveillance. It was shown that low levels of NLRC5 expression correlated with poor prognosis for many solid tumours. The role of NLRC5 in gastric carcinogenesis, however, was not investigated. From Kaplan-Meier plots, we found that high *NLRC5* expression was associated with improved survival in gastric cancer ($p = 0.0053$). The aims of this study were to investigate NLRC5 expression in the gastric epithelium and its association with *Helicobacter pylori*-induced pathology.

Methods: To address the study aims, we analysed *NLRC5* expression in publicly available single-cell RNA sequencing (scRNA-seq) datasets from cases of gastritis (GS), intestinal metaplasia (IM) and gastric cancer (GC) (GSE249874), as well as in gastric-derived 3D organoids and 2D monolayers (GSE255276 and GSE167561). Immunohistochemistry (IHC) and immunofluorescence (IF) were performed on human and mouse gastric biopsies to evaluate NLRC5-positivity and localisation.

Results: NLRC5 expression was detected in epithelial, immune and endothelial cells from GS, IM, and GC tissues. Its expression was significantly upregulated in *H. pylori*-positive gastritis (Wilcoxon, $p<2.2e-16$), particularly in chief cells located at the gastric gland base, but not in surface pit cells (verified by IHC and IF in human gastric samples). As gastric carcinogenesis progressed, however, expression declined in parallel with the loss of chief cells and transdifferentiation into spasmolytic polypeptide-expressing metaplasia (SPEM), a proposed precursor of gastric cancer.

Conclusion: NLRC5/*NLRC5* expression in response to *H. pylori* infection appears to be localised specifically to chief cells at the gland base rather than the surface epithelial cells. Preliminary findings suggest that chief cell-derived NLRC5 may be involved in modulating gastric epithelial responses during *H. pylori*-associated carcinogenesis. A reduction in NLRC5 expression may potentially facilitate the transdifferentiation of chief cells into SPEM lineages, thereby contributing to metaplastic progression and tumour development.

Cancer-Microbiome interface: Novel Phage Therapy by Targeting *Fusobacterium Nucleatum* in Gastrointestinal Cancer

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Background: Fusobacterium nucleatum is a well-known cancer-associated bacterium linked to tumor growth, immune suppression, and resistance to immunotherapy. While *F. nucleatum* is sensitive to a range of antibiotics, concurrent antibiotic use has been linked to adverse clinical outcomes. Bacteriophages offer a promising alternative due to their host specificity and minimal off-target effects. In this study, we utilized FNU1, a bacteriophage selective for F. nucleatum, to investigate its potential as a therapeutic effect aimed at limiting the pro-tumorigenic effects of this bacterium but also enhance the efficassy of immunotherapy.

Aims: To evaluet efficacy of FNU1 therapy targeting F.nucleatum on cancer cells.

Method: Co-localization of Gal-GalNac and *F. nucleatum* infection in gastrointestinal cancer cell lines was assessed using flow cytometry. In vitro studies evaluated the effects of FNU1 therapy on cancer cell proliferation and migration. Proliferation and viability were measured using the MTS assay, while wound healing and transwell migration assays assessed cell motility. ImageJ and GraphPad Prism 9.0 software were used for data analysis.

Result: This result showed that *F. nucleatum*-infected cancer cells exhibited significantly increased proliferation compared to uninfected cells. However, post-infection treatment of cancer cells with FNU1 bacteriophage led to significant decrease in cancer cell proliferation in vitro. Additionally, infection with *F. nucleatum* significantly increased wound closure compared to the uninfected control group with or without FNU1, indicating that the bacteria had increased the migratory activity of cancer cells significantly. Notably, there was no significance difference between uninfected cells and infected cells treated with FNU1 bacteriophage.

Conclusion: The FNU1 bacteriophage effectively infected and eliminated *F. nucleatum* or limited its effect, thereby attenuating the bacterial-dependent increase in cancer cell migration. The results suggest that further evaluation of FNU1 using pre-clinical model is warranted.

Keywords: Gastrointestinal cancer, Gal-GalNac, F.nucleatum, FNU1, microbiome

Sex-specific immune resilience to DSS-induced colitis in the *Nlgn3*^{R451C} mouse model of autism

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Gastrointestinal (GI) disruptions and an increased prevalence of immune-related conditions such as inflammatory bowel disease (IBD) are frequently reported in children with autism and are often associated with altered gut microbiota. Administration of dextran sodium sulfate (DSS) is a well-established model of ulcerative colitis used to study gut inflammation and systemic effects in rodents. However, little is known about the trajectory of sickness behaviour and behavioural outcomes post-recovery from DSS-colitis, especially in the context of autism and sex-specific responses. Adult male and female *Nlgn3*^{R451C} mice expressing an autism-associated variant in the Neuroligin-3 synaptic protein received 2%-DSS for 7 days prior to 2-weeks recovery. Mice were monitored using the disease activity index (DAI). Locomotion and anxiety-like behaviours were assessed via open field testing every other day from day 7 to 21. Mice were culled at day 21 and anatomical measurements obtained.

Interestingly, DSS-treated *Nlgn3*^{R451C} female mice had lower DAI scores and milder colitis symptoms compared to WT males at day 7. Open field tests at 2 and 4 days post-DSS treatment revealed reduced mobility in DSS-treated WT and *Nlgn3*^{R451C} male mice when compared to SHAMs, but not in WT female mice. One week post DSS-treatment, WT but not *Nlgn3*^{R451C} male mice spent more time in the OF centre zone compared to SHAM male mice. After 2 weeks recovery, colon length was unchanged in DSS-treated and SHAM-treated mice across groups. Despite the recovery period, DSS-treated male *Nlgn3*^{R451C} and WT mice showed increased spleen weights compared to male SHAM *Nlgn3*^{R451C}. In contrast, spleen weight post recovery was unchanged in WT and *Nlgn3*^{R451C} DSS-treated female mice.

Female *Nlgn3*^{R451C} mice show resilience to DSS-induced colitis both during and post treatment compared to wild-types. Given that locomotor impairments during active colitis reflect sickness behaviour, increased time spent in the centre of the open field during recovery suggests that inflammation differentially affects behaviour in male *Nlgn3*^{R451C} and WT mice. Further neuroinflammatory assessments are needed to understand the potential role of *Nlgn3*^{R451C} on long-term colitis-related sickness behaviour.

Functional Assessment of the NOD2 Signalling Pathway in Patients with Inborn Errors of Immunity

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Background: Inborn errors of immunity (IEIs) are rare, inherited disorders characterised by infection susceptibility and immune dysregulation, with causal genetic variants unidentified in 70% of cases. This lack of genetic diagnosis impedes use of targeted therapeutics that could prevent severe organ damage and early death due to difficult-to-treat, comorbid non-infectious complications. In predominantly antibody deficiency (PAD), causal variants function within specific immune signalling pathways, including the NOD2 signalling pathway. To advance diagnostics, we developed an ex-vivo functional assay to evaluate NOD2 signalling, enabling validation of variants of unknown significance, and providing functional insights in patients without a causal defect.
Methods: Blood monocytes were evaluated by flow-cytometry for L18-MDP-induced (NOD2-dependent) TNF-α production, and phosphorylated (p-) p38 and p-p65. LPS-stimulated (NOD2-independent) and unstimulated samples were run concurrently. The assay was performed on 14 healthy-donors, 27 genetically unexplained PAD patients, and 3 patients with a hemizygous defect in X-linked Inhibitor of Apoptosis (encodes for essential pathway protein XIAP). All patients had WES analysed using our in-house bioinformatics pipeline.
Results: In healthy-donors, L18-MDP induced TNF-α production in 58% (range 22.2-90.1%) of monocytes, and a fold-change in median fluorescence intensity (MFI) of 1.04 (range 0.69-1.3) and 0.80 (range 0.67-0.98) for p-p38 and p-p65, versus LPS-stimulated cells. XIAP patients had 0% (0%-0.63%) of monocytes producing TNF-α, and MFI fold-change of 0.64 (0.5-0.79) and 0.50 (0.27-0.64) of p-p38 and p-p65, respectively. No significant difference was observed between healthy-donor and genetically unexplained patients. Of the 27 patients, 15 had at least one read-out outside of the healthy-donor range. Initial WES analysis showed these 15 patients had a median of 8 rare (minor allele frequency, MAF <0.01), or 3 ultra-rare (MAF <0.001) variants in the 397 NOD2 signalling pathway genes.
Conclusion: Here we show assessment of NOD2-dependent TNF-α, p-p38 and p-p65 can identify patients with potential abnormal pathway function, providing a rationale to explore gene variants associated with the NOD2 pathway. The use of a pathway-focused approach could streamline candidate gene variant identification beyond the currently known IEI genes and increase genetic diagnosis rates in patients.

Temporal proteomic profiling reveals novel IRAK3 interactors in Toll-like receptor signalling

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Introduction. Chronic systemic inflammation increases with age and drives the onset and progression of many conditions including cancer, cardiovascular disease, and autoimmune disorders. Patients living in rural and remote communities often face additional barriers to treatment access, highlighting the importance of identifying novel therapeutic targets. Acute inflammation is normally protective, but excessive or repeated activation of innate immune pathways contributes to chronic disease. Interleukin-1 receptor–associated kinase 3 (IRAK3) is a key negative regulator of Toll-like receptor (TLR)-mediated inflammation and a central mediator of immune tolerance. However, its broader molecular interactors and functions remain incompletely defined.

Aim. This study aimed to identify putative interactors of IRAK3 and to map its role in regulating acute and chronic inflammatory responses, with the long-term goal of informing new drug targets for inflammatory conditions.

Methods. THP-1 monocytes were stimulated with lipopolysaccharide (LPS) or mock-treated at early (30 min) and intermediate (2 h) timepoints, modelling acute and repeated inflammatory challenges. Proteins were extracted and analysed using label-free LC-MS/MS. Quantitative proteomic data were further examined using clustering, t-SNE dimensionality reduction, and gene ontology enrichment to identify differentially regulated proteins and enriched biological pathways.

Results. Approximately 2,600 proteins were identified, providing a comprehensive proteomic landscape of innate immune activation. Distinct temporal signatures separated LPS-treated from control conditions. At 30 minutes, nuclear transport and cytoplasmic regulatory proteins were enriched, while at 2 hours immune effector proteins were predominantly upregulated. Gene ontology analysis confirmed enrichment in immune system processes under repeated LPS challenge. Among these, IRAK3 itself was elevated, alongside nuclear-associated proteins such as CARM1 and ELAVL1. Clustering analyses positioned these proteins within functional interaction networks, and comparison with prior proteomic datasets suggested potential direct or indirect association with IRAK3.

Conclusion. Our findings highlight IRAK3 as a hub integrating cytoplasmic and nuclear pathways during repeated inflammatory challenge. This proteomic landscape identifies novel candidate interactors and extends our understanding of how IRAK3 contributes to immune tolerance. Ongoing work will validate these interactions and explore their potential as therapeutic targets for chronic inflammatory conditions, which remain a pressing health concern, particularly in rural populations.

The protective effect of breastfeeding on infant inflammation: a mediation analysis of the plasma lipidome and metabolome

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PUBLISH CONSENT WITHHELD.

Immune Cell Necroptosis Restrains Cutaneous Inflammation: Implications for Therapeutic Targeting

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Necroptosis is a lytic, pro-inflammatory form of programmed cell death typically associated with tissue damage and immune activation. Several inhibitors are in clinical development as potential therapeutics for inflammatory and degenerative diseases. However, our recent work indicates a counterintuitive and clinically relevant role for necroptosis in restraining cutaneous immune overactivation.

While performing excision wound studies to investigate a previously observed healing advantage in MLKL KO mice, we unexpectedly discovered that the MLKL-deficient animals were more prone to dressing-associated dermatitis. To separate tissue-intrinsic from immune-intrinsic effects, we created bone marrow (BM) chimeras. Transplanting *Mlkl*$^{-/-}$ BM into either WT (*Mlkl*$^{-/-}$→WT) or *Mlkl*$^{-/-}$ (*Mlkl*$^{-/-}$→*Mlkl*$^{-/-}$) recipients consistently worsened dermatitis. In contrast, WT BM transplanted into *Mlkl*$^{-/-}$ hosts (WT→*Mlkl*$^{-/-}$) reduced dermatitis towards WT levels while retaining the tissue-driven healing advantage. WT→WT chimeras showed only mild reactions comparable to un-transplanted WT mice. Thus, loss of MLKL in the immune compartment amplified inflammatory skin responses, irrespective of keratinocyte genotype, suggesting immune cell necroptosis has a protective role in limiting skin inflammation.

A similar pattern was seen in a toxic epidermal necrolysis (TEN) model, where necroptotic KO mice consistently recover faster than WTs. In the bone marrow transplant scenario, *Mlkl*$^{-/-}$→*Mlkl*$^{-/-}$ chimeras showed delayed recovery compared with untransplanted *Mlkl*$^{-/-}$ controls, despite both groups being MLKL deficient in all cells. This suggests the protective effect of immune cell necroptosis is particularly important following immune reconstitution. Notably, WT→*Mlkl*$^{-/-}$ chimeras recovered even faster than untransplanted *Mlkl*$^{-/-}$ mice, indicating that MLKL-deficient immune cells may interfere with the tissue-intrinsic healing advantage of MLKL deficiency.

Mechanistically, we propose that sub-lethal necroptotic signalling in keratinocytes triggers a differentiation program that protects barrier integrity, at the cost of proliferative repair, while immune cell necroptosis limits activation, survival, or accumulation of pro-inflammatory populations at injury sites. These findings challenge the prevailing view of necroptosis as purely pro-inflammatory with important implications for therapeutic targeting. Systemic inhibition of necroptosis could inadvertently remove beneficial immune-intrinsic restraints and exacerbate immune-mediated skin pathology, whereas approaches targeting necroptosis in a tissue-restricted or cell type-specific manner may preserve immune control while promoting epithelial repair.

Structural Insights into KvrA: A MarR Homologue that Senses and Responds to Copper Stress

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Bacteria utilise a suit of transcription factors to alter the regulation of their genome in response to a range of stimuli, be it changes in nutrient availability, host cell infection or the presence of antibiotics. One key set of bacterial transcription factors are the multi-antibiotic resistance regulator proteins (MarR proteins) which regulate gene products associated with antibiotic resistance mechanisms. While emphasis has been placed on the function of these proteins and uncovering which operons they regulate, less research has focused on understanding what molecular mechanisms cause the recruitment or dissociation of these transcription factors. Previous research has shown that *E. coli* MarR forms disulphide mediated tetramers [1] which suggests a possible mechanism of regulation whereby an influx of cellular copper or the presence of antibiotics causes the oligomerisation of MarR and thus changes its ability to bind to DNA. However, whether this mechanism is conserved across other MarR homologues is unknown. To this end, we present the structural and biophysical characterisation of a MarR homologue from *Klebsiella pneumoniae ;* KvrA, *t*hat has been tied capsule formation in both hyper virulent and classical *K. pneumoniae* strains [2]. Ongoing work aims to structurally and functionally characterise KvrA in its dimeric and tetrameric form to determine potential oligomeric and conformational changes that may serve as KvrA regulatory mechanisms and identify potential KvrA binding sequences in *Klebsiella*. This work not only broadens our understanding of the mechanisms of transcriptional regulation but, given the MarR proteins' involvement in resistance mechanisms, may offer a novel avenue for antibiotic development.

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The *Burkholderia* T6SS-5 and oxidative stress responses during infection

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Burkholderia pseudomallei is a facultative intracellular Gram-negative bacterium and the causative agent of melioidosis, a severe infectious disease endemic to southeast Asia, northern Australia, and many tropical countries worldwide. *B. pseudomallei* invades the cytosol of eukaryotic cells, such as macrophages, using the *Bsa* type III secretion system, and subsequently employs the type VI secretion system-5 (T6SS-5) to mediate intercellular spread, resulting in the formation of characteristic multinucleated giant cells (MNGCs). The T6SS-5 is also required for optimal virulence during infection of mammalian hosts. However, it has thus far not been possible to identify specific biochemical activities mediated by the T6SS-5, largely due to an inability to inactivate the single known effector protein secreted by the T6SS-5 without inactivating the secretion system itself.

Here, we are identifying the roles for the T6SS-5 by examining the host response to infection. We infected RAW 264.7 macrophages with wild-type and T6SS-5 deficient (∆T6SS) strains of *B. thailandensis*, a BSL-2 model organism sharing ~93% genetic similarity with *B. pseudomallei*. We conducted bulk RNA barcoding and sequencing (BRB-seq) after infection. The differentially expressed genes were detected using the DESeq2 R package and used for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. Inflammatory and oxidative damage-related signalling pathways were enriched in the comparison between infected and uninfected groups. Infection with the ∆T6SS strain showed metabolic dysregulation compared to the wild-type infection at the transcriptional level. Notably, genes associated with glutathione (GSH) metabolism, a key antioxidant pathway in mammalian cells, exhibited altered expression.

Based on these findings, we hypothesize that the T6SS-5 in *B. pseudomallei* and *B. thailandensis* might have undescribed role(s) in modulating GSH metabolism, presumably to mitigate ROS accumulation and facilitate intracellular survival.

Investigating the role of O-GlcNAcylation in regulating Dendritic cell maturation

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O-GlcNAcylation is the posttranslational modification of serine and threonine residues of nuclear, cytoplasmic and mitochondrial proteins with the sugar β-N-acetylglucosamine (GlcNAc). The modification is governed by the reciprocal activity of two enzymes only – O-GlcNAc Transferase (OGT) and O-GlcNAcase (OGA) which catalyses the addition and removal of GlcNAc respectively. O-GlcNAcylation is highly dynamic and reversible – linking nutrient sensing and stress to regulation of essential cellular processes such as gene expression and signal transduction.

Cumulative literature has addressed the multifaceted role that O-GlcNAcylation plays in maintaining immune cell homeostasis. However, very few studies have addressed the role of O-GlcNAcylation in an *in vivo* setting as both germline and cell specific ablation of either enzyme involved in O-GlcNAcylation is associated with lethality.

Using the Cre/LoxP strategy, we successfully established a dendritic cell specific O-GlcNAcylation deficient mouse model by knocking out the *O-GlcNAc transferase (Ogt)* gene in CD11c expressing cells. Mice harbouring *Cd11c*-specific ablation of *Ogt* exhibited defective conventional DC (cDC) cellularity and function *in vivo*. Most notably, we observed a substantial reduction in splenic cDC2A cellularity accompanied with cell intrinsic defects. This was marked by substantial reduction in the $ESAM^{+}$cDC2A cellularity and concomitant accumulation of ESAM- cDC2A immediate precursors. The loss of O-GlcNAcylation in splenic cDC2A also resulted in altered antigen presentation ability. The accumulative defects resulted in diminished germinal centre responses marked by reduced T-follicular helper cell and germinal centre B cell generation after immunisation with a model antigen (Sheep Red Blood Cells). While *Ogt* ablation did not impact total cDC1 cellularity, a significant decrease in homeostatically mature $CCR7^{+}$ splenic cDC1 was noted. Recently, homeostatic maturation of cDC1 has been proposed to be primarily driven efferocytosis *in vivo*. Our data suggests that the loss of O-GlcNAcylation did not affect cDC1 efferocytosis capability but did result in the dysregulated processing of engulfed apoptotic material *in vivo.* Together, our ongoing findings demonstrate the importance of O-GlcNAcylation in regulating DC function and immunity *in vivo*. Ongoing work is directed to the identification of O-GlcNAcylated proteins that are critical for DC function and immunity.

Role of Ubiquitination in Toxoplasma Differentiation

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A quarter of the global population is chronically infected with *Toxoplasma gondii*. Following infection, *T. gondii* switches from fast-growing tachyzoites which invade virtually any nucleated cell, to slow-growing bradyzoites with tropism towards muscle and central nervous system tissues. Bradyzoites are characteristic of chronic infection, impervious to clearance via the human immune system. Current therapies are not only inefficient in controlling latent parasite burden, but cause several serious side effects, necessitating the hunt for novel therapeutics targeting the chronic stage. BFD1, a myb-like transcription factor regulates the initiation of differentiation from tachyzoites to bradyzoites. BFD1 is shown to be regulated post-transcriptionally, where protein levels accumulate upon a differentiation signal. Through a CRISPR screen, we identified an E3 ubiquitin ligase complex called the GID complex that regulates differentiation, named after the glucose-induced degradation deficient protein identified in yeast. We showed that loss of GID resulted in a loss of expression of known bradyzoite proteins, in a similar manner to loss of BFD1, suggesting a strong link of this E3 ligase in regulation of differentiation. We identified that GID acts upstream of BFD1 translation and regulates differentiation through the 3'-UTR of the BFD1 transcript, likely by modulating translation repression of this master regulator. We used mass spectrometry coupled with transcriptomics to map, for the first time, the global post-translational regulation during differentiation. We also plan to use orthologonal methodologies to reveal substrates of the GID complex. Overall, this study will provide the first evidence of and the mechanism of the ubiquitin signaling system during differentiation in *Toxoplasma* and other apicomplexan parasites.

Understanding metabolic sensing during *Toxoplasma* differentiation

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Toxoplasma gondii is an intracellular parasite found globally that establishes untreatable latent infection in muscle and neural tissue. It is not known what triggers the differentiation into this latent form or why it is resistant to current therapies. Our laboratory undertook a CRISPR screen to determine which genes were required for the utilization of glutamine or glucose *in vitro*. The results uncovered that a switch of carbon source can trigger differentiation, and identified several genes in central carbon metabolism, suggesting a link between these two processes. I utilised a newly reported human muscle cell line (KD3) to study the link between host and parasite central carbon metabolism and differentiation. Overall, we found that KD3 cells as compared to the canonical 'alkaline stress' model have significant benefits and reveal novel phenotypes. We generated several parasite mutant strains lacking genes involved in central carbon metabolism, multiple showed defective differentiation which was most pronounced in the KD3 myotubes. In particular we show that a glutamate dehydrogenase (GDH2) is particularly important for differentiation and localises to the parasite nucleus, suggesting a link to gene regulation. Together this work suggests that central carbon metabolism is linked to *Toxoplasma* differentiation in unappreciated ways.

Mapping protein complexes to reveal new functions in the malaria parasite

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Malaria is a mosquito-borne disease caused by the *Plasmodium* parasite, with sub-Saharan Africa disproportionately accounting for 90% of global cases. Novel treatments are urgently needed to combat the emergence of resistance to all antimalarials. However, identifying promising drug targets from the parasite's complex lifecycle has been challenging due to the incomplete understanding of malaria biology. While genomic and transcriptomic studies have provided the foundation for understanding its underlying biology through comparison to well-understood model eukaryotes, the large number of unannotated genes limits further comprehension.

To overcome this barrier, this project will use Protein Correlation Profiling (PCP). PCP identifies interacting proteins by mass spectrometry analysis of complexes that co-elute in chromatographic fractionates. The co-elution of unannotated non-conserved proteins with well-characterised proteins will provide crucial insights into their functions through 'guilt by association'. Various combinations of chromatographic separation techniques can be implemented to ensure high coverage of these genuine protein interactions.

The project will focus on tracking protein-protein interaction changes throughout the parasite's asexual blood stage, the phase responsible for clinical symptoms. Despite its importance, molecular processes regulating the cell cycle and gene expression during this stage remain poorly understood. By mapping protein complex dynamics across these asexual stages using PCP, the project will uncover unannotated protein functions critical for parasite survival.

This technique has not yet been implemented at a proteome-wide scale across the asexual lifecycle of Plasmodium falciparum. The resulting network of protein–protein interactions will complement other proteomic approaches, guide functional validation of previously unannotated proteins and provide critical insights for the development of novel symptom-targeted therapeutics.

Characterising monoclonal antibodies to placental malaria antigen, VAR2CSA

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Placental malaria, resulting from the sequestration of *Plasmodium falciparum*-infected red blood cells (iRBCs), leads to adverse pregnancy outcomes [1]. The sequestration is mediated by VAR2CSA, a protein that binds to placental chondroitin sulfate A (CSA) [2]. VAR2CSA antibodies protect against adverse pregnancy outcomes [3]; however, no pregnancy-specific vaccine or therapy has been developed to date. We identified and expressed VAR2CSA-specific IgG1 monoclonal antibodies (mAbs) using B cells of exposed Papua New Guinean women. VAR2CSA mAbs were characterised by their ability to recognise eight heterologous CSA-binding *P. falciparum* strains, to neutralise CSA binding and/or induce phagocytosis of iRBCs by THP-1 monocytes. We identified 16 mAbs, all of which targeted just two of the six domains of VAR2CSA. Cross-reactivity varied between mAbs, with 2D9 binding to all eight strains. Although individual mAbs did not promote phagocytosis, combinations targeting distinct VAR2CSA epitopes did. None of the mAbs inhibited iRBCs from binding to CSA. Structural studies using hydrogen-deuterium exchange mass spectrometry (HDX-MS) with 2D9 revealed a broadly conserved epitope on the globular VAR2CSA structure. Our findings revealed a conserved VAR2CSA epitope as a target for strain-transcending immunity, suggesting that effective mAb therapies should combine mAbs targeting distinct epitopes. Altogether, we demonstrated how mAbs can dissect naturally acquired antibody responses to inform vaccine design.

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Cross-reactive Immunity to VAR2CSA Antigen in Placental Malaria

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Placental malaria infections (PM) occur due to infected red blood cells (iRBCs) sequestrating in the placenta by binding CSA via iRBC antigen VAR2CSA. VAR2CSA is the leading anti-PM vaccine candidate but its antigenic variation stymies vaccine development efforts.

My project therefore aims to investigate the development of cross-reactive immunity towards VAR2CSA antigen using antigenically different *P.falciparum* isolates. Plasma samples taken mid-pregnancy and then monthly until delivery from 25 primigravid Malawi Pregnant women as well as samples taken mid-pregnancy from 50 Malawi pregnant women with differing gravidities and 10 non pregnant Malaria naive Melbourne individuals shall be examined using a binding inhibition assay that measures antibodies that block iRBC binding to the placental receptor CSA.

Seropositivity for binding inhibitory antibodies will be defined as 2SD above the mean of the Melbourne individuals. Breadth of antibody responses will be tracked across pregnancy and compared between the different gravidities.

Investigating the effects of amino alcohol antimalarials on artemisinin activity in *Plasmodium falciparum*

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Malaria is a major global health concern with drug resistance to all currently available antimalarials. Infection with *Plasmodium falciparum* parasites is the main cause of malaria-associated morbidity and mortality. Artemisinin-based combination therapies (ACTs) are the recommended first-line treatment against *P. falciparum*, which combine an artemisinin derivative with a partner antimalarial. The most widely used ACT partner drugs globally are the amino alcohol class, including lumefantrine, mefloquine and amodiaquine. However, the mechanism of action of these drugs remains largely unknown. Additionally, resistance has now emerged to the artemisinin component, which manifests in parasites at the very early stage of its asexual lifecycle known as the ring stage, and it is unknown how amino alcohols interact with artemisinins in ring-stage parasites. This work investigates the relationship between amino alcohols and artemisinins, and the implications for artemisinin resistance.

We performed ring stage survival assays with a combination of dihydroartemisinin (DHA) and amino alcohols, as well as a range of other antimalarials. Parasite cultures were treated with 700 nM DHA alone or in combination for 3 hours. Clinical plasma concentrations of the partner drugs were used, which causes no ring-stage parasite killing under these conditions. Results showed a significant survival increase for *Pf*3D7 rings treated with DHA and the amino alcohols mefloquine or quinine compared to DHA alone (16-27% vs 3-5%). This effect was not seen with other antimalarials such as pyrimethamine and chloroquine. In artemisinin resistant parasites (Cam3.II^{R539T}), amino alcohol combinations with DHA further potentiated artemisinin resistance (20% vs 50-70%). As artemisinin activity is dependent on activation by haem released by parasite haemoglobin digestion, we performed haem fractionation assays to determine if amino alcohols decrease the availability of haem for artemisinin activation. Mefloquine-treated *Pf*3D7 parasites showed a 25% decrease in haem levels, suggesting that amino alcohols antagonise artemisinin activity by decreasing activation. Further studies will use high resolution mass spectrometry to investigate whether amino alcohol antimalarials directly impact artemisinin activation in parasites. Future research may be needed to establish whether the antagonistic relationship exhibited between amino alcohols and DHA at the early ring-stage will potentiate further progression of artemisinin resistance in the field.

Improving the capacity of vaccine-induced antibodies to arrest the growth of Plasmodium falciparum

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Plasmodium falciparum, the deadliest of the malaria-causing parasites, remains a major global health threat. While vaccines targeting the liver stage have been developed, they are not fully protective, enabling parasites to break through to the symptomatic stage of infection, the blood stage. This underscores the urgent need for novel vaccines that can effectively target the parasite's blood stage to provide a greater degree of protection. To survive in the bloodstream, *P. falciparum* invades and replicates within red blood cells (RBCs), using its essential *Pf*RH5 protein to bind to the RBC receptor basigin to trigger invasion.

*Pf*RH5 is the current leading blood-stage vaccine candidate. Neutralising anti-*Pf*RH5 monoclonal antibodies (mAbs) bind to the tip of the *Pf*RH5 protein, sterically blocking its interaction with basigin and preventing RBC invasion. However, *Pf*RH5 mAbs do not completely block parasite invasion of RBCs unless used at high concentrations and as mAb concentrations are reduced, their blocking efficiency declines. The World Health Organization recommends a malaria vaccine efficacy threshold of at least 75% against clinical malaria for recommended widespread use in areas with ongoing transmission. The current RH5 vaccine in phase 2b trials only shows 55% vaccine efficacy. Therefore, improving the invasion-blocking efficiency of *Pf*RH5 mAbs is essential to enhance vaccine efficacy to the WHO-recommended threshold for broad implementation and to provide greater protection against disease.

To boost the efficiency of RH5 mAbs, we successfully combined them with invasion-slowing compounds that give the RH5 mAbs more time to inhibit invasion. We have also successfully applied this strategy to polyclonal RH5 IgG from previously vaccinated individuals. These polyclonal RH5 IgGs, when combined with invasion-inhibiting compounds, also show a synergistic inhibitory interaction. Underscoring that this approach is effective not only in the laboratory but also has potential in real-world contexts, particularly if it can be used to boost natural immunity in endemic populations.

Through plate-based assays and live-cell microscopy, this work provides valuable insights into how neutralising mAbs against vaccine candidate *Pf*RH5 disrupt the parasite's invasion of RBCs and informs novel therapeutic interventions against malaria and offers potential strategies for the development of improved blood-stage malaria vaccines.

Antibody-dependent neutrophil phagocytosis of *Plasmodium falciparum* infected erythrocytes is mediated by FcγRIIa

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PUBLISH CONSENT WITHHELD

Metabolic Tracing in *P. falciparum* Using a Stable Isotope Labelling Strategy

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Plasmodium falciparum, the causative agent of malaria, undergoes complex metabolic transformations across its life cycle stages, presenting opportunities for targeted therapeutic interventions. This study employs stable isotope labelling (SIL) with $^{13}C_6$-glucose to investigate metabolic fluxes in infected red blood cells (iRBCs) under different developmental conditions. High-resolution mass spectrometry and bioinformatic analysis enable the identification of both native and labelled metabolites, offering insights into metabolic pathway activities and potential novel metabolite discovery. The approach reveals novel metabolic functions and captures dynamic metabolic changes across various stages, contributing to a comprehensive understanding of *Plasmodium* metabolism and supporting efforts to develop novel antimalarial strategies.

Hydrogel Loaded with Endometrial Mesenchymal Stem Cells Alleviates Birth Injury in a Sheep Model

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Background: Vaginal childbirth is a leading cause of pelvic floor disorders (PFDs), including pelvic organ prolapse (POP), affecting up to 25% of women. While the damage occurs during childbirth, its enduring impact often becomes evident later in life, marked by complications in bladder, bowel, and sexual functions. The increasing demand for preventative treatment approaches for POP after birth injury has led to the exploration of novel biologically active scaffolds such as Aloe Vera-Alginate hydrogel (AV-ALG Hyd) comprising proliferative and regenerative human Endometrial Mesenchymal Stem Cells (eMSC). This study addresses the urgent need for effective birth injury interventions by investigating the therapeutic potential of AV-ALG-Hyd with and without eMSC in promoting healing. The primary aim was to construct and evaluate the efficacy of tissue engineered AV-ALG-Hyd with eMSC in postpartum tissue healing.

Methods: Herein, we selected primiparous ewes and simulated birth injury using a balloon catheter that is clinically used to mitigate post-partum uterine bleeding. AV-ALG-Hyd, with proliferative and regenerative human eMSC was transplanted directly following simulated vaginal birth injury, while control groups underwent injury without AV-ALG-Hyd and/or eMSC injection. Ewes transplanted with AV-ALG-Hyd without eMSCs, and sham injury served as control. Explant analysis at 30 and 90-day timepoints encompassed pre- and post-operative Pelvic Organ Prolapse Quantification system, a standardized method based on vaginal landmark measurements, eMSC retention, tissue healing, immune response, and tissue tensiometry.

Results & Conclusion: AV-ALG-Hyd with eMSC treatment improved postpartum tissue healing, alleviates birth injury, enhancing eMSC retention, and restoring smooth muscle content. Treated tissues showed improved biomechanics, stabilized elastin, and collagen deposition. In summary, the result highlight the potential of AV-ALG-Hyd with eMSC therapy as a preventive approach to POP associated with vaginal childbirth injury.

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