Victorian Infection
& Immunity Network

Young Investigator Symposium

Friday 8th November 2024
Monash Institute of Pharmaceutical Sciences
Royal Parade, Parkville

www.viin.org.au
#viinyis, #YoungVIIN2024
@The_VIIN

Welcome to the 2024 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 2024 Young Investigator Symposium.

Special welcome to our keynote speakers: Dr Danika Hill of Department of Immunology, Monash University, and Prof Stephanie Gras of La Trobe Institute for Molecular Science, La Trobe University.

This is the 17th 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, and remind you to be vigilant about remaining COVID-safe.

We are indebted to many who have been vital to bringing about the 2024 meeting, namely:

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

Catarina Almeida, Peter Doherty Institute & University of Melbourne
Tom Angelovich, RMIT University
Joshua Bourne, Monash Health & Monash University
Aaron Brice, ACDP, CSIRO
Dimitra Chatzileontiadou, LIMS, La Trobe University
Michelle Chonwerawong, Hudson Institute of Medical Research
Sam Davis, CSIRO
Ruby Dawson, University of Adelaide
Emily Eriksson, Walter and Eliza Hall Institute
Paul Gill, Alfred Hospital & Monash University
Gabriela Khoury, Burnet Institute
Robson Kriiger Loterio, Burnet Institute
Kevin Lee, Royal Melbourne Hospital & University of Melbourne
Stella Liong, RMIT University
Christophe Macri, University of Melbourne
Meg Manohar, CSIRO
Joyanta Modak, Deakin University
Linda Reiling, Burnet Institute
Natalia Sampaio, Hudson Institute of Medical Research
Stephany Sanchez, WHO Collaborating Centre for Reference and Research on Influenza
Stephen Scally, Walter and Eliza Hall Institute
Ghizal Siddiqui, Monash Institute of Pharmaceutical Sciences
Caroline Soliman, CSL & University of Melbourne
Praveena Thirunavukkarasu, Monash BDI, Monash University
Ryan Toh, Murdoch Children's Research Institute
Jinxin Zhao, Monash BDI, Monash University

Madeleine Wemyss, Victorian Infection and Immunity Network Project Officer
Rebecca Smith, VIIN Program Manager (Outgoing)

- The 60+ Session chairs and judges for oral presentations, Science Bites and posters, which is a reflection of the ongoing popularity of this event. Thanks to each for your time and expertise.
- The sponsors and advertisers for this symposium. Your support is more and more important to the success of this event.
- The 15 Academic Institutions and government agencies that support VIIN annually through financial contributions. Without your support, our activities would cease.

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

Sincerely,

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

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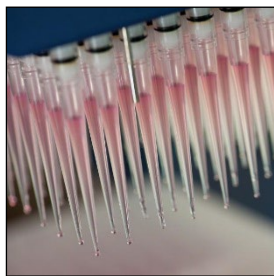
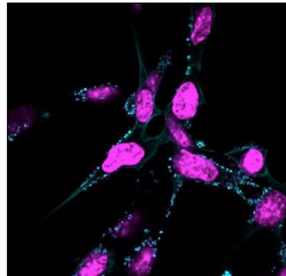
Thank you to our sponsors

For links to exhibitor and sponsor websites:
https://www.viin.org.au/event/viin-young-investigator-symposium-2024

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

VIIN Contributors include:

MONASH University

Burnet

Doherty Institute

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THE UNIVERSITY OF MELBOURNE

LA TROBE UNIVERSITY

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murdoch children's research institute

For enquiries contact: info@viin.org.au

The Hartland Oration at Lorne

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

In honour of Liz's contribution to VIIN, the network is delighted to have established the Hartland Oration. This oration will be delivered at the Lorne Infection and Immunity Conference from 2018 onwards. The Hartland Orator will be selected at this VIIN Young Investigator Symposium and will be the post-doctoral researcher giving the best 10 minute oral presentation. This outstanding young researcher will receive free registration to the 2025 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!

Would you like to join the VIIN Young Investigator Committee in 2025?

We are seeking Expressions of Interest from young investigators* who would like to join the VIIN Young Investigator Committee in 2025 for a 2-year term of commitment**.

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

In this role, you will be required to:

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

Please note that these are voluntary roles.

The deadline to apply is Monday 25 November 2024, 11.59pm. To apply, please see QR code below or click here: https://forms.gle/w6KU7dnqiSpeS2aU6

For more information on committee member responsibilities and the selection process, please see policy linked here, or email info@viin.org.au with any enquiries. Thank you.

We look forward to your involvement!

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

** 2-year commitment is preferred, but flexible depending on professional and personal circumstances.

Program-at-a-Glance 2024

FRIDAY 8 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: Modelling Host-Pathogen Interactions**	Lecture Theatre 1
09:45 – 10:20	**Session 2: Keynote Speaker, Dr Danika Hill – Laboratory Head, Department of Immunology, School of Translational Medicine, Monash University**	Lecture Theatre 1
10:20 – 11:00	**Morning Tea** (Includes transit time from and to Lecture Theatres)	Cossar Hall
11:00 – 11:45	**Session 3: Science Bites I**	Lecture Theatre 1
11:45 – 12:30	**Session 4: Oral Presentations** **Theme: Inflammation and Innate Immunity**	Lecture Theatre 1
12:30 – 13:40	**Lunch and Poster Session I** (Includes transit time from and to Lecture Theatres)	Cossar Hall
13:40 – 14:25	**Session 5: Oral Presentations** **Theme: Immunity at the Host-Pathogen Interface**	Lecture Theatre 1
14:25 – 15:00	**Session 6: Keynote Speaker, Prof Stephanie Gras – Laboratory Head and Deputy Director, La Trobe Institute for Molecular Science (LIMS), La Trobe University**	Lecture Theatre 1
15:00 – 15:40	**Afternoon Tea** – Sponsored by BMG Labtech & QIAGEN (Includes transit time from and to Lecture Theatres)	Cossar Hall
15:40 – 16:25	**Session 7: Science Bites II**	Lecture Theatre 1
16:25 – 17:10	**Session 8: Oral Presentations** **Theme: Adaptive Immunity and Therapeutics**	Lecture Theatre 1
17:10 – 17:15	**Thanks and Announcements**	Lecture Theatre 1
17:15 – 18:25	**Evening Networking and Poster Session II** – Sponsored by QIAGEN & BMG Labtech (Includes transit time from Lecture Theatres)	Cossar Hall
18:25 – 18:45	**Prizes, Acknowledgements and Conclusion** – Including Hartland Oration Prize winner for 2024 **Presented by VIIN Co-Convenors:** **Prof Gilda Tachedjian and Prof Richard Ferrero**	Cossar Hall

Keynote Speakers

Dr Danika Hill, Monash University

What can sore throats teach us about future *Streptococcus pyogenes* vaccines?

Dr Danika Hill leads the Precision Vaccinology laboratory in the Department of Immunology within the School of Translational Medicine at Monash University. Dr Hill's lab uses high-parameter flow cytometry, single cell RNA and immune repertoire sequencing with human clinical cohorts to understand T and B cell responses to Group A Streptococcus, malaria and influenza. Dr Hill completed a PhD at the Walter and Eliza Hall Institute of Medical Research, and a postdoc at the Babraham Institute in Cambridge supported by an NHMRC CJ Martin Fellowship. Danika joined Monash University in 2020 and started her own lab in 2023. Her research is supported by the Michelson Prize from the Human Immunome Project, and has ongoing partnerships with leading vaccine developers (e.g. GSK, Dynavax Technologies).

Prof Stephanie Gras, La Trobe Institute for Molecular Science

Immune signalling from a structural biology perspective

Prof Stephanie Gras is head of the Viral & Structural Immunology laboratory and Deputy Director of the La Trobe Institute for Molecular Science (LIMS) at La Trobe University, Melbourne, Australia. She is an internationally recognised leader in the field of T cell Immunology and Structural Biology with a sustained record of high-quality publications in peer reviewed journals (> 130 publications in *Nature, Science, Cell, Immunity, Nature Immunology, Science Immunology, Nature Communications, PNAS …*), with a successful record of research funding (NHMRC, MRFF, ARC, VMRAF, Commercial contract & philanthropy), and is strongly committed to advance supportive and equitable research environments. Prof Gras is currently an NHMRC Investigator (L2) and has been awarded five fellowships over her career (Monash, ARC Future Fellowship, NHMRC CDF2 and SRFA). She has been awarded the Georgina Sweet Award for Women in Biomedical Science (2017), SCANZ Sandy Mathieson Medal (2022), ASBMB Shimadzu Medal (2023). Prof Gras is also an inventor and co-founder of Resseptor Therapeutics to modify T cells for therapeutics applications.

Prof Gras' research is instrumental on providing a better understanding of the first key event in T cell-mediated immunity towards pathogens: the antigen recognition mechanism. Notably her work has elucidated the link between HLA and asymptomatic COVID profile, link between TCR docking orientation and T cell activation. Understanding antigen recognition using structural biology offers tremendous opportunities to design new therapies that mobilise, reprogram, or boost the immune system.

Notes

COVID-Safe Precautions

Although prevalence of COVID-19 infections in the community currently remain stable at relatively low levels, we encourage all delegates to continue to take 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 upon request.
- Test before arrival: All delegates are strongly encouraged to complete a Rapid Antigen Test at home before departing for the symposium.
- Wear a mask: It is VIIN's position that all delegates are strongly encouraged to wear a closely-fitting mask in the Lecture Theatre block. We similarly encourage you wear a mask as much as possible in Cossar Hall. A small number of masks will be available at the registration desk if required.
- Sanitise frequently: Hand sanitiser will be available throughout the venue.
- If you are concerned about being exposed to SARS-CoV2 and other 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.

TWITTER / X: We encourage you to join the conversation on Twitter / X with #YoungVIIN2024 and #viinyis. Please follow us @The_VIIN where we will be live tweeting 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 Tweet 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

Trade Displays

We are fortunate to have the support of several companies at our Symposium, who will be holding trade displays from morning tea through to the evening poster session. Please visit their tables and ask about their products and services.

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 hosted by Monash Institute of Pharmaceutical Sciences, located at 381 Royal Parade, Monash University Parkville Campus.

On arrival, please register at the desks located in the foyer in Sissons Building 401, to your left as you face the courtyard. Posters, trade 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. Lecture Theatres are accessed through the courtyard, heading in the direction of Mile Lane.

Drink tickets: At registration, please collect a name tag and drinks coupons. These drinks coupons must be retained during the symposium and exchanged during Evening Networking/Poster Session II 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

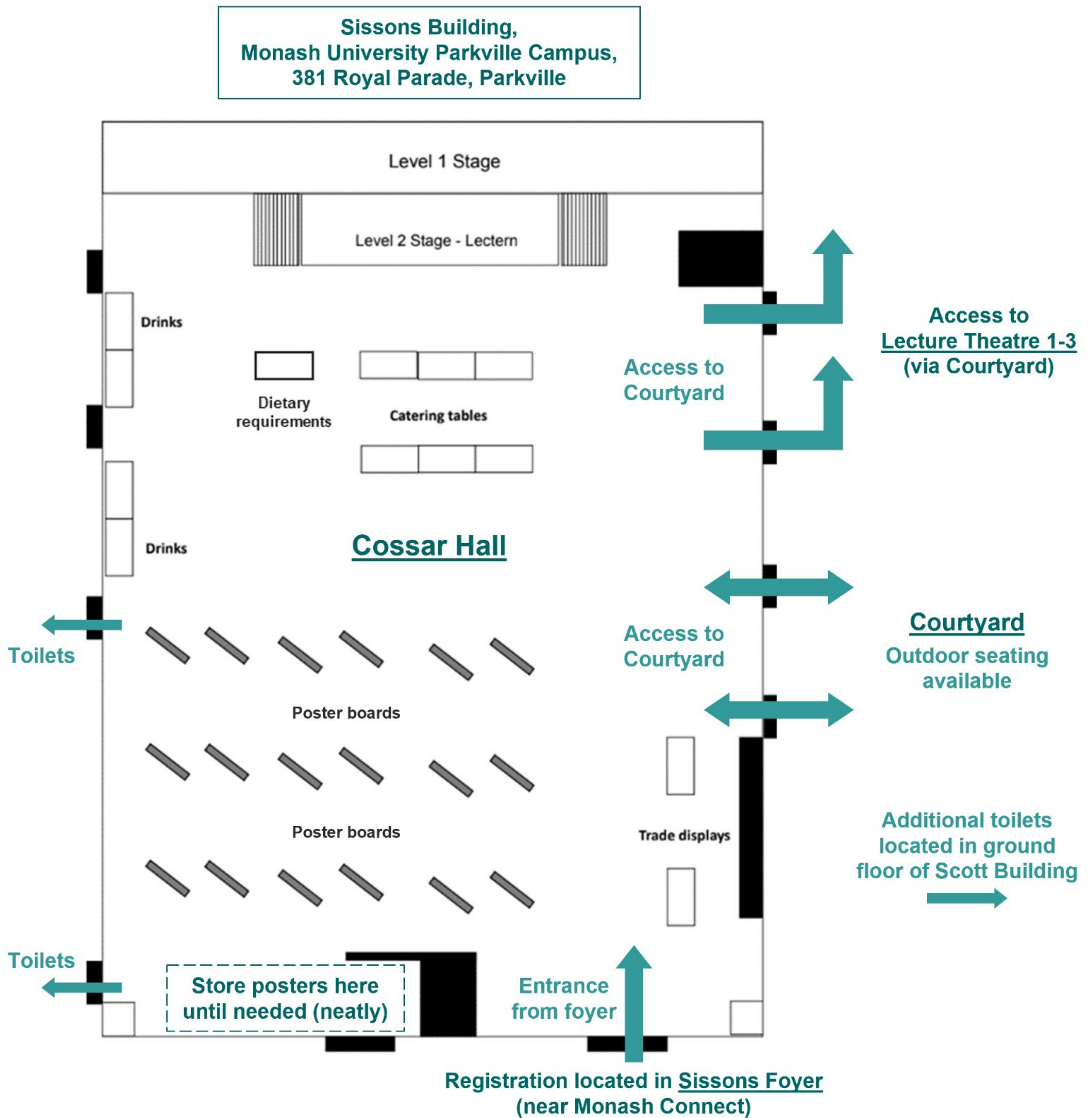
Please provide us with your feedback regarding this year's Symposium using our post-event survey, accessible via the QR code below or at this link: https://forms.gle/qAUoVN3pyNKsHeo38

Your experiences of the event will help inform our planning for 2025, 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 2024 VIIN Young Investigator Symposium.

Venue Map



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Thursday 14th October, 2024 | 10.30am–12.30pm

Ask me anything: Extraction edition

Is your extraction workflow not delivering the results you need? In this Q&A session, ask our expert your questions and join us over coffee to discover best practices and advanced solutions for optimizing your PCR and extraction protocols. Join us at Sammy's Cafe and we'll shout you a cuppa!

Expert details

Dr. Vinod Dagar
Field Application Scientist. QIAGEN

Participate in our extraction quiz on the day and win a limited edition QIAGEN cooler box!

RSVP to Amram Williams at: **amram.williams@qiagen.com**

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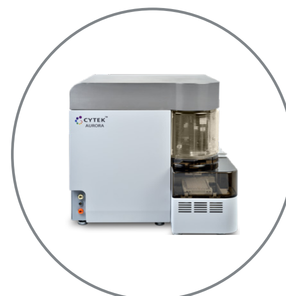

Contact: rhyland@cytekbio.com

Friday 8 November 2024

Friday 8 November: 8.15 am – 6.45 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: Kevin Lee and Gabriela Khoury**	Lecture Theatre 1
09:00 – 09:45	**Session 1: Oral Presentations** **Theme: Modelling Host-Pathogen Interactions** **Chairs: Stella Liong and Christophe Macri**	Lecture Theatre 1
09:00	**Using human intestinal organoids to study EPEC infection** Eva Chan, Hudson Institute of Medical Research; Early Career Researcher	
09:10	**Modelling & targeting cytokine storm in dengue-infected mice** William Clow, Walter and Eliza Hall Institute of Medical Research; PhD Student	
09:20	**Elucidating effects of single and multiple resistance mechanisms on *Pseudomonas aeruginosa* response to meropenem by mechanism-based mathematical modelling** Dominika Fuhs, Monash Institute of Pharmaceutical Sciences; PhD Student	
09:30	**Development of *ex vivo* models of nasal epithelia to elucidate the mechanism of *Bordetella bronchiseptica*-mediated blockade of influenza virus replication in the nasal cavity** Lynn Nazareth, Australian Centre for Disease Preparedness, CSIRO; Early Career Researcher	
09:45 – 10:20	**Session 2: Keynote Speaker – Dr Danika Hill** **Laboratory Head, Dept. of Immunology, Monash University** **Chairs: Josh Bourne and Dimitra Chatzileontiadou**	Lecture Theatre 1
09:50	**What can sore throats teach us about future *Streptococcus pyogenes* vaccines?** Danika Hill, Laboratory Head, Department of Immunology, School of Translational Medicine, Monash University	
10:10	**Q&A**	
10:20 – 11:00	**Morning Tea** (Includes transit time from and to Lecture Theatres)	Cossar Hall
11:00 – 11:45	**Session 3: Science Bites I** **Chairs: Aaron Brice and Stephany Sanchez**	Lecture Theatre 1
11:02	**Protecting Australia's abalone: A herpesvirus (HaHV-1) defence strategy** Jacinta Agius, Department of Microbiology, Anatomy, Physiology and Pharmacology, La Trobe University; PhD Student	
11:06	**Functional assessment of the NOD2 signalling pathway in patients with inborn errors of immunity** Ebony Blight, Department of Immunology, Monash University; PhD Student	
11:10	**Transient inhibition of type I interferon enhances CD8+ T cell stemness and vaccine protection** Benjamin Broomfield, Walter and Eliza Hall Institute of Medical Research; PhD Student	
11:14	**Age-related differences in mRNA vaccine immunogenicity and adjuvancy** Shivali Savita Chinni, School of Health and Biomedical Sciences, RMIT University; PhD Student	

11:18	**Examining the neuropathogenesis of influenza A virus and SARS-CoV-2** Asmaa Hussein, University of Melbourne & Peter Doherty Institute for Infection and Immunity; PhD Student	
11:22	**Female mice exhibit enhanced TLR7-dependent interferon and cytokine responses to respiratory syncytial virus infection** Thomas Huttmann, School of Health and Biomedical Sciences, RMIT University; Honours Student	
11:26	**Anti-inflammatory effects of L-sulforaphane against SARS-CoV-2** Leanne Quah, Murdoch Children's Research Institute; Research Assistant	
11:30	**Machine learning accelerates screening of diagnostic targets for *Neisseria gonorrhoeae*** Andrey Verich, The Kirby Institute, University of New South Wales; PhD Student	
11:34	**Applying pro-apoptotic agents to combat chronic HIV infection *in vivo*** Le Wang, Walter and Eliza Hall Institute of Medical Research; PhD Student	
11:45 – 12:30	**Session 4: Oral Presentations** **Theme: Inflammation and Innate Immunity** **Chairs: Kevin Lee and Linda Reiling**	Lecture Theatre 1
11:45	**The overlap between lipid droplets and extracellular vesicles in viral infection** Irumi Amarasinghe, La Trobe Institute for Molecular Science; PhD Student	
11:55	**Divergent roles of necroptosis in skin inflammation and wound healing** Holly Anderton, Walter and Eliza Hall Institute of Medical Research; Early Career Researcher	
12:05	**Influenza A-induced inflammation in alveolar macrophages, but not epithelial cells, requires Toll-Like Receptor 7** Ameanah El-Hennawi, Centre for Respiratory Science and Health, RMIT University; Honours Student	
12:15	**The plasma metabolome of juvenile idiopathic arthritis differs by subtype and is partially explained by chronic inflammation: a case-control study** Jooa Kwon, Murdoch Children's Research Institute; PhD Student	
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 – 14:25	**Session 5: Oral Presentations** **Theme: Immunity at the Host-Pathogen Interface** **Chairs: Natalia Sampaio and Praveena Thirunavukkarasu**	Lecture Theatre 1
13:40	**Characterization of the immune paralysis of splenic macrophages following systemic inflammation** Laura Bahr, University of Melbourne & Peter Doherty Institute for Infection and Immunity; PhD Student	
13:50	***Helicobacter pylori* Tipα is a novel nucleomodulin that is secreted on extracellular vesicles that target the nucleus** Jack Emery, Hudson Institute of Medical Research; PhD Student	
14:00	**Human unconventional T cells shape the early immune response to Group A Streptococcus** Christopher Menne, Murdoch Children's Research Institute; Early Career Researcher	
14:10	**Type 2 diabetes exacerbates fungal infection in mice, possibly by blunting the immune response** Helen Stölting, Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute, Monash University; Early Career Researcher	

14:25 – 15:00	**Session 6: Keynote Speaker – Prof Stephanie Gras** **Deputy Director, La Trobe Institute for Molecular Science** **Chairs: Dimitra Chatzileontiadou and Josh Bourne**	Lecture Theatre 1
14:30	**Immune signalling from a structural biology perspective** Stephanie Gras, Laboratory Head and Deputy Director, La Trobe Institute for Molecular Science (LIMS), La Trobe University	
14:50	**Q&A**	
15:00 – 15:40	**Afternoon Tea** – Sponsored by BMG Labtech & QIAGEN (Includes transit time from and to Lecture Theatres)	Cossar Hall
15:40 – 16:25	**Session 7: Science Bites II** **Chairs: Stephen Scally and Ghizal Sidiqui**	Lecture Theatre 1
15:42	**Human stem-cell models revealed altered infection responses in AATD** Sahel Amoozadeh, University of Melbourne & Murdoch Children's Research Institute; PhD Student	
15:46	**Disentanglement of tumour-associated exhausted (TEX) and tissue-resident memory T (TRM) cells** Thomas Burn, Department of Microbiology and Immunology, University of Melbourne; Early Career Researcher	
15:50	**Response and resistance to combination immune checkpoint blockade associate with distinct baseline and on-treatment blood T-cell profiles in melanoma patients** Jack Edwards, Department of Immunology, Monash University; PhD Student	
15:54	**HIV transcription persists in the brain of virally suppressed people with HIV** Janna Jamal Eddine, RMIT University; PhD Student	
15:58	**Deciphering and targeting of transcriptional drivers of T cell exhaustion** Sining Li, University of Melbourne & Peter Doherty Institute for Infection and Immunity; PhD Student	
16:02	**Developing precision RNA therapeutics for tuberculosis** Jan Schaefer, Walter and Eliza Hall Institute of Medical Research; PhD Student	
16:06	*WITHDRAWN*	
16:10	**Predicting antibiotic effect on *Pseudomonas aeruginosa* with mechanism-based modelling where PK/PD indices cannot** Alice Terrill, Monash Institute of Pharmaceutical Sciences; PhD Student	
16:14	**Identification of B cell epitopes in serological exposure markers for improved *Plasmodium vivax* surveillance** Hanqing Zhao, Walter and Eliza Hall Institute of Medical Research; PhD Student	
16:25 – 17:10	**Session 8: Oral Presentations** **Theme: Adaptive Immunity and Therapeutics** **Chairs: Paul Gill and Meg Manohar**	Lecture Theatre 1
16:25	**Leveraging the position of lymph node memory CD8+ T cells to enhance protective immunity** Brigette Duckworth, Walter and Eliza Hall Institute of Medical Research & University of Melbourne; Early Career Researcher	
16:35	**Reaction hijacking inhibition of tRNA charging enzymes of malaria parasites and bacteria** Nutpakal Ketprasit, Bio21 Institute, Department of Biochemistry and Pharmacology, University of Melbourne; PhD Student	

16:45	**The Medicines for Malaria Venture Pathogen Box compound MMV687794 impairs blood-stage *Plasmodium falciparum* invasion through potential inhibition of parasite lipid metabolism** Dawson Ling, Walter and Eliza Hall Institute of Medical Research; Early Career Researcher	
16:55	**The characterisation of human Vδ3+ γδT cells and the development of bispecific antibodies to harness their function** Tina Zhang, University of Melbourne & Peter Doherty Institute for Infection and Immunity; PhD Student	
17:10 – 17:15	**Thanks and Announcements** **Chairs: Paul Gill and Meg Manohar**	Lecture Theatre 1
17:15 – 18:25	**Evening Networking and Poster Session II** – Sponsored by QIAGEN & BMG Labtech (Includes transit time from Lecture Theatres) **See below for more information**	Cossar Hall
18:25 – 18:45	**Prizes, Acknowledgements and Conclusion** – Including Hartland Oration Prize winner for 2024 **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 13:25**	Cossar Hall
Poster Number	**Poster Details**	
1	**Precise CRISPR insertion for deciphering immune interactions** Tim Muusse, Manufacturing Research Unit, CSIRO; Early Career Researcher	
2	**Understanding the molecular mechanism of recognition of *Bacteroides fragilis* produced glycosphingolipids by Natural Killer T (NKT) cell receptors** Vasudha Maddali, Department of Biochemistry and Molecular Biology, Monash University; PhD Student	
3	**Crohn's associated invariant T cells recognise small molecules on CD1d** Alison White, Peter Doherty Institute for Infection and Immunity & University of Melbourne; Honours Student	
4	**Exploring trogocytosis between DC and B cells** Laura Almagro, Department of Biochemistry and Pharmacology, University of Melbourne; PhD Student	
5	**Microfluidic solution for evaluating exhausted T cells' response toward PD-1 blockade efficacy** Wei-Che Chang, Integrated Photonics and Applications Centre (InPAC), RMIT University; PhD Student	
6	**Discovering targets of long-lived humoral immunity for Group A Streptococcus vaccine design** Holly Fryer, Department of Immunology, Monash University; PhD Student	
7	**Using rabies virus as a tool to understand the mechanisms of synapse formation in the brain** Steph Olliff, Deakin University & Australian Centre for Disease Preparedness, CSIRO; PhD Student	
8	**Characterisation of novel cytokine interferon epsilon in the murine peritoneal cavity** Jasmine Chuah, Hudson Institute of Medical Research; PhD Student	
9	**Prenatal and early life viral infection synergistically modify expression of cholinergic, dopaminergic and complement C4 genes in the hippocampus and prefrontal cortex in mice** Paneet Dhaliwal, Centre for Respiratory Science and Health, RMIT University; Honours Student	
10	**Identification of metabolic candidates contributing to the comorbidity of diabetes, cardiopulmonary, and cardiovascular diseases** Zeki Ilker Kanbagli, Monash Institute of Pharmaceutical Sciences; Early Career Researcher	
11	**Remodelling of the plasma proteome by sex hormones in a longitudinal model of feminizing gender-affirming hormone therapy** Ngoc Lan Nhi Nguyen, Murdoch Children's Research Institute; Masters Student	
12	**Temporal and cross-serotype analysis of dengue T cell targets to inform vaccine design** Jingjing Liu, Department of Electrical and Electronic Engineering, University of Melbourne; PhD Student	

13	**Immunogenic HLA-B*44:03 restricted peptide does not induce the same response in individuals with B*44 superfamily molecules** Samuel Liwei Leong, La Trobe Institute for Molecular Science; PhD Student
14	**COVID-19 results in broad autoantigen recognition post-infection, with anti-calprotectin autoantibodies associated with better clinical outcomes** Rhiane Moody, School of Health and Biomedical Sciences, RMIT University; Early Career Researcher
15	**Increased spike-specific IgG4 following has variable consequences on FcγR-mediated responses** Carissa Aurelia, Peter Doherty Institute for Infection and Immunity & University of Melbourne; PhD Student
16	**P-cresol sulfate acts on epithelial cells to reduce allergic airway inflammation** Rhiannon Grant, Department of Immunology, Monash University; PhD Student
17	**Association of *Plasmodium falciparum* specific afucosylated IgG with placental malaria protection** HongHua Ding, Peter Doherty Institute for Infection and Immunity; PhD Student
18	**Fc-dependent functional antibody responses in immunity to severe *Plasmodium falciparum* malaria in children** Grace Wright, Burnet Institute; Masters Student
19	**Naturally acquired functional antibody responses to *Plasmodium vivax* vaccine candidates are associated with protection against clinical malaria infections** Pailene Lim, Walter and Eliza Hall Institute of Medical Research; Research Assistant
20	**Developing machine learning models to understand CRISPR-Cas13b silencing principles** Khoa Nguyen, Department of Electrical and Electronic Engineering, University of Melbourne; PhD Student
21	**High throughput antimicrobial screening at CSIRO** Srinivasan Jayashree, Biomedical Program, Manufacturing Research Unit, CSIRO; Research Assistant
22	**Predictive and generative AI for drug discovery: Identification of SARS-CoV2 NSP14 inhibitors** Thomas Coudrat, Manufacturing Research Unit, CSIRO; Mid-Career Researcher
23	**IL-6 as diagnostic and prognostic biomarker meta-analysis studies** Hina Amer, School of Health and Biomedical Sciences, RMIT University; PhD Student
24	**Parasite-host metabolic cross-talk to detect malaria** Teha Gebi, Monash Institute of Pharmaceutical Sciences; PhD Student
25	**How to catch a parasite red-handed: Looking for *Plasmodium falciparum* exported proteins in the infected hepatocyte using proximity ligation** Elena Lantero-Escolar, Walter and Eliza Hall Institute of Medical Research; Early Career Researcher
26	**Defining targets and mechanisms of action of immunity against *Plasmodium vivax* circumsporozoite protein** Rosy Cinzah, Burnet Institute & Department of Medicine, University of Melbourne; PhD Student

27	**A comprehensive analysis of the regulatory mechanisms underlying the enzymes of the TCA cycle-glyoxylate shunt junction in *Mycobacterium tuberculosis* as a novel drug target for the age-old pandemic** Evelyn Huang, School of Chemistry, University of Melbourne; PhD Student
28	**Defining the on-target activity of *P. falciparum* plasmepsin V peptidomimetic inhibitors** Wenyin Su, Walter and Eliza Hall Institute of Medical Research; PhD Student
29	**Proteomics-based drug target identification in *Plasmodium falciparum*** Yijia Ji, Monash Institute of Pharmaceutical Sciences; Honours Student
30	**Interrogating circulating immune cell methylome differences across the TB disease spectrum** David Vincent Romero, Walter and Eliza Hall Institute of Medical Research; PhD Student
31	**Inhibition of type I interferon signalling during *Shigella flexneri* infection** Anita Chaulagain, Monash University & Hudson Institute of Medical Research; PhD Student
32	**Metabolic mysteries of bat urine and faeces: A potential non-invasive tool to monitor flying foxes under different ecological conditions** Avirup Sanyal, Griffith University & Australian Centre for Disease Preparedness, CSIRO; PhD Student
33	**Rifaximin and the evolution of daptomycin-resistant *Enterococcus faecium*** Adrianna Turner, Department of Microbiology and Immunology, University of Melbourne; Early Career Researcher
34	**Establishing novel therapeutics for HTLV-1** Lewis Williams, Walter and Eliza Hall Institute of Medical Research; Early Career Researcher
13:25	**Judging to be finalised**
13:40	**[Return to main program above]**

Poster Session II: 5.15 pm – 6.25 pm

17:15 – 18:25	**Evening Networking and Poster Session II – Sponsored by QIAGEN & BMG Labtech** (Includes transit time from Lecture Theatres) **Poster judging to be finalised by 18:10**	Cossar Hall
Poster Number	**Poster Details**	
35	**Decoding the effector-mediated dialogue between *Coxiella burnetii* and its host during infection** Genevieve Samuel, Department of Microbiology, Monash Biomedicine Discovery Institute, Monash University; PhD Student	
36	**Rational design of live bacterial therapeutics to clear *Klebsiella pneumoniae* from the gut** Sher Maine Tan, Department of Microbiology and Immunology, University of Melbourne; PhD Student	
37	**Exploring bacteriocins in infection-causing *Klebsiella* isolates** Abhinaba Ray, Monash Biomedicine Discovery Institute, Monash University; PhD Student	
38	**Identification and characterisation of *Cryptosporidium* effector proteins in host-pathogen interaction** Lena Chng, Walter and Eliza Hall Institute of Medical Research; PhD Student	
39	***Legionella pneumophila* Dot/Icm effector triggers host heat shock response to facilitate intracellular replication** Rachelia Wibawa, Hudson Institute of Medical Research; Early Career Researcher	
40	**Ecology and diversity of Avian paramyxovirus 1, the causative agent of Newcastle disease, in Australian wild birds** Sebastian Carmody, Australian Centre for Disease Preparedness, CSIRO & Peter Doherty Institute for Infection and Immunity, University of Melbourne; Honours Student	
41	**Characterization of the structure and dynamics of oral polymicrobial biofilms** Bindusmita Paul, Department of Biochemistry and Pharmacology, University of Melbourne; PhD Student	
42	**Aztreonam and ciprofloxacin combination therapy yields synergistic results for resistant *Pseudomonas aeruginosa* strains** Charlotte Picton, Monash Institute of Pharmaceutical Sciences; Honours Student	
43	**The molecular mechanisms of axon degeneration in flavivirus infection** Heather Irving, Australian Centre for Disease Preparedness, CSIRO; Masters Student	
44	**Systemic inflammation in solid tumour malignancy patients impairs generation of *de novo* SARS-CoV-2 vaccine responses** Ruth Purcell, Peter Doherty Institute for Infection and Immunity & University of Melbourne; PhD Student	
45	**Unravelling the impact of changing ionizable lipids on mRNA-LNP vaccine pharmacokinetics and biodistribution** Yuxiang Ren, Monash Institute of Pharmaceutical Sciences; PhD Student	
46	**Developing novel lipid nanoparticles to reprogram lung macrophages** Joshua Iscaro, Centre for Respiratory Science and Health, RMIT University; PhD Student	

47	**Using big data for rational vaccine design to elicit broadly neutralizing antibodies against Hepatitis C virus** Haiyi Ye, Department of Electrical and Electronic Engineering, University of Melbourne; PhD Student
48	**Serological and molecular analyses define the antigenic evolution of the influenza B virus neuraminidase over 81 years** Thi Hoai Thu Do, Peter Doherty Institute for Infection and Immunity & University of Melbourne; PhD Student
49	**Nanospike surfaces: A new frontier in viral infection control** Samson Mah, School of Health and Biomedical Sciences, RMIT University & Manufacturing Research Unit, CSIRO; PhD Student
50	**iNKT cells develop through a 4-stage pathway in human thymus** Naeimeh Tavakolinia, Department of Microbiology and Immunology, University of Melbourne; PhD Student
51	**The influence of repeated influenza exposure on the CD8+ T cell response** Cristina Triffon, Burnet Institute; Early Career Researcher
52	**Age-related changes in T cell early activation events** Anna Iasinskaia, School of Health and Biomedical Sciences, RMIT University; PhD Student
53	**2′-O-Methyl-guanosine 3-base RNA fragments mediate essential natural TLR7/8 antagonism** Sunil Sapkota, Hudson Institute of Medical Research; Early Career Researcher
54	**Mechanisms of mRNA vaccine adjuvancy in aged human dendritic cells** Valeeshah Rashid, School of Health and Biomedical Sciences, RMIT University; Honours Student
55	**Lipidation of Kv1.3 blocking peptide HsTX1[R14A] alters its pharmacokinetics and biodistribution to target tissues** Lihuan Lin, Monash Institute of Pharmaceutical Sciences; PhD Student
56	**Assessing immune competence to SARS-CoV-2 vaccination in patients with inflammatory bowel disease receiving anti-TNF treatment** Lachlan Bradbury, Department of Immunology, Monash University; Honours Student
57	**Evaluating immune response against SARS-CoV-2 in immunocompromised children** Leanne Quah, Murdoch Children's Research Institute; Research Assistant
58	**Uncovering the design principles of CRISPR/Cas13d as an effective antiviral strategy** Emily Hann, CSIRO & Deakin University; PhD Student
59	**Metabolic tracing in *P. falciparum* using a stable isotope labelling strategy** Junwei Tang, Monash Institute of Pharmaceutical Sciences; PhD Student
60	**Dual plasmepsin IX and X inhibitors are refractory to resistance** Paola Favuzza, Walter and Eliza Hall Institute of Medical Research; Early Career Researcher
61	**Association of novel IgG3 allele with malaria infections in children from Sepik region of Papua New Guinea** Maria Saeed, Peter Doherty Institute for Infection and Immunity & University of Melbourne; PhD Student
62	**Antibody responses in children given the RTS,S malaria vaccine with and without drug chemoprevention** Alexander Harris, School of Translational Medicine, Monash University; PhD Student

63	**Investigating antibodies against cerebral malaria in children** Yuchi Ji, Peter Doherty Institute for Infection and Immunity; Masters Student	
64	**Dissecting germinal centre B cells induced by infection and vaccination during malaria** Jessica Canning, Burnet Institute & Department of Immunology, Monash University; PhD Student	
65	***Plasmodium falciparum*-infected erythrocytes inhibit neutrophil extracellular trap formation** Akachukwu Onwuka, Department of Infectious Diseases, University of Melbourne; PhD Student	
66	**Blood biomarker discovery: High-dimensional blood immune-profiling in children with different disease settings showed major age-related changes in proportion of immune cells** Sedi Jalali, Murdoch Children's Research Institute, Early Career Researcher	
67	*WITHDRAWN*	
18:10	**Judging to be finalised**	
18:25 – 18:45	**Prizes, Acknowledgements and Conclusion** **Presented by VIIN Co-Convenors:** **Prof Gilda Tachedjian and Prof Richard Ferrero**	Cossar Hall

ABSTRACTS
SESSION 1

Using human intestinal organoids to study EPEC infection.

Eva Chan[1, 2*], Cristina Giogha[1, 2], Garrett Ng[1, 2], Ruo Wang[1, 2], Paul Hertzog[1, 2] and Elizabeth Hartland[1, 2]

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[2]Department of Molecular and Translational Science, Monash University, Clayton, Victoria, Australia.

The gastrointestinal tract is lined by a single layer of epithelial cells that forms a barrier to the external environment. This barrier is exposed to several environmental stressors, and on occasion, encounter gastrointestinal pathogens, such as enteropathogenic *Escherichia coli* (EPEC). This bacterium can disrupt the epithelial lining leading to symptoms such as diarrhoea, fever, vomiting, and in extreme cases death. This is possible due to the bacterial effector proteins that are injected into the host cell, resulting in disruptions to host cell cytoskeleton and cell signalling. Such findings have been possible due to the use of immortalised cell lines in the laboratory, however, immortalised cell lines are not representative of the cellular heterogeneity exhibited in the human gastrointestinal tract.

AIM: To establish an organoid co-culture model to allow efficient infection of human intestinal epithelial cells with EPEC, to study epithelial response.

METHODS: Human intestinal organoid cultures grown as 2-dimensional cultures (monolayers) have been established to allow access to the apical cell surface by EPEC for infection. Immunofluorescent staining and imaging, Western blotting, and cytometric bead array, were performed following infection.

RESULTS: Immunofluorescent staining and imaging confirmed infection, through the visualisation of EPEC attaching to the epithelial surface, leading to reorganisation of host actin. The degradation of host proteins by EPEC effector proteins was detected by Western blotting and measurement of cytokines secreted by organoid derived monolayers, through cytometric bead array, further confirmed epithelial response to infection.

CONCLUSION: By establishing a bacteria-organoid co-culture system with human intestinal organoid derived monolayers, this will facilitate the study of host cell response and epithelial cell function following infection.

Modelling & Targeting Cytokine Storm in Dengue-infected Mice

William Clow[1*], Merle Dayton, Liana Mackiewicz, Lewis Williams, Reet Bhandari, Marc Pellegrini[2] and Marcel Doerflinger[1]

[1]Walter & Eliza Hall Institute of Medical Research, [2]Centenary Institute of Cancer Medicine & Cell Biology

* = presenting author

Dengue is the most common human mosquito-borne viral infection, and the causative agent of many epidemics annually across the globe. A proportion of infections present with debilitating symptoms of systemic inflammation and vascular leakage, which can prove deadly unless closely managed in hospital. Severe disease is characterized by a cytokine storm: an abnormal host immune response in which overabundant immune signaling contributes to host pathology. Treatment is limited to fluid replacement therapy to replace lost blood volume, and Dengue vaccines have proven problematic, leading to lowered public uptake. As this disease continues to expand across the globe, novel therapeutic options are urgently needed.

To address this, I've optimized a Dengue mouse model to recapitulate the inflammatory symptoms seen in humans, where viral replication occurs alongside symptom onset and release of serum cytokines. Type I interferon (IFN)-deficient mice infected with Dengue virus type 2 (DENV2) develop peak viremia at 2 days post-infection, with growing weight loss and immune activity up until 4-5 days post-infection, at which point viral load becomes low or undetectable and mice recover by 9 days post-infection. 17/26 cytokines tested were upregulated in infected mouse plasma, including key inflammatory agents observed in human Dengue such as IL-6, TNF and IL-1β.

Interestingly, Dengue shares many risk factors, immune signatures and inflammatory symptoms with COVID-19. During the COVID-19 pandemic, many clinical trials were performed to identify effective treatments against COVID-19-mediated cytokine storm. I'm screening some of these strategies in my Dengue mouse model to identify potential host targets to mitigate immunopathology during Dengue and severe Dengue. TNF-depleting monoclonal antibody significantly reduces Dengue-induced weight loss independent of viral load. In comparison, the glucocorticoid Dexamethasone inhibits weight loss to a lesser degree, at the cost of inhibited viral clearance. Neutralization of IL6R, IL1β or GMCSF had no effect on weight loss. I'm also trialing the JAK inhibitor Baricitinib for the first time in Dengue-infected mice. This work will provide crucial insight to developing therapeutic strategies against moderate/severe Dengue. A strategy developed in this pipeline may also have great potential in treating the significantly deadlier Dengue/COVID-19 coinfection.

Elucidating effects of single and multiple resistance mechanisms on *Pseudomonas aeruginosa* response to meropenem by mechanism-based mathematical modelling

Dominika T. Fuhs[1*], Sara Cortés-Lara[2], Jessica R. Tait[1], Kate E. Rogers[1], Carla López-Causapé[2], Wee Leng Lee[1], Roger L. Nation[1], Antonio Oliver[2], Cornelia B. Landersdorfer[1]

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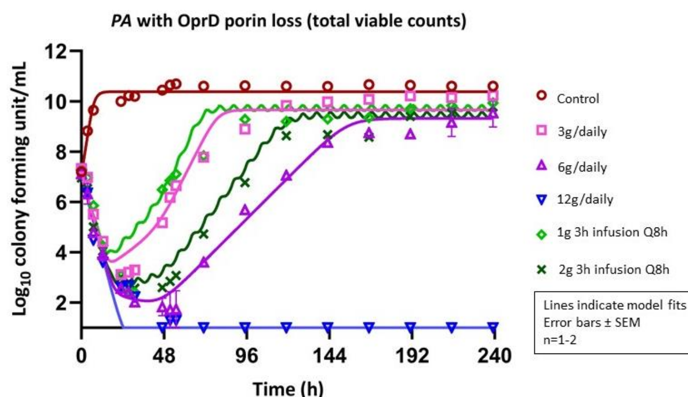
Introduction: Meropenem (MEM) is used against *Pseudomonas aeruginosa* (*PA*) infections, but resistance mechanisms reduce its effectiveness. Mechanism-based mathematical models (MBMs) address limitations of PK/PD indices, such as the time free antibiotic concentration exceeds the pathogen's minimum inhibitory concentration ($fT_{>MIC}$).

Aims: Characterise the effects of different baseline resistance mechanisms on bacterial killing and resistance emergence; evaluate whether $fT_{>MIC}$ can predict these effects; develop a novel MBM.

Methods: We conducted 10-day hollow-fibre infection model (HFIM) studies using seven isogenic *PA* strains with OprD porin channel loss, MexAB-OprM efflux pump over-expression, AmpC β-lactamase over-expression, and the combinations thereof. The HFIM simulated MEM PK for critically-ill patients with normal renal function ($t_{1/2,MEM}$=1.5h). All viable counts on drug-free, 3×MIC and 5×MIC MEM-containing agar across all strains, five clinically relevant regimens and control (n=90 profiles) were modelled simultaneously.

Results: $fT_{>MIC}$ could not explain the differences in bacterial response between strains. For example, regimens achieving ≥98% $fT_{>1xMIC}$ suppressed regrowth and resistance of one strain, while even 100% $fT_{>5xMIC}$ failed to achieve this against two other strains, despite all three of these having the same MIC. In contrast, the MBM well characterised all bacterial outcomes of all seven strains with the same model structure and without estimating strain-specific drug effect parameters (observed *vs* individual-fitted r^2=0.98, observed vs population-fitted r^2=0.96, Fig. shows one strain).



Discussion: The resistance mechanisms were described by their effects on the estimated MEM concentration in the periplasmic space, which led to different bacterial outcomes, even for strains with the same MIC. The developed MBM is the first model to directly translate all major mechanisms of MEM resistance in *PA* and their complex interplay. This model represents a first necessary step towards personalised therapy adapted to the individual and the infecting pathogen.

Development of *ex vivo* models of nasal epithelia to elucidate the mechanism of *Bordetella bronchispetica*-mediated blockade of influenza virus replication in the nasal cavity.

Lynn Nazareth[1*], Megha Manohar[1], Matthew Neave[1], Duane Walter[1] , Jasmina M Luczo[1]

[1]*CSIRO Australian Centre for Disease Preparedness*

* = presenting author

Pathogen interference describes the capacity of a colonising pathogen to interfere with the ability of an incoming pathogen to establish infection. *Bordetella bronchiseptica* is a gram-negative bacterium that has been shown to block influenza virus infection in the murine nasal cavity, although, the mechanism for this blockade is unknown[1]. Here, we established *ex vivo* models of the nasal epithelia to examine host-pathogen interactions and elucidate the mechanism of influenza blockade.

The nasal epithelia is composed of two distinct populations - the respiratory and olfactory epithelium. Nasal respiratory and olfactory epithelia were harvested from C57BL/6 mice, each tissue type was expanded before differentiating at air-liquid-interface (ALI). At day 28 post-ALI, trans-epithelial electrical resistance demonstrated robust respiratory and olfactory epithelial barrier integrity. Histological analysis verified the cellular architecture of the nasal respiratory and olfactory ex vivo models and the presence of key epithelial cell populations was confirmed by confocal microscopy.

To elucidate the mechanism of *B. bronchiseptica*-mediated blockade of influenza infection, nasal respiratory and olfactory epithelial ALI cultures were inoculated with *B. bronchiseptica* for 30 hours prior to influenza infection. At 24 hours post-influenza infection, bacterial and virus titres were enumerated, RNA extracted for RNAseq analysis and monolayers fixed for to examine cellular architecture by histology and confocal microscopy. Using these *ex vivo* models, we demonstrate that *B. bronchiseptica* colonisation blocks influenza replication in nasal respiratory and olfactory epithelial cells, recapitulating previously described *in vivo* results[1]. Confocal microscopy and histological analysis via Alcain Blue-PAS staining revealed an upregulation of mucins, a known inhibitor of influenza infection and a possible mechanism of influenza A virus blockade. RNAseq analysis of *B. bronchiseptica*-colonised and influenza infected nasal respiratory and olfactory cells is underway to provide further insights of influenza blockade in the nasal cavity.

Collectively, we have developed ex vivo models of the upper respiratory system to dissect host pathogen interactions and elucidate mechanism of *B. bronchispetica*-mediated blockade of influenza virus. Understanding the mechanism of influenza blockade has major implications for controlling influenza virus transmission events and can be used to develop countermeasures against respiratory viruses with pandemic potential.

[1] Luczo et al. 2023 PMID: 36728413

ABSTRACTS
SESSION 3

Protecting Australia's abalone: A herpesvirus (HaHV-1) defence strategy

Jacinta Agius[1*], Danielle Ackerly[1], Monique Smith[2], Travis Beddoe[1] and Karla Helbig[1]

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* = presenting author

HaHV-1 is a re emerging viral pathogen characterised by mass mortality events in wild and farmed Australian abalone, with no current treatment options. This project sought to examine whether immune priming could protect abalone from lethal HaHV-1 exposure and if this protection is transgenerational.
Naive abalone of alternate ages and species were exposed to HaHV-1 via immersion in infectious water. Real-time PCR was utilised to detect both HaHV-1 in nerves and track immune response genes in haemocytes. Immune priming was performed via injection of abalone with non-specific synthetic nucleic acid or protein, prior to HaHV 1 infection or spawning.

Following optimisation of immune primers and their delivery methods, we established that primers directly administered to abalone at least 5 days prior to infection, provided complete protection against lethal HaHV-1 challenge. Protection lasted at least 121 days and was associated with the transcriptional upregulation of antiviral interferon stimulated genes in abalone haemolymph. In collaboration with 3 Victorian abalone farms and to explore if this protection was transgenerational, female abalone (greenlip and blacklip) were primed between 3 days and 8 weeks prior to spawning to produce blacklip, greenlip and hybrid progeny. The progeny was challenged with HaHV-1 at both 6 and 12-months of age and although it was clear that transgenerational immune priming was not protective, juvenile abalone (<1yo) were found to be significantly less susceptible to HaHV-1 infection when compared to older abalone. This contrasts from what is known in respect to oyster herpesvirus (OsHV-1) infection and our work has therefore added crucial knowledge into the currently poorly understood HaHV-1 field.

This work provides the first insight into a protection strategy against HaHV-1 in collaboration with the Australian abalone industry and informs our current research involving the development of a feed and immersion immune priming approach against HaHV-1 in large-scale aquacultural settings.

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

Ebony G. Blight*[1,2], Samar Ojaimi[2,3], Julian J. Bosco[2,4], Pei M. Aui[1,2], Robyn E. O'Hehir[1,2,4], Emily S.J. Edwards[1,2], Menno C. van Zelm[1,2,4,5]

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* = presenting author

Background: Inborn errors of immunity (IEIs) are rare immunological disorders characterised by susceptibility to infection and immune dysregulation. Despite advances in genomics, many IEI patients remain genetically undiagnosed, limiting access to targeted therapeutics. In predominantly antibody deficiency (PAD), 70% of affected genes function in one of 5 critical immune signalling pathways. One pathway is downstream of nucleotide-binding oligomerization domain-2 (NOD2), a receptor for muramyl dipeptide (MDP). We here developed functional assays to evaluate the pathway, enabling screening of genetically undiagnosed patients for abnormal signalling responses. This will be used to help interpret identified variants of unknown significance (VUSes) in the pathway, or help guide analysis of exome data for identification of novel candidate variants.

Methods: Immune cells of healthy controls, a patient with a hemizygous XIAP mutation and a patient with a heterozygous XIAP VUS were evaluated by flow cytometry for L18-MDP-induced (NOD2-dependent) production of intracellular TNF-α, and phosphorylation of p38 (p-p38) and p65 (p-p65). LPS-stimulated (NOD2-independent control) and unstimulated (negative control) samples were run concurrently.

Results: In healthy donors (n=7), L18-MDP induced TNF-α production in 50.0% (range 34.0-72.7%) of monocytes and induced a fold-change of 4.92 (range 4.06-7.62) and 3.84 (range 2.67-10.29) for median fluorescent intensity (MFI) of p-p38 and p-p65 when compared to unstimulated cells. The patient with a hemizygous XIAP mutation had complete absence of NOD2-dependent TNF-α production and phosphorylation of p38 and p65, whereas the patient with a heterozygous XIAP VUS had 7.32% of monocytes producing TNF-α, but p-p38 and p-p65 MFI change within range (4.27 and 2.5, respectively).

Conclusion: Here we show assessment of NOD2-dependent TNF-α production is able to identify patients with complete loss-of-function phenotypes, and show potential of assessing fold-change in MFI of p-p38 and p-p65 to identify pathway defects. In future, we will assess patients lacking a genetic diagnosis, alongside more healthy donors to increase sample size and help guide interpretation of variant impact. This *ex vivo* functional evaluation of immune pathways, such as NOD2, could provide rapid insights into pathogenicity of variants of unknown significance, and give insight into mechanisms of disease, thereby expediting genetic diagnosis and treatment in PAD patients.

Transient inhibition of type I interferon enhances CD8$^+$ T cell stemness and vaccine protection

Benjamin J. Broomfield[1,2,*], Chin Wee Tan[1,2,3], Raymond Z. Qin[1,2], Brigette C. Duckworth[1,2], Carolina Alvarado[1], Lennard Dalit[1,2], Jinjin Chen[1,2], Liana Mackiewicz[1], Hiromi Muramatsu[4], Marc Pellegrini[5], Kelly L. Rogers[1,2], Woohyun J. Moon[6], Stephen L. Nutt[1,2], Melissa Davis[3,7,8], Norbert Pardi[4], Verena C. Wimmer[1,2], and Joanna R. Groom[1,2]

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The protective capacity of TCF-1$^+$ stem cell-like memory CD8$^+$ T (T_{SCM}) cells lie in their long-term persistence, potent proliferative capacity and ability to generate effector T cells upon rechallenge. Thus, T_{SCM} cells are key cellular targets for vaccination and immunotherapy. Yet how this cell population can be therapeutically promoted is unclear. Here, we report that T_{SCM} cell generation is directed with early and transient inhibition of the type I interferon (IFN) receptor (IFNAR) during viral infection and vaccination. Using multiparameter flow cytometry and scRNA-seq, we identified T_{SCM} cells generated with early IFNAR blocking were distinct from exhausted T cell subsets and remained increased following viral clearance. Three-dimensional light sheet imaging of intact lymph nodes revealed elevated T_{SCM} cell differentiation correlated with T cell retention within the lymph node paracortex. Inhibition of IFNAR signaling drove a compensatory increase in IFNγ production, which increased CXCR3 chemokine abundance and disrupted gradient formation. In the absence of both IFNAR and IFNγ, chemokine expression was lost, which again ablated gradient formation to promote paracortex location and T_{SCM} cell fate. Applying these findings, we combined transient, early IFNAR inhibition with mRNA-LNP vaccination to specifically generate T_{SCM} cells and confer enhanced prophylactic protection against chronic infection. Our findings support the design of vaccine and adjuvant approaches that elicit T_{SCM} cells for protection against infectious pathogens or for therapeutic cancer vaccination.

Age-Related Differences in mRNA Vaccine Immunogenicity and Adjuvancy

Shivali Savita Chinni[1*], Patrick Leung[1], Gemma S Trollope[1], Jonathan L McQualter[1], Leonard Lim [2], Harry Al-Wassiti [2], Colin Pouton [2], Kylie M Quinn[1,3]

[1] School of Health and Biomedical Sciences, Royal Melbourne Institute of Technology (RMIT) University, Bundoora, VIC, Australia,

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[3] Department of Biochemistry, Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia.

Older individuals are less likely to mount robust adaptive immune responses to mRNA vaccines, leaving them more vulnerable to a range of infections. To design better vaccines for older people, we first need to understand how current formulations perform in an aged setting. We therefore used a mouse model to examine age-related differences in mRNA vaccine immunogenicity and adjuvancy.

To assess adaptive immune responses, we vaccinated young (<5 months-old) and aged (19 months-old) C57BL/6 mice with an mRNA vaccine encoding SARS-CoV-2 Spike at day 0 and 28 and assessed antigen-specific adaptive responses at peak (day 42) and memory (day 91) timepoints. CD8 and CD4 T cell responses were significantly reduced in aged mice at both timepoints, and antibody titers were also markedly reduced in aged mice, consistent with differences seen in older humans.

To define age-related differences in adjuvancy mechanisms, we vaccinated young and aged mice with mRNA vaccines loaded either with DiD to track vaccine uptake or with mScarlet mRNA to track antigen expression. Draining lymph nodes (dLNs) and sera were harvested after 5, 16 and 48 hours, dendritic cells (DCs) were magnetically enriched and profiled by flow cytometry. DC numbers were dramatically reduced by at least 10-fold in aged mice before and after vaccination. Rates of vaccine uptake in young and aged DCs were comparable (88% *vs* 87.7%) but the frequency of antigen-expressing DCs increased with age (12.33% *vs* 18.3%). Ageing accelerated expression of inflammatory cytokines (IL-1, IL-6) in sera and/or dLNs.

Collectively, this suggests that ageing results in a loss of synchronisation between the innate response and antigen presentation by DCs, which could undermine adaptive immunity. Strategies that circumvent these age-related deficits could improve vaccine outcomes for older people.

Examining the neuropathogenesis of influenza A virus and SARS-CoV-2

Asmaa Hussein[1*], Julie McAuley[1] and Victoria A Lawson[1]

[1]Department of Microbiology and Immunology, The Peter Doherty Institute of Infection and Immunity, Melbourne, Australia

* = presenting author

PUBLISH CONSENT WITHHELD

Female mice exhibit enhanced TLR7-dependent interferon and cytokine responses to respiratory syncytial virus infection

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Respiratory syncytial virus (RSV) is a common respiratory pathogen responsible for global infections. In healthy individuals, RSV typically causes mild, cold-like symptoms. However, young children and the elderly are at higher risk of developing severe lower respiratory tract infections, such as bronchiolitis, which may lead to hospitalization. Notably, there is a sex difference in the severity of RSV infection in early life, with males more frequently experiencing severe respiratory disease compared to females. One factor that might contribute to this difference is the higher expression of X-linked toll-like receptor 7 (TLR7) in female immune cells. TLR7 detects viral RNA and triggers the transcription of antiviral and proinflammatory genes, helping to suppress viral replication and establish immunity.

Although TLR7-dependent sex differences have been observed in other respiratory viral infections, its role in RSV infection remains underexplored. To address this, we compared the inflammatory response to acute RSV infection in male and female mice, as well as in alveolar macrophages (AM) and bone-marrow derived macrophages (BMDMs) from both sexes. 10–14-week-old C57Bl/6 wild-type (WT) and TLR7 knockout (TLR7 KO) mice of both sexes were infected with RSV (A Long strain), and airway inflammation, lung pathology, and inflammatory cytokine expression were assessed after 7 days.

Our findings showed that RSV infection induced similar levels of lung histopathology in both sexes, but female mice had a greater presence of macrophages in their airways according to differential cell staining. Gene expression analysis revealed higher upregulation of type I interferon (IFN), IL-1β, and IL-6 in the lungs of infected female mice compared to males. Viral transcripts were also higher in female lungs. In contrast, these cytokine responses were dampened in TLR7 KO mice, regardless of sex, indicating that TLR7 plays a pivotal role in the inflammatory process during infection. Furthermore, TLR7 mRNA expression was higher in the lung tissue. Ex vivo RSV infection of BMDMs resulted in a stronger cytokine response in male cells compared to female cells. These results emphasize a TLR7-dependent sex difference in RSV infection in mice, suggesting that female mice may exhibit a more robust inflammatory response.

Anti-Inflammatory Effects of L-Sulforaphane against SARS-CoV-2

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PUBLISH CONSENT WITHHELD

Machine Learning Accelerates Screening Of Diagnostic Targets for Neisseria gonorrhoeae

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Background: *Neisseria gonorrhoeae* is a high-priority global health burden. It's the second most common sexually transmitted bacterial infection with emerging resistance to last-line treatments. *N.gonorrhoeae* infections are typically asymptomatic and these persistent and/or resistant infections are a key contributor to infertility – an innovative solution is needed (1).

Aims: Determine potential resistance-associated gene variants as part of a pilot project aiming to improve molecular target selection by combining novel, CSIRO-developed machine learning and epistatic interaction analysis programs (2). This analysis aims to facilitate faster molecular target *in-silico* pre-screening for molecular tests to rapidly diagnose *Neisseria gonorrhoeae* while recommending treatments; ultimately, aiming to improve health outcomes.

Methods: Downloaded publicly available *N.gonorrhoeae* whole-genome sequencing, paired with ciprofloxacin resistance data. Trimmed 3,297 fastq files with trimmomatic, then generated variant call files using bwa mem. Analysed variant files with machine learning by VariantSpark, utilising random forest algorithms generating 1000 trees to investigate resistance-associated gene variants. The 250 most important variants were analysed using BitEpi for epistatic interactions contributing to resistance (2).

Results: Variantspark algorithms identified known ciprofloxacin resistance-related variants in *GyrA* and *ParC*. tRNA synthesis pathway genes, primarily *IleS*, *Tgt*, and *MiaB*, had resistance-associated variants. BitEpi analysis determined variants of the known genes required no interaction, while tRNA genes participated in extensive (up to 4-level) epistatic interactions to contribute to resistance (3).

Conclusions: VariantSpark and BitEpi highlighting known resistance genes serves as an important validation of the analysis. Thus, this analysis will be further adapted to other bacterial datasets and other antibiotic related associations. Notably, to our best understanding, no studies implicate tRNA synthesis gene variants in ciprofloxacin resistance in *N.gonorrhoeae* (3). Therefore, these variants and their network can be further investigated as panel for testing for novel molecular diagnostics tests aiming to diagnose *N.gonorrhoeae* while recommending antibiotic treatments.

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Applying Pro-apoptotic Agents to Combat Chronic HIV Infection *in Vivo*

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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. Patients will suffer from life-long side-effects and chronic inflammation. A functional cure for chronic HIV infection is urgently needed. Previous *ex vivo* research has shown resistance to cell death as a feature of the HIV reservoir, which serves as a therapeutic target.

To study this hypothesis *in vivo*, we established a humanized immune system mouse model (HIS mice) by transplanting human hemopoietic stem cells into 48 hours-old immunodeficient (NSG) mice. After 16 weeks of reconstitution, we infected these mice with HIV and suppressed their viremia with ART. We then test single/ combination treatment with SMAC Mimetics and BH3 mimetics. During the experiment, human CD4 T cell and HIV viremia was being monitored by flow cytometry and qPCR. We evaluated our treatment's efficacy by analytical treatment interruption (ATI), in which we interrupt all treatment including ART and compared the time to HIV rebound between groups. We also characterized the tissue HIV reservoir by Intact Proviral DNA Assay (IPDA) and Multiplexed RNA Assay through digital PCR.

We witnessed a delay in HIV rebound after 4 weeks of SMAC Mimetic treatment compared to vehicle, and the effect is enhanced after extending the treatment to 6 weeks. We also mechanistically investigated how SAMC Mimetic diminished HIV reservoir. In the future we plan to test the combination of SMAC Mimetics and BH3 Mimetics, and apply the newly established dPCR protocol to test efficacy of different therapeutics.

ABSTRACTS
SESSION 4

The overlap between lipid droplets and extracellular vesicles in viral infection

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Cell-to-cell communication is essential for coordination of biological processes such as viral infections, and this is often facilitated by the exchange of small particles. Lipid enclosed particles, including extracellular vesicles (EVs), mediate intercellular communication through transfer of their cargo and are actively explored for their role in various diseases and potential therapeutic and diagnostic applications. Our lab has shown that lipid droplets (LDs) are integral intracellular organelles in the early host response and are required for an efficient interferon response.

We can now show that LDs leave cells in vitro and can directly influence the antiviral response of neighboring cells against ZIKV. To elucidate LD transport mechanisms, we mined published databases to reveal an overlap of up to 96% in the proteomes of EVs and LDs, with both groups sharing proteins involved in biogenesis, cargo recruitment and transport. To understand if LDs are hijacking EV secretion pathways, proteomic comparative analysis using a primary immortalised astrocyte model was performed with and without ZIKV infection. Western blot and super resolution microscopy were used to confirm protein targets. There was approximately a 75% proteome overlap; with 48 of the 50 common EV protein markers present in astrocyte EVs also observed in their LD proteome. Notably, proteins involved in EV secretion pathways, ALIX and TSG101 were found to be localised to LDs with an increased association during Zika virus infection.

To date, there has been little research investigating the similarities of protein cargo between LDs and EVs and their potential interactions, which will be important in determining the antiviral mechanisms of LDs. Here, we have demonstrated for the first time that LDs carry proteins involved in EV biogenesis and release which may allude to their transport between cells.

Divergent Roles of Necroptosis in Skin Inflammation and Wound Healing

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Apoptotic cell death is essential for resolving acute inflammation during skin injury and infection. However, lytic forms of cell death, such as necroptosis, can directly promote inflammation, ensuring an adequate tissue response or exacerbating an already inflammatory situation. This study examines the impact of losing the necroptotic effectors RIPK3 and MLKL on skin healing in three distinct injury models: (1) Smac-Mimetic induced Toxic Epidermal Necrolysis (TEN), (2) tamoxifen-induced genetic loss of cFLIP leading to disrupted epidermal apoptosis, and (3) mechanical injury via full-thickness excision wounds.

Our findings demonstrate that RIPK3 and MLKL knock-out (KO) mice exhibit comparable severity in TEN and cFLIP-deficient models as wild-type (WT) mice. Remarkably, KO mice recover from lesions faster across all models, including mechanical injury. Specifically, KO mice showed significantly reduced wound areas by day 6 post-excision (WT vs MLKL, P=0.0011; WT vs RIPK3, P=0.0002), indicating that this effect is not simply an artefact of innate immune signalling disruption.

Bone marrow transplantation experiments revealed that KO mice reconstituted with WT bone marrow before wound excision retained their enhanced healing, suggesting that the benefit is not linked to the haematopoietic system. Notably, epidermal-specific MLKL KO mice also maintained their wound-healing advantage. Surprisingly, mice reconstituted with MLKL KO bone marrow showed delayed wound healing and heightened sensitivity to inflammatory skin reactions, reminiscent of cutaneous Graft vs Host Disease.

These findings indicate that loss of necroptotic effectors in keratinocytes promotes faster epidermal recovery, while their absence in immune cells may exacerbate inflammation. This research provides critical insights into the dual role of necroptosis in epidermal health and highlights potential considerations for targeting necroptosis in therapeutic interventions.

Influenza A-induced Inflammation in Alveolar Macrophages But Not Epithelial Cells Requires Toll-Like Receptor 7

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Influenza A virus (IAV) is a highly contagious respiratory pathogen that causes considerable morbidity and mortality worldwide. Following infection, IAV viral RNA is sensed by host pattern recognition receptors (PRRs) such as toll like receptor 7 (TLR7) and retinoic acid inducible gene-I (RIG-I). The activation of these PRRs instigates a robust antiviral immune response that leads to the production of type I interferons (IFNs) and pro-inflammatory cytokines to block viral replication and aid in viral clearance. While it is acknowledged that TLR7 and RIG-I trigger the production of inflammatory genes in response to IAV, the interaction between these pathways in different cell types is not fully understood, particularly since a dysregulated inflammatory response can exacerbate disease. In this study, we compared the responses of established murine alveolar macrophage (MHS) and epithelial (MLE12) cell lines to infections with Hk-X31 (H3N2) or PR8 (H1N1) strains of IAV. Reverse transcriptase quantitative PCR was used to measure changes in inflammatory cytokine gene expression. Our results showed that macrophages were less sensitive to cell death induced by IAV infection but exhibited a stronger inflammatory response compared to epithelial cells. This heightened response was due to the higher expression and activation of TLR7 or RIG-I in macrophages. We also employed CRISPR/Cas9 gene editing to genetically ablate TLR7 and RIG-I expression in these cells. In macrophages, the deletion of TLR7 but not RIG-I significantly blunted inflammation to IAV infection. In contrast, TLR7 or RIG-I deficiency did not dramatically alter the inflammatory response to IAV infection in epithelial cells. These findings indicate that the differential expression of TLR7 and RIG-I in these cell types contributes to their distinct responses to IAV infection, with macrophages inducing a strong TLR7-dependent inflammatory response. This study enhances our understanding of how different host receptors contribute to IAV-induced inflammation and may help identify new therapeutic targets for treating IAV infections.

The plasma metabolome of juvenile idiopathic arthritis differs by subtype and is partially explained by chronic inflammation: a case-control study

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Abstract

Background: Juvenile idiopathic arthritis (JIA) is challenging to disease classification and monitoring due to the lack of specific molecular markers. A molecular signature may therefore be useful for subtype classification in JIA, and targeted plasma metabolomics profiling has the potential to provide such a signature. This study aimed to characterise the differences in the plasma metabolome between JIA patients and non-JIA controls, and differences between JIA subtypes, and to determine the extent to which these differences are due to chronic inflammation measured by glycoprotein acetyls (GlycA) or/and acute inflammation measured by the high-sensitivity C-reactive protein (hs-CRP).

Methods: Nuclear magnetic resonance (NMR) metabolomics of plasma of 73 children with JIA and 18 sex- matched controls was assessed cross-sectionally. Associations between 71 metabolomic biomarkers and JIA, four JIA subtypes, and chronic and acute inflammation were assessed using multivariable linear regression models.

Results: Three biomarkers were different between the control and JIA group, with acetate reduced in JIA (mean difference -0.98 standard deviations, [95% confidence interval -1.49, -0.47], *Padj* =0.015), while docosahexaenoic acid (DHA) (1.01 [0.47, 1.55], *Padj* =0.015) and GlycA (0.91, [0.36, 1.46], *Padj* =0.041) were elevated in JIA. Subtype analysis revealed that systemic JIA (sJIA) samples accounted for these changes, with no significant metabolic differences identified in oligoarticular and polyarticular (rheumatoid factor positive and rheumatoid factor negative) JIA relative to controls. A total of 24 of 71 biomarkers were significantly different (*Padj* <0.05) in systemic JIA compared to controls, including acetate, DHA and GlycA. Of the 24 biomarkers, only 6 were significantly associated with levels of the inflammatory marker GlycA. hsCRP showed weaker and fewer associations with metabolomic changes compared to GlycA, with HDL measures distinctively inversely correlated with hsCRP unlike its positive correlation with GlycA.

Conclusion: The variation of plasma NMR metabolome of systemic JIA is the most pronounced relative to non-JIA controls and other JIA subtypes, which show limited evidence of metabolomic disruption. Only a small number of metabolomic profile differences in sJIA were associated with levels of GlycA, and fewer markers were correlated with hsCRP, indicating a complex relationship between JIA, metabolic disruption, and inflammation.

ABSTRACTS
SESSION 5

CHARACTERIZATION OF THE IMMUNE PARALYSIS OF SPLENIC MACROPHAGES FOLLOWING SYSTEMIC INFLAMMATION

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Infections caused by pathogens can lead to systemic inflammatory response syndrome (SIRS). This hyperinflammatory state is often followed by an immune suppressed phase. During this time, patients are highly susceptible to secondary infections. Initial studies revealed striking alterations in the immune system post-infection. In a pneumonia model, lung macrophages displayed long-term reduced phagocytosis capabilities and epigenetic alterations. Studies on blood-borne pathogens have shown that splenic dendritic cells are functionally impaired in antigen capture and presentation. However, if splenic macrophages exhibit any similar features of immune paralysis following blood-borne infections remain unknown. Splenic macrophages are a heterogeneous population and have a major role in the clearing of pathogens and apoptotic material from the blood, as well as in the resolution of inflammation. Thus, it is of great interest to understand their contribution to SIRS.

In the systemic model of immune paralysis, C57BL/6 mice were injected with 20 nmol of the TLR-9 ligand CpG oligodeoxynucleotide (ODN). Up to 28 days after this inflammatory challenge, macrophage activation was monitored by detecting the markers CD86, CD40, MHC-II and CD163 by flow cytometry. In addition, the total number of tissue macrophages were enumerated using counting beads. Lastly, the phagocytosis capability of splenic macrophages was studied in vivo via the uptake of either intravenously injected dye-labelled cells or 1 µm fluorescent microbeads.

Systemic inflammation triggered by CpG ODN caused splenic macrophage activation towards a proinflammatory phenotype for at least 7 days. At the same time, the total tissue macrophage number decreased shortly after the inflammatory stimulus, and then increased above baseline at day 5. Our functional studies showed that splenic macrophage phagocytosis of both dye-labelled cells and beads were significantly impaired compared to untreated controls.

These results indicate that severe inflammation causes functional restrictions of splenic macrophages for at least 7 days. Our work provides the first insights on how systemic inflammation triggered by blood-borne stimuli contributes to impairment of pathogen clearance by macrophages and to susceptibility to secondary infections.

Helicobacter pylori Tipα is a novel nucleomodulin that is secreted on extracellular vesicles that target the nucleus.

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Helicobacter pylori produces a novel nucleomodulin, tumour necrosis factor (TNF)-α-inducing protein (Tipα), which was reported to bind eukaryotic DNA, upregulate *TNF* expression and promote carcinogenesis. Tipα is secreted in a soluble form by the bacterium, but how it enters host cells and traffics to the nucleus was not clear. As Tipα was identified in the proteome of *H. pylori* extracellular vesicles (EVs), we hypothesised that these membrane "blebs" may mediate the secretion and delivery of Tipα to the nucleus, resulting in perturbed nuclear processes that promote carcinogenesis. To address this, *H. pylori tipA* and *tipA/tipA*+ mutant strains were generated and their EVs characterised by Western blotting, Nanoparticle Tracking Analysis and electron microscopy. We confirmed that *H. pylori* EVs harbour Tipα. We also identified strain-specific differences in the amounts of Tipα secreted by the bacteria, but contrary to a previous report, there was no correlation between the amounts secreted and disease outcome. Importantly, we demonstrated that most of the Tipα in culture supernatants was associated with EVs, suggesting that this is the main secretion mechanism for the protein. Following co-incubation of EVs with AGS epithelial cells, we could detect Tipα in the nuclear compartment at 4-hours post-incubation. Consistent with previous work, recombinant Tipαinduced TNF production in THP-1 cells. Conversely, EVs lacking Tipα induced significantly more TNF (p = <0.0001) and interleukin-8 (IL-8; $p = 0.0439$) than WT or *tipA/tipA*+ EVs. In AGS cells, *tipA* EVs also induced significantly more IL-8 than those from WT EVs ($p = 0.0129$). These data show that soluble and EV-associated Tipα may have different biological effects on host cells. In conclusion, we propose that EVs are the major mechanism for the secretion and delivery of Tipα to the nucleus, thereby modulating host immune responses and promoting gastric carcinogenesis.

Human unconventional T cells shape the early immune response to Group A Streptococcus

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Group A streptococcus (GAS) is a human-restricted pathogen that remains a major worldwide health burden due to the lack of an effective vaccine. Vaccine development has been impeded by various factors including incomplete knowledge of host immunity and correlates of protection against naturally occurring GAS infections.
A recently established controlled human infection model of GAS mediated pharyngitis provided unprecedented insight into the immune response against GAS suggesting an involvement of unconventional T cells (UTC) in the acute inflammatory response.
To define the role of unconventional T cells in anti-GAS immunity, we comprehensively analysed innate and adaptive immune responses of healthy adult PBMC and cord blood cells challenged with irradiated GAS. Using spectral flow cytometry and multiplex cytokine analysis, we identified a discrete cytokine signature after GAS stimulation that was driven by MAIT cell and Vδ2 gamma delta T cell activation, identifying them as major contributors to the early immune response against GAS. This prompt engagement of unconventional T cells was driven by the rapid release of proinflammatory cytokines by monocytes and dendritic cells upon GAS challenge. Furthermore, our work identifies variations in the quality of the response of innate myeloid cells as a significant factor contributing to differences in unconventional T cell function between GAS naïve and experienced individuals which contributes to our understanding of host protection against GAS throughout life.
These results underline a pivotal role of unconventional T cells in the context of GAS infection and advocates the use of these cells as targets in future human vaccine studies.

Type 2 diabetes exacerbates fungal infection in mice, possibly by blunting the immune response

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PUBLISH CONSENT WITHHELD.

ABSTRACTS
SESSION 7

Human stem-cell models revealed altered infection responses in AATD

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Alpha-1 Antitrypsin Deficiency (AATD) arises from mutations in the *SERPINA1* gene, leading to early-onset chronic obstructive pulmonary disease, characterised by rapid lung function decline and frequent exacerbations, often triggered by respiratory viral infections. In patients with the PiZZ genotype of AATD, alpha-1 antitrypsin (AAT) protein misfolds and accumulates within the endoplasmic reticulum. In this study we aim to understand the pathogenic cellular and molecular mechanisms underlying infections in AATD. We hypothesise that AATD mutations disrupt appropriate immune responses to viral infections. AAT is mainly produced by type 2 alveolar epithelial cells and alveolar macrophages in the lung. Accessing primary sources of these cells poses challenges; therefore, to overcome these limitations we employed induced pluripotent stem cells (iPSCs) from an AATD donor (PiZZ genotype) and an isogenic control (PiMM genotype). These iPSCs were then differentiated into type 2 alveolar epithelial cells (iAT2s) or macrophages (iMacs) using established protocols. Respiratory syncytial virus (RSV) responses were profiled daily for five days in PiZZ and PiMM iAT2s cultured at an air-liquid interface. In comparison to PiMM iAT2s, PiZZs exhibited increased cell death and reduced trans-epithelial electrical resistance. Notably, viral transcription and RSV shedding were significantly altered in AATD iAT2s. Additionally, expression of antiviral interferons, interferon-stimulated genes, cytokines and chemokines were significantly different following infection. To assess how AATD mutations affect iMacs, we measured their ability to phagocytose fluorescently-tagged bacteria, a critical function in alveolar macrophage defence. PiZZ iMacs displayed a significant impairment in phagocytosis compared to PiMMs. Our results indicate that AATD mutations alter infection responses in both iAT2 and iMacs. Future studies will compare these findings with responses to other viral or bacterial respiratory infections, aiming to unravel new therapeutic strategies for AATD.

Disentanglement of tumour-associated exhausted (T_{EX}) and tissue-resident memory T (T_{RM}) cells

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$CD8^+$ T cells serve as a key weapon in the therapeutic armamentarium against cancer. While $CD8^+$ $CD103^+$ T cells with a tissue-resident memory (T_{RM}) phenotype have been favourably correlated with patient prognoses, the tumour microenvironment comprises dysfunctional exhausted T (T_{EX}) cells that exhibit a myriad of T_{RM}-like features, leading to conflation of these cell types. Here, we deconvolute T_{RM} and T_{EX} cells within the intratumoural $CD8^+$ $CD103^+$ T cell pool across human cancers, ascribing markers and gene signatures that distinguish these $CD8^+$ populations and enable their functional distinction. We found that T_{RM} cells exhibit superior functionality and are associated with long-term survival post tumour resection, whereas T_{EX} cells are required for responsiveness to immune checkpoint blockade. Importantly, we show that tumour-associated T_{EX} and T_{RM} cells are clonally and developmentally distinct with the latter predominantly comprised of low affinity and tumour-independent bystanders. While tumour-associated T_{RM}-like cells can be forced towards a T_{EX} cell fate if chronically stimulated with their cognate antigen, T_{EX} cannot revert to a T_{RM}-like cell fate. This suggests that the majority of bona fide T_{RM} within the vicinity of tumour antigens are likely tumour-independent bystanders. Thus, approaches to induce the activation of tumour associated T_{RM} as bystanders may serve to complement current immunotherapeutic approaches that aim to enhance the functionality of T_{EX} cells.

Response and resistance to combination immune checkpoint blockade associate with distinct baseline and on-treatment blood T-cell profiles in melanoma patients.

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Introduction

Despite the success of immune checkpoint blockade (ICB), a majority of melanoma patients fail to respond or experience severe treatment-related toxicity. Currently, there are no reliable biomarkers available to predict these events and guide treatment choices. Additionally, the reliance on proportional rather than absolute immune cell counts, and the inclusion of heterogenous patient cohorts and analysis techniques have limited translation of previous research. We here evaluated the peripheral T-cell compartment to identify immune features associated with ICB outcome.

Methods

Blood samples were collected from 41 advanced melanoma patients at baseline and after one cycle of combination PD-1 + CTLA-4 ICB. Patients were classified as responders or non-responders based on radiographic best overall response to treatment. Absolute immune cell counts were obtained and PBMCs cryopreserved prior to spectral flow-cytometric T-cell immunophenotyping.

Results

19 patients (46%) failed to respond to treatment. At baseline, these patients had fewer T cells than age-matched healthy controls (median 780 vs. 1297 cells/µL, p=0.00012), mostly due to reduced naive $CD4^+$ (p=0.00203) and $CD8^+$ (p=0.00149) T cells, and showed an increased prevalence of a highly immunosuppressive T regulatory (Treg) cell phenotype and higher expression of the proliferation marker $Ki67^+$ across major T cell subsets compared to responders. One cycle of ICB expanded T memory, helper, and regulatory, but not naive, subsets, and responders showed greater Ki67 upregulation in $CD4^+$ central memory (Tcm) (p=0.0086), stem cell-like memory (p=0.045), and regulatory (p=0.0257) T cells compared to non-responders. Compared to $Ki67^-$ cells, these $Ki67^+$ cells expressed a higher proportion of PD-1 at baseline, expanded to a greater degree on-treatment, and generally co-expressed higher amounts of TIGIT, TIM-3, CD39, and ICOS. The fold change of Ki67 expression in $CD4^+$ Tcm cells after one cycle of treatment differentiated responders and non-responders (AUC=0.7545, p=0.0094).

Discussion

Response to ICB was associated with distinct T-cell profiles before and after one cycle of treatment, and significantly differentiated responders and non-responders. Further work using combinations of immune features promises to improve predictive capacity.

HIV Transcription Persists in the Brain of Virally Suppressed People with HIV

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Background: Over 38 million people live with human immunodeficiency virus (HIV) worldwide. HIV persistence in the brain is a barrier to cure, and potentially contributes to HIV-associated neurocognitive disorders, which affects ~30% of people with HIV (PWH) despite viral suppression with antiretroviral therapy. We have previously identified the persistence of a reservoir of intact, potentially replication competent HIV DNA in the brain of virally suppressed PWH. However, whether HIV transcription and protein production also persists in the brain of virally suppressed PWH is unclear.

Methods: HIV transcripts produced during early and late-stage viral transcription were detected in human autopsy frontal cortex brain tissue from virally suppressed (n=12, undetectable HIV RNA viral load in plasma) and non-virally suppressed (n=13; 61,223 HIV RNA copies/mL in plasma) PWH using a nanowell digital PCR based assay. Multiplex immunofluorescence was used to identify the presence of the HIV p24 protein within C68+ myeloid cells and CD3+ T cells in matched brain tissue. Associations between levels of HIV transcripts, clinical parameters, and levels of the intact and defective HIV DNA as measured by the intact proviral DNA assay were assessed by correlative analysis.

Results: Early-stage transcripts were produced in 23/24 PWH. Late-stage transcripts were evident in 12/13 non-virally suppressed PWH and 5/11 virally suppressed PWH. HIV p24 was detected within myeloid cells of all PWH with late-stage transcripts, demonstrating production of viral proteins in these individuals. Levels of all HIV transcripts correlated with levels of total and intact HIV proviruses ($P<0.05$ for all), demonstrating that the level of HIV transcription is associated with the HIV reservoir in the brain.

Conclusions: These findings suggest that the brain is a transcriptionally active reservoir of HIV in virally suppressed PWH which may contribute to ongoing neuroinflammation and HIV-associated neurocognitive disorders.

Deciphering and targeting of transcriptional drivers of T cell exhaustion

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CD8+ T cells responding to chronic infections and tumours become functionally impaired, a state known as 'exhaustion' that facilitates disease progression. Despite advancements in immunotherapy, such as with immune checkpoint blockade, not all patients respond, and these treatments do not overcome the functional limitations linked to T cell exhaustion. Thus, there is an urgent need to identify new targets for immunotherapy that can reinvigorate exhausted CD8+ T cells to enhance clinical outcomes. Taking advantage of the murine Lymphocytic Choriomeningitis Virus (LCMV), we previously developed a novel chronic infection that allowed us to adjust antigen presentation of the immunodominant gp33-41 epitope without altering overall inflammation or infection. CD8+ T cells exposed to low antigen levels during chronic infection did not acquire fundamental hallmarks of exhaustion. Instead, they sustained low expression levels of PD-1 and TOX and elevated expression of the effector marker CX3CR1 and high ability to produce effector cytokines. Critically, exposing exhausted T cells from high antigen wild-type chronic LCMV infections to our low antigen stimulation reversed the critically aspects of T cell exhaustion including TOX and PD-1 expression. Finally, Preliminary transcriptional profiling from single cell RNA sequencing identified potential regulators of T cell function that might serve as therapeutic targets. Overall, we developed a unique model to identify critically regulators of exhaustion in T cells responding to chronic infection. Targeting of these newly identified regulators might facilitate the design of novel therapeutic strategies to rescue the previously irreversible exhaustion phenotype, offering new insights for therapeutic interventions.

Developing precision RNA therapeutics for Tuberculosis

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Over centuries, Mycobacterium tuberculosis (Mtb), the causative agent of Tuberculosis (TB), has evolved alongside humans, developing sophisticated strategies to evade our immune response. Mtb primarily infects alveolar macrophages and creates a hidden replicative niche inside what is supposed to be our first defense against respiratory infections[1]. Antibiotics are currently our only treatment for TB, but toxic side effects and skyrocketing rates of antimicrobial resistance (AMR) urgently necessitate novel host-directed therapeutic approaches (HDT)[2]. Targeted delivery of HDTs that act at the host-pathogen interface to induce apoptosis of infected host cells holds great promise to clear the pathogen and promote adaptive immune responses without eliciting systemic side effects and limiting AMR[3].

Lipid nanoparticles (LNPs) have revolutionised the targeted delivery of RNA therapeutics. We have developed LNPs that enable precise RNA delivery to pulmonary macrophages *in vivo* without targeting the surrounding cell populations. These LNPs allow for direct RNA delivery to the lung *in vivo,* with luciferase mRNA expression in mouse lungs, 16h post intranasal instillation. Leveraging this versatile delivery platform, we were able to kill Mtb-infected primary human macrophages using siRNAs against key regulators of host cell apoptosis, cellular inhibitors of apoptosis (cIAP1/cIAP2). Additionally, we raised nanobodies against the major Mtb virulence factor, ESAT-6, which acts by limiting host cell apoptosis. LNP-encapsulated mRNA coding for these nanobodies has great potential to complement the host-directed siRNA approach to further boost apoptosis. We currently assess the potential of these LNP-delivered combination RNA therapeutics in a clinically relevant mouse model of TB infection.

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Predicting antibiotic effect on *Pseudomonas aeruginosa* with mechanism-based modelling where PK/PD indices cannot

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PK/PD indices are based on minimum inhibitory concentrations (MICs) and link bacterial response to antibiotic exposure. The index for ciprofloxacin (CIP) is the ratio of free drug area under the concentration-time curve to MIC over 24h (*f*AUC/MIC) and for meropenem (MER) it is the percentage of time the free concentration remains above the MIC (or a multiple of MIC) over 24h (%$fT_{>MIC}$).

The study evaluated if the effect of CIP and MER alone and in combination on isogenic strains of *P. aeruginosa* could be predicted by PK/PD indices or depended on resistance mechanisms present.

Seven isogenic *P. aeruginosa* strains: PAO1 (wild-type reference strain), PAΔAD (*ampD* knockout/ampC overexpression), PAOD1 (*oprD* mutation/loss of porin OprD), PAΔmexR (*mexR* knockout/MexAB-OprM upregulation), PAΔADΔmexR, PAOD1ΔmexR and PAOD1ΔAD (combinations of these resistance mechanisms) were used. MICs were determined in triplicate. Strains were exposed to MER (1-64mg/L) and CIP (0.5-4mg/L) alone and in combination, in static concentrations over 72h. Mechanism-based mathematical modelling (MBM) was performed.

MICs were 1-16 mg/L for MER and 0.125-1 mg/L for CIP. PK/PD indices did not predict bacterial response. 1-4x MIC and *f*AUC/MIC of 48-384 for MER and CIP, respectively, were required to suppress regrowth across strains. PK/PD indices also could not predict combination therapies. An MBM was developed that described the bacterial response to antibiotic based on the resistance mechanisms, predicting mono- and combination therapies of MER and CIP. Including controls and biological replicates, 292 treatments were modelled.

PK/PD indices alone did not predict the MER or CIP exposure required to suppress bacterial regrowth, indicating mechanisms of resistance should be considered when optimizing dosing. An MBM, accounting for resistance mechanisms could predict the impact of double mutations and combination therapies.

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

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Abstract:

Malaria is a global epidemic and imposes a significant public health burden. *Plasmodium* is the malaria pathogen. Among the *Plasmodium* species that infect humans, *Plasmodium vivax* (*P.v.*) affects the largest population and poses a substantial challenge to global malaria elimination efforts due to its ability to cause asymptomatic and low-density infections. Detecting *P.v.* in routine surveillance has been challenging due the low sensitivity of diagnostic tools. Recent studies have focused on serology, a novel surveillance strategy based on antibody detection. Our lab previously screened and developed a panel of 8 *P.v.* antigens as serological exposure markers (SEMs). The *P. v.* SEMs have high specificity and sensitivity for detecting recent *P.v.* exposure in cohort studies in multiple geographic areas[1]..

In this project, we advanced the previous researchers` outcomes by performing epitope screening within the *P.v.* SEMs. We utilized *P.v.* SEM data sources[1,2] and bioinformatic tools to predict linear and conformational B cell epitopes, selecting around 320 peptides. We collected cross-sectional *P.v.* samples from the China-Myanmar Border (CMB) and cohort study *P.v.* samples from *P.v.* endemic areas as previously described[1]. These samples were used to validate the seroprevalence of the predicted epitopes.

Several epitopes in the *P.v.* Merozoite Surface Protein 9 (MSP9) and Apical Membrane Antigen (AMA1) demonstrated good seroprevalence and specificity for current infection individuals on the CMB. Our results provide insights into the precise immnuogenic regions of the existing *P.v.* SEMs and contribute to the development of improved serological surveillance tools.

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ABSTRACTS
SESSION 8

Leveraging the Position of Lymph Node Memory CD8+ T Cells to Enhance Protective Immunity

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The primary goal of vaccination is to achieve long-lasting, protective immunity. Most vaccine platforms require boosting for memory cell maintenance. Current vaccine boosting strategies repeat the primary challenge, without regard for critical differences between naïve and memory cells. Here, we have used 3D light-sheet fluorescence microscopy and intravital multi-photon imaging to discover that $CD8^+$ memory T cells reside long-term in a distinct lymph node niche. Unlike naïve cells that position in the T cell paracortex, $CD8^+$ central memory T cells (T_{CM}) occupy the lymph node cortical ridge. This distinct T_{CM} location was conserved following multiple diverse infection models, including LCMV, HSV-1 and *Listeria*. We propose that this memory niche supports the long-term survival of memory T cells.

To explore cell-cell contacts regulating T_{CM} location, we used high-resolution confocal microscopy and spatial transcriptomics to identify specific dendritic cells (DC) and stromal cells which interact with T_{CM} within the memory niche. We have developed antibody targeting and LNP formulations to generate novel boosting vaccine approaches that specifically target T_{CM}-DC interactions, testing whether these improve memory cell maintenance and recall capacity. Our findings suggest a paradigm shift in vaccine boosting that leverages the distinct location and interactions of T_{CM} to enhance long-lasting protective immunity.

Reaction hijacking inhibition of tRNA charging enzymes of malaria parasite and bacteria

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The rise of drug-resistant pathogens is a threat to global health and sustainable development. This includes resistance to current antimalarial and antimicrobial compounds. Development of novel antimalarials and antibiotics with a breakthrough mechanism of actions is a high priority. AMP mimicking, nucleoside sulfamate pro-inhibitors have recently been shown to target *Plasmodium* aminoacyl-tRNA synthetases, via a novel reaction hijacking mechanism. The target enzyme catalyses the linkage of the pro-inhibitor to an amino acid, thus forming an adduct that tightly binds to and inhibits the enzyme.

To identify additional hijacking-susceptible targets in Plasmodium and bacteria, this project investigated a natural product nucleoside sulfamate, from a soil bacterium of the genus Streptomyces. Combining mass spectrometry, enzyme biochemistry, parasitology, and structural biology, we showed that dealanylascamycin is a potent pro-inhibitor of *P. falciparum* aspartyl-tRNA synthetase. Using the same pipeline, we identified prolyl-tRNA synthetase as one of the targets of the Streptomyces pro-inhibitor in *Enterococcus faecium*, which is listed by the World Health Organisation as a high-priority pathogenic bacterium. Our discovery of reaction-hijacking of P. falciparum and *E. faecium* enzymes will aid the design of potent and selective compounds, with the potential to expand to other human pathogens.

The Medicines for Malaria Venture Pathogen Box compound MMV687794 impairs blood-stage *Plasmodium falciparum* invasion through potential inhibition of parasite lipid metabolism

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Malaria is a deadly infectious disease caused by the Apicomplexan parasite of the *Plasmodium* genus and transmitted to humans by the *Anopheles* mosquito. There were 608,000 mortalities reported in 2022, and 97% were accounted for by the deadliest species, *Plasmodium falciparum*. Responsible for clinical symptoms of malaria and parasite proliferation, parasite invasion of erythrocytes represents an attractive drug target. This process involves the lysis of infected erythrocytes by mature parasites (schizonts), which release daughter parasites (merozoites) to invade new erythrocytes. Here, we discovered the compound MMV687794 that blocked invasion when added to schizonts but not purified merozoites, suggesting the compound impaired merozoite development within schizonts. Genomic analysis of MMV687794-resistant parasites revealed two mutations to the alpha/beta hydrolase protein containing *N*-myristoylation and lipid-binding motifs, which we termed ABH-83 after its size. One of the mutations, C36W, were inserted into wild-type parasites using CRISPR/Cas9 along with an epitope tag and a GlmS riboswitch. This allowed for the validation of ABH-83 as a drug target and downstream examination of its biological role. While transgenic parasites containing the C36W mutation (C-C36W) recapitulated the MMV687794-resistant phenotype, knockdown of ABH-83 expression did not alter C-C36W parasites' susceptibility to MMV687794. Furthermore, knockdown of ABH-83 expression in transgenic parasites containing the wild-type ABH-83 (C-WT) did not increase their sensitivity to MMV687794 but rather conferred MMV687794 resistance to a similar degree of that observed in C-C36W parasites. This suggests MMV687794 may not directly target ABH-83, but the C36W mutation served as a resistance/compensatory mechanism. Time-course western blot series on the transgenic parasites show that ABH-83 is most highly expressed in schizonts, consistent with a role in invasion. Microscopy and carbonate extraction data demonstrate ABH-83 is associated with the membranes of the rhoptries, organelles that secrete crucial invasion-related ligands during erythrocyte invasion. Lipidomics results indicate that C-C36W parasites have perturbed phospholipids when compared to C-WT, validating ABH-83's role in parasite lipid metabolism. Together, this points to ABH-83 having a functional role in rhoptry lipid metabolism, and ongoing work seeks to further dissect ABH-83's localisation and MMV687794's direct target(s).

The characterisation of human Vδ3⁺ γδT cells and the development of bispecific antibodies to harness their function

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Human γδT cells are innate-like lymphocytes that demonstrate powerful anti-tumour properties. In humans, γδT cells are classically divided into dominant subsets defined by their T cell receptor usage with namely, the Vδ1, 2 and 3⁺ γδT cell populations. Here, Vδ2⁺ γδT cells are abundant in the periphery and are associated with enhanced innate-like functions whereas Vδ1⁺ γδT cells localise in mucosal tissues and are suggested to contribute to adaptive-like stress surveillance. In contrast, little is known regarding the biology and functional potential of Vδ3⁺ γδT cells compared to their Vδ1 and 2⁺ γδT cell counterparts, largely due to a lack of reagents to efficiently isolate these cells. To address this, our laboratory has developed a monoclonal antibody (mAb) that binds the Vδ3⁺ T cell receptor. Utilising a combination of flow cytometry and single cell RNA sequencing techniques we show that Vδ3⁺ γδT cells are phenotypically more similar to Vδ1⁺ γδT cells as exhibited by their CD45RA and CD27 co-expression profile. This adaptive-like Vδ3⁺ γδT cell phenotype is accompanied by decreased innate-like surface and functional marker expression compared to Vδ2⁺ γδT cells such as surface CD161 and intracellular PLZF. We have furthermore engineered this anti-Vδ3 mAb to generate bispecific antibodies that bind to both Vδ3⁺ γδT cells and the model tumour antigen Her2. Utilising these bispecific antibodies, *in vitro* expanded Vδ3⁺ γδT cells demonstrate efficient killing of Her2⁺ human breast cancer cells with comparable efficacy to both Vδ1 and 2⁺ γδT cell killing of the cancer cells.

Accordingly, we show that Vδ3⁺ γδT cells can be phenotypically and functionally similar to Vδ1⁺ γδT cells and we have developed bispecific antibodies that specifically harness the functionality of this subset. Considering the emerging interest and utility of Vδ1⁺ γδT in cancer immunotherapy, this study highlights the potential of Vδ3⁺ γδT cells as a novel immunotherapeutic target.

ABSTRACTS POSTER SESSION I

Precise CRISPR insertion for deciphering immune interactions.

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Protein-protein interactions are pivotal to successful signal transduction in response to infection and inflammation. Some of these proteins demonstrate multifaceted mechanisms of control across multiple signaling pathways, which are often poorly understood. One such protein is macrophage migration inhibitory factor (MIF), a pleiotropic immunomodulatory protein that has been implicated in numerous inflammatory signaling cascades, including NF-κB- and NLRP3-dependent pathways. MIF is constitutively present in the cytosol and extracellular space, yet its mode of secretion is unclear. MIF is cytoprotective in response to a range of inflammatory stimuli, but potentially permissive to some viruses. Moreover, deletion or inhibition of MIF has been shown to decrease severity and/or susceptibility to a number inflammatory and autoimmune diseases, including rheumatoid arthritis, lupus and inflammatory bowel disease. Proteomics data has revealed a number of potentially interesting interacting partners for MIF that could explain its pleiotropic nature. In addition to MIF, we focus on proteins that demonstrate anti-viral or anti-inflammatory activity and inhibitory molecules to said proteins. We are utilising the CRISPR-cas9 technique, CRISPaint, to tag these proteins endogenously, either with a fluorescent protein, biotinylation enzyme or destabilization domain, providing novel pathways to investigate transient/stable protein-protein interactions, cellular localization and protein function. CRISPaint does not require homology arms and reduces the occurrence of deletions and frameshift mutations. It utilises universal donors, making it a highly adaptive system that only requires the generation of target-specific targeting vectors. Covalently tagged endogenous protein holds potential to decrease the number of non-target-specific hits currently seen with immune-precipitation and mass spectrometry approaches. Validation of protein interactions and mechanisms will be conducted in biologically relevant cell lines. This approach will not only help us to uncover the biological mechanisms by which target proteins (i.e. MIF) influence inflammatory responses but will also provide a pipeline for other immune protein interactome studies.

Understanding the Molecular Mechanism of Recognition of *Bacteroides fragilis* produced glycosphingolipids by Natural Killer T (NKT) cell Receptors

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Abstract

The gut microbiome consists of symbiotic microorganisms, which play an essential role in regulating immune function through T cell-mediated immune response. Studies have shown that Bacteroides fragilis, which comprises over 50% of the species found in the colon, produces a suite of α-galactosylceramides termed as BfaGCs. These BfaGCs are glycosphingolipids that are presented by CD1d leading to the activation of Natural Killer T (NKT) cells. This interaction between the CD1d - BfaGCs complex and the NKT T cell receptor promotes mucosal healing, enhance epithelial barrier function, and restores immune homeostasis in gut-related disorders. Although, these BfaGCs are structurally similar to the NKT cell prototypical antigen α-galactosylceramide, they possess distinct structural features, which make these BfaGCs unique CD1d presented lipids.

To date, only two crystal structures have been reported on how these BfaGCs are positioned within the antigen binding pocket of CD1d and interact with the NKT TCR. Here, we report one novel structure showing the interaction between the CD1d-BfaGCs and the NKT TCR, wherein it was seen that the BfaGC anchors within the binding pocket of CD1d and headgroup is protruded out which is recognized by the NKT TCR. Furthermore, through Surface Plasmon Resonance, we have identified that CD1d-BfaGC complexes have a nanomolar affinity for the NKT TCR. Overall, we have been able to provide valuable insights on the affinity of the interaction between the NKT TCR and four BfaGCs, complementing our structural studies. This study provides further insights on the symbiotic relationship between the host and the gut microbiota producing lipids which serves as a promising new therapeutic approach for treating various human diseases.

Oh, S.F., Praveena, T., Song, H. et al. Host immunomodulatory lipids created by symbionts from dietary amino acids. Nature 600, 302–307 (2021). https://doi.org/10.1038/s41586-021-04083-0

Crohn’s associated invariant T cells recognise small molecules on CD1d

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Natural killer T-cells (NKTs) belong to the large unconventional T-cell family, which are not restricted to peptide-major histocompatibility complex (MHC) recognition. Instead, NKTs recognise lipid antigens presented by the non-polymorphic antigen-presenting molecule CD1d via their T-cell receptors (TCRs). Type I NKTs are the best characterised population, with a semi-invariant TCR (TRAV10-TRAJ18 in humans) conferring specificity to their common antigen, α-galactosylceramide (αGalCer).

Type II NKTs are less well understood, expressing different TCRs and recognising lipids presented by CD1d, but not αGalCer. Our lab has described a population of type II NKTs that responds to a family of non-lipid antigens – benzofuran-sulfonate small-molecules, termed ‘PPBFs’ – when presented by CD1d in conjunction with specific self-lipids (Van Rhijn et al, 2004; Almeida et al, 2021). In the latter study, PPBF-reactive T-cells isolated from healthy donors showed limited TCR-gene usage, preferentially TRAV12 or TRAV14.

Recently, a T-cell clonotype consisting of semi-conserved TRAV12-TRAJ6 rearrangements was found to be highly expanded in people with Crohn’s disease, termed Crohn’s associated invariant T-cells (‘CAITs’, Rosati et al, 2022). Their TCRα, including the CDR3α-motif, is remarkably similar to that of PPBF-reactive type II NKTs, suggesting the same type II NKT cells play a role in the disease (Almeida et al, 2021; Rosati et al, 2022; Minervina et al, 2024).

Crohn’s disease is one of two major forms of inflammatory bowel disease, a debilitating and incurable autoimmune condition of unclear aetiology, affecting the gastrointestinal tract.

We have used a selection of CAIT TCR-sequences derived from Crohn’s patients to produce TCR-transduced reporter cells. With these CAIT-TCR-transduced cells we show that PPBF leads to CD1d-dependent activation when co-cultured with CD1d+ antigen-presenting cells, and that they directly bind, through their TCRs, PPBF-CD1d tetramers, enhanced by co-loading with specific self-lipids (sphingomyelin 34:1), and inhibited by others (sphingomyelin 42:2). This shows CAITs are CD1d-PPBF-reactive type II NKTs.

Our results support a role for type II NKTs in Crohn’s disease and a possible role for PPBF-like small molecules altering recognition of CD1d-bound lipids, driving CAIT activation and expansion. Exploring this mechanism of this poorly understood disease may lead to new diagnostics or treatments targeted towards these cells.

Exploring trogocytosis between DC and B cells

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Trogocytosis is a cellular process in which one cell extracts membrane fragments and membrane-embedded molecules from another, potentially incorporating them into its own membrane. This process plays a significant role in a wide range of biological contexts, such as neuronal axon pruning or embryonic development. This phenomenon also occurs between two important types of immune cells: B cells and dendritic cells (DC) (Schriek et al., 2022). B cells recognise complement component C3 attached to MHC II on the DC surface, which initiates the transfer of DC membrane proteins to the B cells. In this study, we sought to determine the extent and mechanisms of trogocytic material transfer, which could provide deeper insights into the regulation of the immune system.

To investigate this, splenic dendritic cells were purified from mice with cytoplasmic Venus fluorescent protein in their cDC1, and B cells were isolated from mice that lacked Venus expression. These cells were co-cultured and analysed by flow cytometry and microscopy. The results showed that up to 90% of the B cells contained detectable levels of the fluorescent protein, indicating the transfer of cytoplasmic contents. To further explore the molecular mechanisms driving this process, a range of inhibitors targeting different molecular pathways that regulate membrane dynamics, including actin polymerisation and endocytosis, were tested. However, these inhibitors did not prevent the transfer, suggesting that trogocytosis may be a passive process or involve redundant pathways.

This study reveals a novel aspect of trogocytosis between DC and B cells, indicating that cytoplasmic contents are transferred in addition to membrane and membrane-anchored molecules. This finding suggests a more complex interaction during trogocytosis than previously understood. Further research into the underlying mechanisms of this transfer could provide deeper insights into the functional implications and potential physiological roles of trogocytosis between DC and B cells. Understanding these mechanisms could also have broader implications for immunological research and potential therapeutic applications.

Microfluidic solution for evaluating exhausted T cells' response toward PD-1 blockade efficacy

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Ovarian cancer (OC) is still one of the deathliest cancers and there is still no effective treatment(1). Immunotherapies based on immune checkpoint inhibitors (ICIs) have shown favorable outcomes in lymphomas (>60%), but the overall response rate in OC remains modest (<10%)(2). Long-term exposure to OC cells can lead to T cell exhaustion, and research indicates that extremely exhausted T cells (T_{ex}) can be one of the main reasons to the poor ICI outcomes(3). While it is known that T cells in ovarian tumors become exhausted, the variability in exhaustion levels and their impact on treatment outcomes remain poorly understood(4). In this research, we want to study how we can optimize the ICIs administration timing depending on the exhaustion level. The hypothesis is that PD-1^{high} T_{ex} cells need more time to be reinvigorated by ICIs and that it should be possible to identify which T cell exhaustion states are critical during ICI treatment and predict response to ICIs. We have dynamically evaluated different exhaustion levels of Immune cells' response toward ICIs using a microfluidic single-cell system that can isolate around 100 live PD-1^{+} Jurkat cells. PD-1 expression profiles have been assessed in real time to learn what are the proper time and dosages of immunotherapies that can effectively revert those states. Our research shows how we can assess more precisely ICIs and learn why some OC patients do not respond efficiently to ICIs.

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Discovering targets of long-lived humoral immunity for Group A *Streptococcus* vaccine design

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There is an urgent need for a vaccine against Group A *Streptococcus* (*Streptococcus pyogenes*, GAS), a bacteria that causes over 600 million pharyngitis cases and 160,000 deaths from invasive infectious disease every year. Post-infectious autoimmune sequelae including rheumatic heart disease disproportionately affect First Nations Australians. The current lack of knowledge about immunity in humans is a barrier to vaccine development. To bridge this knowledge gap and improve vaccine design and evaluation, we have comprehensively mapped the memory B cell responses specific for 7 GAS candidate vaccine antigens in human tonsils.

Using spectral flow cytometry, we have quantified and sorted memory B cells specific for GAS protein and carbohydrate antigens from current vaccine candidates in tonsils from paediatric donors with and without GAS colonisation. Subsequently, single-cell RNA sequencing is performed to phenotype the antigen-specific memory B cell response in detail and identify B cell receptor clones that may generate high-affinity monoclonal therapeutics.

We have quantified tonsil memory B cells specific for all studied GAS antigens. Flow cytometry data reveals different isotypes and activation phenotypes of antigen-specific B cells, and a variation in response magnitude based on GAS colonisation status. We have performed single cell sequencing on GAS antigen-specific memory B cells sorted from these donors, and this dataset enables detailed analysis of the subsets of vaccine antigen-specific B cells as well as their B cell receptor repertoire and degree of clonal expansion.

This study has revealed that memory B cells specific for multiple GAS vaccine antigens are present in human secondary lymphoid tissue. Furthermore, we have observed that most antigens can elicit germinal centre responses – the process that generates high affinity and long-lasting antibody responses. Our study provides critical knowledge about immunity induced by natural infection to GAS, to inform development of immunogenic vaccines and new therapeutics that can reduce the devastating global burden of GAS diseases.

Using rabies virus as a tool to understand the mechanisms of synapse formation in the brain

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Synapses are the small junctions between neurons that allow them to communicate. Loss of these synaptic junctions results in neurological disease and disorders such as Alzheimer's and/or autism. Synapses are integral for the functioning of our body and mind, but the underpinning processes that lead to synapse formation are extremely complex and still widely unknown.

Rabies (lyssavirus) has the unique ability to transfer exclusively throughout the nervous system across synaptic junctions. Our lab has recently discovered that the viral protein (X) derived from a highly neuroinvasive strain of rabies virus can manipulate the biology of synapse formation, facilitating its efficient spread throughout the nervous system. This viral protein was found to inhibit axonal degeneration and increase the production of synapses.

My research focuses on using the X viral protein as tool to gain new insights into the intricate molecular mechanisms and signalling pathways involved in synapse formation. Through a combination of interactome and mass spectrometry analyses, I have successfully identified a range of synaptic proteins that interact directly with the X viral protein in infected neurons. These are now being further characterised through biochemical assays and western blot analyses with hopes of unravelling novel mechanisms in synapse formation. Advanced imaging techniques such as confocal and super-resolution microscopy are also being employed to examine the precise localisation of these proteins on the synaptic membrane. This comprehensive approach aims to expand our understanding of the complex processes governing synapse development and provide valuable knowledge about the intricate neural networks that comprise the brain.

Through decoding the unique properties of the rabies X viral protein, we have identified a new tool to unravel the molecular mechanisms underlying synapse formation. Furthermore, our research endeavours to develop next-generation therapeutics inspired by the X viral protein, aiming to restore synaptic functioning and combat neurodegeneration. With further optimisation, our work aims to improve neuronal health and connectivity, bringing hope to those affected by these debilitating conditions.

Characterisation of Novel Cytokine Interferon Epsilon in the Murine Peritoneal Cavity

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The novel type I interferon, interferon epsilon (IFNε) possesses a unique manner of spatiotemporal expression and regulation. Largely studied in the female reproductive tract (FRT), it has been shown to maintain homeostatic conditions and mediate protective immunity against common FRT pathogens. Many FRT pathologies including cancer, endometriosis, and infection can extend detrimentally into the peritoneal cavity, and are characterised by dysregulated immune responses. Therefore, it is hypothesised that IFNε may have protective immunoregulatory effects that influence the phenotype, activity and composition of peritoneal immune cells under both steady state and inflammatory conditions.

Previous research utilizing syngeneic mouse models of high grade serous ovarian cancer (HGSOC) has indicated that IFNε administration alleviates tumour burden and ascites development, accompanied by changes in the activity of peritoneal immune cell populations. Following these observations, the peritoneal immune cells present in female wild-type (WT) and *Ifnε-/-* mice in steady-state conditions and during inflammation were investigated via immunophenotyping. The results indicated that in homeostatic states, endogenous IFNε maintains a basal immunity in the peritoneal cavity by regulating the peritoneal myeloid cells through their recruitment into the peritoneal cavity, differentiation and proliferation, as well as through the modulation of the activation states in the peritoneal lymphoid compartment.

Intraperitoneal administration of exogenous IFNε prior to the induction of peritonitis in WT mice showed that IFNε may act similarly to IFNβ in the peritoneal cavity during infection, potentially by regulating the migration and activation of the peritoneal immune cells in response to infection. Collectively, these data underline the role of IFNε in maintaining the basal immunity of the murine peritoneal cavity, which may prompt the peritoneal immune response during bacteria-induced inflammation. Thus, IFNε may have potential as a future immunotherapy for peritoneal pathologies that induce aberrant activity in the peritoneal immune cells, primarily peritonitis and HGSOC metastases.

Prenatal and early life viral infection synergistically modify expression of cholinergic, dopaminergic and complement C4 genes in the hippocampus and prefrontal cortex in mice.

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Introduction: Influenza A virus (IAV) infection during pregnancy has been shown to disrupt foetal brain development and has been implicated in neurodevelopmental disorders (NDDs) such as schizophrenia, autism spectrum disorder and bipolar disorder (1). Dopaminergic and cholinergic neuronal signaling, particularly in the hippocampus (HC) and prefrontal cortex (PFC) are involved in regulating motivation, learning and memory; their dysregulation has been linked to NDDs (2). Synaptic pruning refines neural circuits to promote healthy brain maturation, however recent genome-wide association studies have shown that complement C4, which is involved in activating microglia-mediated synaptic pruning, was significantly elevated in schizophrenic patients (3). Therefore, this study aims to recapitulate the “two-hit” hypothesis of NDDs, which postulates that prenatal infections heightens susceptibility, and subsequent early-life environmental insults such as respiratory infections (i.e. respiratory syncytial virus (RSV)) in childhood can lead to NDD onset.

Methods: Pregnant C57BL/6 mice were infected with IAV (HKx31; H3N2 strain;10^3PFU) or with PBS (vehicle control). At 3-weeks old the offspring were subsequently infected with RSV-long strain ($2x10^7$PFU) or with PBS and culled 6-weeks post infection (equivalent to late adolescence). Gene expression changes in dopaminergic and cholinergic systems in the PFC and HC were assessed by qPCR. Flow cytometry was used for immune cell profiling in whole brains. Sex differences were also examined.

Results: Adult mice that experienced an early-life “single hit” (IAV or RSV alone) did not show significant gene expression changes in the brain compared to uninfected mice. In contrast, mice that received the “two-hit” insult (IAV/RSV) showed alterations in expression of genes linked to regulators of dopaminergic and cholinergic systems in the HC and PFC. Moreover, C4 was only significantly upregulated in the PFC of male but not female mice. Flow cytometry data shown significantly increased infiltrating CD4+ T cells in IAV/RSV mice.

Conclusion: This study demonstrates that mice exposed to at least two viral insults in life (prenatal and early-life) are more likely to develop dysregulated dopaminergic and cholinergic systems, as well as increased synaptic pruning activity. Furthermore, our “two-hit” model disproportionally affects male mice, suggesting that males may be more susceptible to developing NDDs triggered by viral infections than females.

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Identification of metabolic candidates contributing to the comorbidity of diabetes, cardiopulmonary, and cardiovascular diseases

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The global rise in morbidity and mortality is driven by the prevalence of diabetes, cardiopulmonary diseases (CPDs), and cardiovascular diseases (CVDs) [1]. The comorbidity of these conditions exacerbates disease severity and complicates treatment, necessitating integrated research to uncover shared molecular mechanisms and therapeutic targets [2, 3]. Mitochondrial dysfunction in particularly immune cells, T-cells and macrophages, common in diabetes, CPDs, and CVDs, impairs energy production, increases oxidative stress, contributing to insulin resistance, hyperglycemia, inflammation, cardiomyopathies, and pulmonary hypertension (PH) [4, 5, 6, 7]. NADPH oxidase (NOX) proteins, key players in oxidative stress, interact with proteins in metabolic pathways, highlighting their role in mitochondrial and cellular functions [8]. Metabolic targets were identified using Thermo Scientific™ Orbitrap™ Astral™ mass spectrometry (MS) and liquid chromatography (LC-MS). Data from control-fat (CFD) and high-fat (HFD) fed mice showed distinct expression of metabolic proteins and metabolites, including the NOX and NAD stem groups. Future research exploring the interplay between NOX proteins and mitochondrial targets could provide insights into novel therapeutics. These insights may enhance therapeutic efficacy through personalized approaches in co-developing treatments for these diseases. Advanced technologies like CRISPR could lead to innovative treatments targeting NOX-related pathways.

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Remodelling of the plasma proteome by sex hormones in a longitudinal model of feminizing gender-affirming hormone therapy

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Abstract

Background

The plasma proteome is a measure of thousands of proteins in the circulation, reflecting the physiological state and disease susceptibility. The proteome is crucial for understanding reproductive health, cancer risk, and immune function. We know that sex, and sex hormones, can influence the proteins in the circulation. In this study we examine the longitudinal changes in the plasma proteome in transgender women undergoing gender-affirming hormone therapy (GAHT).

Methods

Blood samples from 20 transgender women were collected at baseline and six months post-feminizing GAHT. The GAHT regiment consisted of estradiol, a type of estrogen, and an anti-androgen to reduce testosterone concentrations. OLINK proteomics measured 5,329 proteomic biomarkers in plasma using a sequencing-based approach. Changes in protein concentration were determined using the 'nlme' package for mixed linear models in R. Gene Ontology and KEGG databases were used to identify pathways that GAHT changed. Immune-related protein changes were further validated using Luminex technology to evaluate 31 cytokines in a larger cohort of 49 transgender women undergoing GAHT.

Result

Feminizing GAHT significantly altered 857 proteins in the circulation ($p<0.05$), with nearly 94% showing a decrease. The top three affected proteins, SPINT3, INSL3, and EDDM3B, were related to male spermatogenesis. Decreased testosterone levels were associated with a reduction in prostate cancer biomarkers, while estradiol concentration correlated with cytokines, related to immune function. Among these cytokines, Eotaxin exhibited the most significant alteration after GAHT ($p<0.0001$). This change in cytokine profile was confirmed in the larger cohort of 49 transgender women on GAHT.

Conclusion

Our results show that feminizing GAHT changes the concentration of more than 15% of measured proteins in the circulation. These changes reflect the loss of male fertility and potential alterations in the immune protein profile. This study provides new insights into how sex hormones influence the plasma proteome.

Temporal and Cross-serotype Analysis of Dengue T Cell Targets to Inform Vaccine Design

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Context: Dengue is experiencing a significant geographical expansion, with over 3.9 billion people in more than 128 countries at risk and an estimated 100 to 400 million infections annually, underscoring the urgent need for an effective vaccine. Extensive research indicates the protective role of T cells in dengue immunity, suggesting the importance of designing vaccines that elicit a robust T cell response. Given the distinct antigenic characteristics of the four dengue serotypes and the potential immune evasion due to viral evolution, inducing a stable T cell response against multiple serotypes over time presents a challenge. This study aims to identify T cell epitopes that are cross-serotypically conserved and resistant to temporal variability, with the goal of guiding the design of next-generation T cell-based vaccines that target these stable regions.

Method: We utilized experimentally validated DENV T cell epitopes and patient-isolated sequences along with metadata to calculate the epitope conservation across different serotypes over various years, thereby assessing the cross-serotypical and temporal variation in conservation.

Result: We integrated epitope data from IEDB comprising 1817 epitopes across the dengue proteome, along with sequence data from multiple databases, including NCBI (total 79,339 protein sequences) and GISAID (total 89,039 protein sequences). Through the analysis of cross-serotype epitope conservation from 2013 to 2023, we identified 43 T cell epitopes that have consistently remained highly conserved (>90%) across at least three dengue serotypes. Additionally, we reported 311 epitopes that have exhibited genetic variability in recent years, which are worth further experimental test to evaluate their potential for T cell escape.

Conclusion: The results enhance our understanding of DENV T-cell epitope landscape over time, providing insights for developing broadly protective dengue vaccine as well as for evaluating potential T cell escape.

Immunogenic HLA-B*44:03 restricted peptide does not induce the same response in individuals with B*44 superfamily molecules

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The influenza virus is responsible for yearly epidemics that account for approximately 650,000 deaths worldwide, despite vaccines being widely available. Novel vaccine technologies targeting $CD8^+$ T cells are attractive as they are known to recognise conserved peptides of influenza viruses. This is significant as we can target several influenza strains in single a vaccine.

Activating $CD8^+$ T cells require peptides to be presented by the highly polymorphic Human Leukocyte Antigen class I (HLA-I) molecules. These different HLA-I molecules have different peptide-binding preferences, making peptide selection for inclusion in a vaccine difficult. However, several HLA-I molecules have similar peptide presenting preferences and these are grouped into superfamilies.

Targeting peptides presented by several HLA-I molecules in future vaccines may enhance our capacity to induce protection across several broad and diverse populations. We investigated this phenomenon in the HLA-B44 superfamily using the $NS1_{195-203}$, which is known to induce CD8+ T cell responses in HLA-B*44:03 individuals.

Using *in silico* computer predictions, we first visualised how the different HLA-I molecules were presenting the peptide. We identified that HLA-B*44:02 and HLA-B*44:03 were most likely to present $NS1_{195-203}$ to T cells.

We then used T cell activation assays to assess whether the $NS1_{195-203}$ peptide induces CD8+ T cell responses in individuals with the different HLA-I molecules. We found that only HLA-B*44:03$^+$ and HLA-B*44:01+ individuals had any measurable CD8+ T cell response, in line with our structural predictions.

These data suggests that although peptides can be presented by multiple HLAs in the same HLA-I superfamily, it may not induce any, or the same level of response. This therefore warrants further investigation into other peptides to assess whether we should target HLA superfamilies or future therapeutics.

COVID-19 results in broad autoantigen recognition post-infection, with anti-calprotectin autoantibodies associated with better clinical outcomes

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COVID-19, caused by the SARS-CoV-2 virus, can result in autoimmune and autoimmune-like complications. As viral infections have known associations with autoimmunity, autoantibodies to a variety of targets have been reported during acute COVID-19. However, their persistence after 6 months and their correlation with sustained anti-SARS-CoV-2 immunity, is still controversial. In the present study, antibodies to 6 SARS-CoV-2 antigens and 102 human proteins with known autoimmune associations were measured in plasma from Tasmanian healthcare workers 8 months post a single SARS-CoV-2 exposure event (n=31 with confirmed COVID-19 disease and n=21 uninfected controls). IgG antibody responses to SARS-CoV-2 antigens were significantly higher in the COVID-19 convalescent cohort in comparison to the uninfected cohort, highlighting lasting responses. These responses were also found to cross-react to the Omicron variant spike protein. A range of autoantibody reactivities were found in both the uninfected and convalescent cohorts. Individuals with COVID-19 recognised a greater number of the human protein targets, including a common set of autoantigens specific to this group, in comparison to the uninfected controls. Additionally, this breadth of autoreactivity was associated with the long-term level of anti-Spike IgG responses. The most common target for autoantibody responses was Calprotectin, recognized by 22.6% of the overall convalescent cohort. As well as being the most common autoantigen, anti-calprotectin antibodies were associated with return to healthy normal life 8 months post infection. These findings suggest, counter-intuitively, autoantibodies may play a protective role in the pathology of long-COVID-19.

Increased spike-specific IgG4 following has variable consequences on FcγR-mediated responses.

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Antibody Fc-mediated responses, including antibody dependent cellular cytotoxicity (ADCC) and cellular phagocytosis (ADCP), help maintain protection against SARS-CoV-2 variants. Several factors modulate Fc functions, including IgG subclass (IgG1-4) and Fcγ receptor (FcγR) class and polymorphisms (FcγRIIaH/R, FcγRIIIaV/F). Here we characterised spike-specific subclass and Fc-mediated responses following repeated mRNA vaccination and examined the mechanisms driving FcγR-mediated responses.

Plasma (n=24) collected four weeks post-second and third dose of mRNA vaccination were assessed for spike-specific IgG subclass titres and FcγR binding via multiplex assays. Spike-specific IgG4 significantly increased ($p<0.001$) between the second and third mRNA vaccination, and inversely correlated with binding to all tested FcγRs ($r=-0.72$ to -0.77, $p<0.001$), suggesting that IgG4 may hinder FcγR-mediated functions. To confirm this, we added a cocktail of spike-specific IgG4 monoclonal antibodies (mAbs) into second dose mRNA Vaccine samples. High concentration (0.2 nM) IgG4 addition significantly reduced FcγRIIIaV and FcγRIIIaF binding for all vaccinees ($p<0.0001$), the main FcγRs associated with ADCC. This was confirmed using mAbs in cell based ADCC assays, suggesting that excessive IgG4 is detrimental for ADCC induction.

In contrast, IgG4 addition improved FcγRIIaH and FcγRIIaR binding in 38% and 67% of vaccinees respectively, the main FcγRs associated with ADCP, and this was confirmed with cell-based ADCP assays. Upon closer examination, vaccinees with reduced ADCP had significantly higher spike-specific total IgG titres ($p<0.0001$), suggesting that elevated IgG4 competes with more functional subclasses when titres are high, but work in synergy with other subclasses to improve ADCP when titres are low. This trend was more pronounced with more recent SARS-CoV-2 variants where vaccination induced lower spike-specific titres. Finally, in silico mathematical models allowing simultaneous modulation of IgG1 and IgG4 concentrations provided quantitative confirmation of the dual role of IgG4, whereby it can enhance or reduce ADCP. Moreover, model dissection confirms that this is driven by changing levels of competition between IgG1 and IgG4, depending on pre-existing IgG1 levels.

Collectively, we illustrate that the impact of elevated IgG4 titres on Fc functions is dependent upon multiple interconnected antibody, FcγR and antigen factors which should be taken into consideration when dissecting the mechanisms driving an effective Fc-mediated response.

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

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Association of *Plasmodium falciparum* specific afucosylated IgG with placental malaria protection

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Malaria is a life-threatening disease that causes over 600,000 deaths annually, with the most severe form caused by *Plasmodium falciparum*. In terms of naturally acquired immunity, Immunoglobulin G (IgG) antibodies serve a critical role in the activation of immune-protective functions via the Fc-gamma receptors (FcγR). The recent focus on IgG fucosylation highlighted the heightened binding affinity of afucosylated IgG to FcγRIIIa compared to the normally fucosylated IgG, due to the absence of fucose on the highly conserved N-linked glycan located in the Fc domain of IgG, resulting in enhanced protection function activation such as antibody dependent cellular cytotoxicity (ADCC). In this study, we utilized the Fucose-sensitive Enzyme-linked immunosorbent assay (ELISA) for Antigen-Specific IgG (FEASI), an immunoassay that is capable of quantifying Fc fucosylation of antigen-specific IgG antibodies. FEASI consists of two ELISA assays; the first is to measure the levels of antigen-specific IgG independent of fucosylation using total IgG, while the second gives FcγRIIIa specific binding readouts which is highly sensitive to IgG fucosylation. The output of both ELISAs is converted into a ratio that represents the level of afucosylated IgG in a given sample. Here we examined the plasma from N=163 *P. falciparum* infected pregnant Malawian women with or without evidence of placental malaria at delivery using FEASI. Our results showed significantly higher levels of antigen-specific afucosylated IgG in women without evidence of placental malaria at delivery ($p < 0.001$). This finding suggests that afucosylated IgG levels could be a marker for protection and further experiments that explore the association between the levels of afucosylated IgG with neutrophil phagocytosis and NK cell activation against malaria infected erythrocytes are planned. These results have important implications in the understanding of naturally acquired protection to malaria in pregnant women.

Fc-dependent functional antibody responses in immunity to severe *Plasmodium falciparum* malaria in children

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Plasmodium falciparum malaria is a major cause of morbidity and mortality worldwide with young children being particularly susceptible to severe and fatal forms of the disease. Vaccines are a key intervention to help reduce the global burden of malaria. However, currently licensed vaccines have suboptimal efficacy, particularly against severe malaria, and do not target the blood stage of infection, which is associated with clinical symptoms and severe disease. A limited understanding of the targets and mechanisms of action of immunity against severe malaria hinders progress towards vaccines with greater efficacy. Antibodies play a key role in immunity and may mediate their protective function through different mechanisms, including direct neutralization and Fc-dependent functional antibody responses. Functional antibody responses include cross-linking with Fcγ receptors on different immune cells to mediate opsonic phagocytosis, cellular cytotoxicity or cellular inhibition. While these Fc-dependent functional antibody responses have been shown to be important in protective immunity against uncomplicated malaria, their role, along with the specific antigenic targets, in severe malaria remains unknown. In this study, we aimed to identify blood stage parasite antigens targeted by Fc-dependent functional antibodies and evaluate their association with protection against severe malaria in a cohort of children from Papua New Guinea (severe; n=201 and uncomplicated; n=163). Using a high throughput bead-based multiplex assay, we evaluated the functional activity of antibodies to interact with Fcγ receptors I, IIa, and IIIa/b, as well as IgG and IgM, for 33 *Plasmodium falciparum* antigens. We found that an increase in Fcγ receptor binding antibody responses to several antigens was associated with a substantially reduced risk of severe malaria. This included antibody responses to current vaccine candidate antigens and new or less-studied antigens. Furthermore, an increase in antibody responses to a breadth of both antigens and Fcγ receptors was associated with a greater reduction in the risk of severe malaria compared to individual responses. Overall, we identified antigens that were targeted by Fc-dependent functional antibody responses and associated with protection against severe malaria. These findings will inform the development of highly efficacious vaccines to reduce the burden of malaria, specifically the huge toll of severe malaria.

Naturally acquired functional antibody responses to *Plasmodium vivax* vaccine candidates are associated with protection against clinical malaria infections

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Malaria infections caused by *Plasmodium vivax* continue to be a major global health burden. Unique features of *P. vivax* biology compared to other *Plasmodium* species have greatly hindered efforts toward malaria elimination. *P. vivax* infections have undetectable dormant liver-stage infections known as the hypnozoite which can reactivate years after initial infection, contributing to the widespread presence of *P. vivax*. This highlights the importance of an effective vaccine that can specifically prevent *P. vivax* infection. However, compared to *P. falciparum* there have been no successful vaccine candidates to *P. vivax*, partly due to the inability to culture *P. vivax* long-term *in vitro.* By studying naturally acquired immunity to *P. vivax* in endemic populations, a better understanding of the target antigens and immune mechanisms required for an effective vaccine can be gained. Aside from direct inhibition of parasite invasion, antibodies also have important roles in adaptive and innate immunity through the activation of the complement pathway and engagement of Fcγ-receptors (FcγRs) which mediate clearance and development of immune memory against the parasite.

In this study we aimed to determine if functional antibody responses to potential blood and liver-stage *P. vivax* vaccine candidates were associated with protection against clinical malaria infection. We utilised a multiplexed Luminex assay to measure functional antibody responses (IgG, IgG1/IgG3 levels, C1q fixation, and FcγR binding) to 6 *P. vivax* antigens including PvEBPII, PvCSP and PvDBPII in plasma samples from a well-described longitudinal cohort study of children aged 1 – 3 years old from Papua New Guinea (n=264)[1]. Statistical methods were then used to assess the association of functional antibody responses with protection against subsequent clinical malaria infections during 16 months follow-up.

We observed that total IgG, IgG1, IgG3 responses to PvEBPII and PvDBPII were all associated with protection against clinical disease whilst FcγR responses only to PvEBPII were protective. These results suggest that although PvDBPII and PvCSP are the most advanced *P. vivax* vaccine candidates thus far, there is potential in studying PvEBPII further. Additionally, our work highlights that vaccine candidates to *P. vivax* that can induce protective functional antibody responses should be a priority during vaccine development.

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Developing Machine Learning Models to Understand CRISPR-Cas13b Silencing Principles

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CRISPR-Cas13b uses guide RNAs (or 'guides') to silence target RNA transcripts without causing permanent damage to their host genome. This technology has been successfully reprogrammed to silence SARS-CoV-2 replication in human cells, providing opportunities to develop Cas13b-based antiviral drugs. However, the reprogramming procedure involves randomly selecting effective guides from many candidates. Such a procedure lacks a thorough understanding of the molecular principles determining the guides' silencing efficacy, which is crucial to identifying highly effective guides. Thus, there is a need for an informed approach to identifying these principles. This approach will facilitate a more rapid selection of highly effective guides.

We developed various data-driven Machine Learning (ML) models to learn the mapping between the Cas13b guides and their silencing efficacy. We trained the models using a dataset of 236 30-nucleotide sequences of guides targeting various RNA transcripts, with their silencing efficacy quantified *in vitro*. Preliminary evaluation of the models' performance showed that simpler and interpretable models outperformed complex ones. Moreover, we analysed these models' behaviour and identified novel silencing principles of the guides compared to previous studies; for example, Adenine bases at the middle region and Guanine bases at the end region of a guide sequence enhance its efficacy. We aim to evaluate the models by comparing the predicted guides' efficacy against *in vitro* results not included in the training data. We also aim to improve the models' performance by enriching the training dataset with additional Cas13b guides matching the identified principles and quantified *in vitro*.

High throughput antimicrobial screening at CSIRO

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Antimicrobial resistance is one of humanity's greatest rising threats, killing more than a million people every year. Diseases that previously resulted in minor infections can become deadly because disease-causing microbes (bacteria and fungi) are acquiring resistance to previously effective antibiotics. This is a growing health crisis and is predicted to wreak catastrophic impacts on global health and economy if left unaddressed. CSIRO is tackling this problem by enabling new discoveries and accelerating research in this area. Recently, we have developed an in-house system for quickly and accurately testing a large number of compounds for antimicrobial properties against various bacterial and fungal strains, including antibiotic resistant ones. This capability has been supporting ongoing work using Artificial Intelligence to discover antimicrobial compounds and peptides. This work sits within the wider Biomedical Program which has capabilities in many areas, including drug discovery, production of biologics (from process development stage to pre-clinical and clinical scale) and development of biomedical materials.

Predictive and Generative AI for Drug Discovery: Identification of SARS-CoV2 NSP14 Inhibitors

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Virtual screening (VS) uses computer simulation and artificial intelligence to identify potential small molecule hits by searching molecule libraries. This *in silico* early drug discovery hit identification technique complements classical high-throughput physical screening and has the advantage of being much cheaper and faster. Recently, novel docking software were developed to run on graphical processing units (GPUs)[1]. These GPU-accelerated docking software have enabled critical speed improvements for the prediction of small molecule binding to protein targets, and therefore enabled VS on much larger libraries in shorter timeframes. In parallel, ultra-large virtual libraries provide new opportunities to identify novel chemical matter[2]. However, they are not amenable to VS even with GPU-accelerated docking as they number billions of compounds. Fortunately, active learning enables VS on ultra-large libraries[3] by iteratively training a predictive machine learning model as a surrogate to GPU-accelerated docking to further speed up VS[4]. Alternatively, generative modelling is an emerging paradigm for *in silico* hit identification[5], where the desired characteristics for a small molecule hit are defined, and a generative model is iteratively guided using reinforcement learning to generate compounds which meet these characteristics[6]. We will describe the development of both predictive and generative chemistry workflows at CSIRO and showcase their application to the SARS-CoV2 NSP14 methyltransferase, for which novel hits were predicted and experimentally validated.

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IL-6 as diagnostic and prognostic biomarker meta-analysis studies

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Background: Interleukin 6(IL-6) is a pleiotropic cytokine that activates various physiological immune responses to eliminate inflammation. However, its chronic dysregulation contributes to cancer initiation and sustains interaction with immune cells in the tumor microenvironment, which further facilitates cancer progression, metastasis, and chemoresistance.
Methods: Interleukin-6's meta-analysis was conducted to identify the strength of existing data in the current literature on its utility as a potential diagnostic and prognostic biomarker of ovarian cancer (OC).
Results: Metanalysis shows the diagnostic utility of IL-6 serum levels with 76.7% sensitivity (95% CI:0.71–0.92) and 72% specificity (95% CI: 0.64–0.79) compared to healthy and benign conditions. These levels of IL-6 were much higher in ascites, with a sensitivity of 84%(95% CI: 0.710–0.919) and specificity of 74% (95% CI: 0.646–0.82.[1] Preliminary data from our IL-6 prognostic meta-analysis also highlights the efficacy of IL-6 levels in predicting OC outcomes. Studies also indicated the association of blood IL-6 levels with disease-free survival, showing a hazard ratio of 1.60,95%CI. [2]
Conclusion: The meta-analysis studies suggested IL6 as a diagnostic and prognostic biomarker and provided future insight into including IL-6 as a targeted therapy for better management of OC. Further research will further validate the IL-6 inhibitors as a potential therapeutic target.

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Parasite-Host Metabolic Crosstalk to Detect Malaria

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Abstract

Current malaria diagnostic methods have inherent practical and diagnostic limitations, often lacking the required sensitivity. Previous research in our laboratory has revealed a fascinating phenomenon known as the bystander effect. This effect leads to alterations in glycolysis within bystander uninfected RBCs when co-cultured with infected RBCs in same culture dish. Given the relatively low count of infected RBCs in malaria patients, harnessing the potential impact of the bystander effect on the metabolism of the much more abundant uninfected RBCs could offer a highly sensitive technique for detecting the presence of malaria.

Bystander uninfected RBCs exposed to conditioned media from both asynchronous and synchronous cultures showed a similar increase in phosphofructokinase activity, suggesting that the signaling factors derived from the parasite might not necessitate the rupture of RBCs. To identify these factors responsible for the bystander effect, we employed size-based fractionation, followed by exposure to bystander RBCs and subsequent metabolomics analysis. The analysis revealed that the enhancement of glycolysis is driven by small molecules rather than proteins or extracellular vesicles. Clinical DBS samples were obtained from malaria patients across a spectrum of parasite infection intensities, as well as from healthy individuals in a malaria-endemic region of Ethiopia. In our in vitro validation study, most blood metabolites remained detectable in the DBS samples. A DBS metabolomics analysis will assess metabolic disturbances in uninfected RBCs due to malaria and explore its potential as a diagnostic marker. Diagnostic accuracy will be evaluated using ROC analysis of individual and combined metabolites, with sensitivity tested across different parasitemia levels, including asymptomatic cases.

How to catch a parasite red-handed: Looking for *Plasmodium falciparum* exported proteins in the infected hepatocyte using proximity ligation.

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Plasmodium falciparum is the intracellular parasite that causes most cases and deaths from malaria disease. It is inoculated into the human body through an infected bite from *Anopheles* mosquito, and firstly invades human hepatocytes, where it develops and multiplies. Only after the parasites are released from hepatocytes and invade red blood cells does malaria occur. *P. falciparum* dramatically modifies the host red blood cell by exporting numerous proteins into the cell, but the role of such export during infection of the hepatocyte is unexplored. However, malaria parasites effectively alter host cellular immune responses to develop within the hepatocyte without being killed. We hypothesise that this occurs through the export of parasite effector proteins that are exported to the host cell and vacuole membrane interface between host and parasite, that subvert host innate immune signalling.

To identify parasite proteins exported into or facing towards the hepatocyte, we are using a proximity ligation approach. This methodology uses the biotin ligase, TurboID, to biotinylate proteins with close proximity, approximately 40 nm. We have generated hepatocytes expressing TurboID in two locations: one localised in the nucleus and another in the cytoplasm. Infection of these transgenic hepatocytes with *P. falciparum* sporozoites expressing mScarlet will allow the purification of biotinylated parasite and host proteins from infected cells by mass spectrometry. Such proteins will be validated as exported by immunofluorescence microscopy using antibodies and functionally characterized by generated isogenic parasites lacking the genes of interest. Identification of exported liver stage proteins is important for potential target malaria interventions including pre-erythrocytic vaccines.

Defining targets and mechanism of action of immunity against *Plasmodium vivax* circumsporozoite protein.

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Despite global efforts for elimination there is no significant decline in cases and deaths of malaria annually. *P. vivax* is one of the most prevalent *Plasmodium* species causing malaria in humans. Increases in the proportion of *P. vivax* malaria cases in some populations highlights the inadequacy of current control measures and the need for a vaccine that specifically targets *P. vivax*. A major bottleneck in developing *P. vivax* vaccines has been limited knowledge of the targets and mechanisms of action of protective immunity.

A promising vaccine target is the *P. vivax* circumsporozoite protein (*Pv*CSP) which is essential for establishing a successful infection. Antibodies play a key role in immunity against malaria and have been shown in *Plasmodium falciparum* to act through different mechanisms including complement-fixation and engaging Fcγ-receptors (FcγR) which are expressed on immune cells to mediate phagocytosis and cellular cytotoxicity. However, knowledge is limited on the functions of antibodies targeting *P. vivax* and *Pv*CSP specifically. To address these gaps, we quantified the magnitude and functional activity (complement-fixation, binding of FcγRI, FcγRIIa, FcγRIIIa/b, opsonic phagocytosis) of antibodies to *Pv*CSP, and investigated the specific targets of these antibodies, including the central repeat region of the two major allelic variants, and the conserved N- and C-terminal regions. We used antibody samples from malaria-exposed adults in Papua New Guinea and those generated in rabbits following vaccination with *Pv*CSP.

Antibodies were acquired against both major variants of *Pv*CSP. Antibody-mediated FcγRI binding was consistently high across all regions of *Pv*CSP. There was significant binding of FcγRIIIa/b by antibodies, whereas FcγRIIa binding and complement-fixation were generally low. Overall, antibody magnitude and function were higher against the C-terminal and central repeat region compared to the N-terminal region. Antibodies could also promote opsonic phagocytosis by monocytes. These results provide new insights into the important roles of different regions and variants of *Pv*CSP as targets of *P. vivax* antibody responses, including mechanisms of action involved. This knowledge will inform future design of *Pv*CSP vaccines to maximize the induction of protective antibodies.

A comprehensive analysis of the regulatory mechanisms underlying the enzymes of the TCA cycle-glyoxylate shunt junction in Mycobacterium tuberculosis as a novel drug target for the age-old pandemic

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Tuberculosis is an ancient disease, yet today, it still kills over 1 million people every year. The responsible pathogen – *Mycobacterium tuberculosis* (*Mtb*) – has a unique metabolism that enables it to evade our immune system instead of being eradicated by it. One of the metabolic pathways highlighted as essential to the survival of *Mtb* was the glyoxylate shunt. This shunt creates a junction point in the tricarboxylic acid (TCA) cycle, and studies have indicated that a tight regulation of this junction is required for the pathogen's survival, but it is not known how this regulation occurs. This study aims to investigate the regulation of this junction by looking at the two gatekeeping enzymes – isocitrate lyase (ICL) for the glyoxylate shunt and isocitrate dehydrogenase (ICD) for the TCA cycle. The regulation of these enzymes was studied at both the gene- and protein-level. At the gene level, quantitative PCR of *Mtb* cultures revealed that ICL responds significantly to the nutritional environment. At the protein level, screening of metabolites from central carbon metabolism uncovered novel inhibitors and activators of ICL and ICD. Kinetic analysis showed that ICD is more regulated at the protein level by allosteric effectors and that its activity increases in the presence of an active ICL. This comprehensive analysis of the junction at different levels provides a multi-pronged approach for eradicating tuberculosis once and for all.

Defining the on-target activity of *P. falciparum* plasmepsin V peptidomimetic inhibitors

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The prevalence of resistance of malaria parasites against drugs in the field leads to a need for novel antimalarial discovery and development. Plasmepsin V is an aspartyl protease which is essential for the export of proteins from the parasite to the host erythrocyte during the asexual stage of parasite, making it an ideal target for novel antimalarial development. Peptidomimetics that mimic the substrate of plasmpesin V have been designed and shown to elicit parasite death via blocking protein export. While the peptidomimetics have been used to validate plasmepsin V as a bone fide antimalarial target, disparities between biochemical and parasite activity have questioned their on-target activity.

Here, we generated a parasite line with reduced sensitivity to the peptidomimetics. A single nucleotide polymorphism (SNP) located in the plasmepsin V gene was identified through whole genome sequencing. Reverse genetics and biochemical assays using immunoprecipitated plasmepsin V were used to validate the resistance-causing SNP. This data was supported by methods such as cellular thermal shift assays that showed target engagement with plasmepsin V in parasites. This data supports the previous evidence that the peptidomimetics kill the malaria parasite by targeting plasmepsin V and further establishes plasmepsin V as a promising antimalarial drug target.

Proteomics-based Drug Target Identification in *Plasmodium falciparum*

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Malaria continues to be a significant global health threat, largely due to the emergence of drug-resistant strains of *Plasmodium falciparum*, the most lethal of the malaria parasites. With the efficacy of current treatments under threat from resistance, there is a critical need to identify novel drug targets to combat malaria. Proteomics-based approaches are increasingly recognised as powerful tools for drug target identification in *P. falciparum*, offering insights into the parasite's complex proteome and its interactions with antimalarials [1].

In this study, the Halo-tag TurboID method and the Stability of Proteins from Rates of Oxidation (SPROX) technique represent complementary approaches for target identification. The Halo-tag TurboID method utilises Halo-tag that covalently binds to a chemically modified ligand, allowing for biotin labelling by TurboID, thus detecting proteins of interest [2]. This technique facilitates high specificity in studying ligand-protein interactions in live cells or lysates. In contrast, SPROX measures analyses the rate of oxidation, drawing on ligand binding's impact on stability [3]. Combined, these methods offer a comprehensive framework for investigating protein functions, interactions, and stability in Plasmodium parasites.

Initially, we validated these methods using well-established mammalian cell systems and known compounds, such as JQ1, a bromodomain inhibitor. Following validation, we will adapt the optimised approach to study protein–ligand interactions in a more complex system: *Plasmodium falciparum* parasites, specifically focusing on phosphatidylinositol 4-kinases (PI4K) and its inhibitors. This phase represents the core of our method development, aiming to refine the techniques for application in less-characterised and more challenging biological contexts.

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Interrogating circulating immune cell methylome differences across the TB disease spectrum

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Inhibition of type I interferon signalling during *Shigella flexneri* infection

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Shigella flexneri is a gram-negative bacterium that causes shigellosis, an intestinal infection that can lead to bloody diarrhoea. The bacteria replicate intracellularly in epithelial cells and spread from cell to cell by inducing actin polymerization at one pole of the bacterial cell. The ensuing inflammation leads to destruction of the intestinal epithelium. The ability of *Shigella* species to infect host cells is dependent on a type III secretion system (T3SS) that translocate effector proteins into the host cytosol that manipulate cellular functions to promote bacterial survival. Many effector proteins target innate immune signalling pathways, including interferon signalling. Interferons are cytokines that trigger host immune responses via the activation of interferon stimulated genes (ISGs). Unusually, different T3SS effector proteins of *Shigella* have been associated with inhibition of the type I interferon pathway, which is normally involved in viral defence. Our preliminary data has shown that the *S. flexneri* effector proteins, OspD2 and OspD3 inhibit a type I interferon induced ISRE reporter. Both OspD2 and OspD3 are predicted to be cysteine proteases based on the homologous effector EspL from EPEC.

Metabolic mysteries of bat urine and faeces: A potential non-invasive tool to monitor flying foxes under different ecological conditions

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Flying foxes (Pteropus spp) often roost in large aggregations, favouring habitats near abundant nocturnal foraging areas rich in native flowering eucalypts (Granek 2002; Gulraiz et al. 2015; Oleksy et al. 2015). As a result, locally available foraging sources influence patterns of roost occupancy and abundance. However, there has been a shift in their foraging habitats to urban landscapes dominated by a mix of native and exotic plants (Plowright et al. 2011, Timmiss et al. 2020). Downstream metabolic pathways of flying fox individuals are expected to be impacted by this spatial variation in tree community composition as well as the flowering phenology of tree species.

Moreover, recent changes in flying fox ecology driven by climate-driven changes in food availability (acute food shortages) and land use change, have been associated with the timing and number of Hendra virus spillover events. While current models provide a framework to predict Hendra virus spillover clusters through monitoring of proxies of bat fitness and climate, the mechanistic links between these environmental risk factors and physiological outcomes in bats that precipitate spillover are not elucidated. Understanding how flying fox metabolism changes with seasonal and interannual differences in food availability could be key to linking their foraging habits with virus shedding dynamics. To address this, we optimised a protocol for non-invasive under roost sampling of excreta from wild bat populations across four sites and subsequent metabolomics profiling using Nuclear Magnetic Resonance. We demonstrate significant changes in the profile of almost fifteen polar metabolites in urine of wild flying fox populations between summer, when an acute food shortage was observed and the following autumn. In turn, it is hypothesised that nutritional shifts across seasons or food shortages may affect downstream energy stress pathways or resource allocation to immunity investments. This work builds on a recent laboratory study performed on captive bats subjected to different diets and appears to be a promising approach in understanding the mechanisms of bat borne virus shedding in nature.

Rifaximin and the Evolution of Daptomycin-Resistant *Enterococcus faecium*

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Bacterial pathogens such as vancomycin-resistant *Enterococcus faecium* (VREfm) that resist almost all antibiotics are a public health threat. Daptomycin is a last-resort antibiotic, but for which resistance has surprisingly been reported. The objectives of this study were to define the mechanisms of daptomycin resistance in VREfm. Clinical VREfm (n=1000) collected during surveillance studies in Australia underwent daptomycin susceptibility testing and whole-genome sequencing. Mutations associated with daptomycin resistance were identified using a genome-wide association study (GWAS) and confirmed with molecular approaches. A blinded retrospective patient cohort study was used to understand the association between daptomycin resistance in VREfm with the unrelated antibiotic, rifaximin. Daptomycin-susceptibility testing indicated 18.9% (n=189/1000) of VREfm were daptomycin-resistant, giving Australia one of the highest rates of resistance globally. A GWAS suggested daptomycin-resistant VREfm contained novel mutations, including within the rifampicin-resistance determining region of RpoB, typically associated with resistance to the class of rifamycin antibiotics. Isogenic mutants containing the identified RpoB mutations displayed resistance to rifamycins and cross-resistance to daptomycin. An analysis with international VREfm suggested the novel RpoB mutations were globally distributed, with three co-circulating lineages having emerged soon after the rifamycin, rifaximin, was approved for clinical use. In patients, recent rifaximin exposure was significantly associated with the presence of RpoB mutations in VREfm ($P<0.001$) and associated with the presence of a daptomycin-resistant VREfm isolate ($P<0.001$). In a murine model of VREfm gastrointestinal colonization, rifaximin exposure resulted in the *de novo* emergence of VREfm resistant to rifaximin and daptomycin, due to the presence of specific mutations in RpoB. Our study has identified a novel and globally prevalent daptomycin resistance mechanism in VREfm and highlights the serious, unanticipated “collateral damage” that may arise in patients colonised with VREfm following rifaximin use.

Establishing novel therapeutics for HTLV-1

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HTLV-1c is an endemic infection of Central Australia where it infects up to 50% of the First Nations community. Complications from infection include fatality from an aggressive Adult T Cell Leukaemia (ATL), chronic pulmonary disease and bronchiectasis amongst a myriad of other complications. Despite these health implications and despite being first isolated 45 years ago there remains no cure for HTLV-1 infection. We have characterised a humanised mouse model of HTLV-1c infection that recapitulates important aspects of human disease in a preclinical model. Through this model, we have identified MCL-1 as a pivotal target to HTLV-1 survival and have used this knowledge to test novel combinations of cell-death inducing compounds for their efficacy in viral elimination.

ABSTRACTS POSTER SESSION II

DECODING THE EFFECTOR-MEDIATED DIALOGUE BETWEEN *COXIELLA BURNETII* AND ITS HOST DURING INFECTION

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Coxiella burnetii is an obligate intracellular bacterium and the causative agent of Q fever in humans. In contrast to most intracellular bacteria, *C. burnetii* is a member of an exclusive subset of pathogens distinguished by their capacity to persist within a lysosome-derived vacuole. Crucial to the establishment of this niche and the subsequent intracellular success of *C. burnetii* is the expression and activation of a Dot/Icm Type IV secretion system (T4SS). This system facilitates the translocation of approximately 150 bacterial effector proteins directly from the bacterial cytosol into the infected host cell cytoplasm. Through the collective action of these effectors, *C. burnetii* can modulate multiple host cellular processes to promote bacterial replication, maintain host cell viability and avoid immune detection. Despite the significance of this dynamic, a comprehensive profile of the underlying molecular mechanisms at the host-pathogen interface remains largely incomplete.

In this study, we have employed phosphoproteomics to examine the influence of *C. burnetii* on phospho-signaling pathways in macrophages. Multiple pathways including innate immune signaling, lysosome regulation and autophagy are influenced by *C. burnetii* infection. Comparison between wildtype and Dot/Icm-deficient infections has enabled a global snapshot of the influence the *C. burnetii* effector cohort has on phosphorylation and provides a map through which the actions of individual effector proteins can be elucidated.

Rational design of live bacterial therapeutics to clear *Klebsiella pneumoniae* from the gut

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Introduction

Antimicrobial resistance and multidrug resistant organisms (MDROs) pose a significant threat to global health. With few new antibiotics in the development pipeline, novel strategies to combat MDROs are urgently needed.

The gastrointestinal microbiome plays a critical role as a chemical and physical barrier against invasion of pathogenic microorganisms. Disruption of the gut microbiota, primarily after antimicrobial usage, is recognised as a key factor leading to increased susceptibility to gastrointestional colonisation and overgrowth with MDROs such as carbapenem-resistant *Klebsiella pneumoniae* (CRE-Kp). Modulation of the microbiome is a promising approach to prevent of clear MDRO gut colonisation.

Methods

1200 human gut commensal bacteria were isolated using YCFA-based culturomics. All isolates were screened using miniaturised, high throughput, co-cuture assays to identify isolates that inhibit the growth of CRE-Kp. Selected commensal isolates were also screened using agar overlay assays. An *in silico* tool (SHIELD) to predict commensal communities with CRE-Kp inhibitory activity has been developed and used to design communities comprised of commensals from our biobank. Several of these *in silico* designed communities have been tested for anti-CRE-Kp activity using *in vitro* co-culture assays

Results

We have successfully isolated 1200 human gut commensal bacteria from healthy stool samples and used these isolates to develop an accessible human gut commensal biobank. Using high throughput screening we identified 127 commensals that inhibit the growth of CRE-Kp and 53 commensals that completely kill CRE-Kp under *in vitro* co-culture conditions. *In silico* design of microbial communities made up of commensals from our biobank has resulted in the development of potential live bacterial therapeutics (LBTs) with significant *in vitro* anti-CRE-Kp activity.

Discussion and conclusions

Our analyses have identified a range of commensal species that inhibit CRE-Kp using a diverse array of mechanisms including nutrient blocking and the production of bioactive primary and secondary metabolites such as short chain fatty acids and bacteriocins. Our development of *in silico* tools to predict commensal communities that can inhibit CRE-Kp is a promising approach that will facilitate the development of LBTs that can be tested under *in vivo* conditions to determine their efficacy in clearing MDROs from the gut.

Exploring bacteriocins in infection-causing *Klebsiella* isolates

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Bacteriocins are toxic proteins produced by bacteria to outcompete closely related species for resources such as nutrients and space. Due to their potent antibacterial properties, bacteriocins are becoming increasingly valuable in food and health industries. Traditional large-scale phenotypic screening methods to hunt bacteriocins are often time-consuming and costly. Therefore, we present an *in silico*-based search to identify highly divergent and uncharacterized bacteriocins in *Klebsiella* genomes. Using the published resources such as BAGEL database and current literature, we curated a database bacteriocins containing representative cluster sequences. We then used HHblits, to develop HMM profiles for these bacteriocins and then scanned 452 clinical *K. pneumoniae* genomes collected from patients in Alfred Hospital, to identify the bacteriocins present in the infection causing isolates. To minimize false positives, we applied a stringent 50% cut-off for the alignment with known bacteriocins and accounted for potential bias caused by genomes belonging to transmission clusters. Additionally, we analysed the genomic context of the bacteriocins and investigated their distribution across different *Klebsiella* lineages. Our preliminary results reveal that bacteriocins, especially those with colicin V-like structure, are present in ~83% of *Klebsiella* genomes. Colicin V-like bacteriocins display 42 different sequence variation or “alleles” and significant structural diversity that correlate with lineage classification. Structural analysis identified functional hot spots in these bacteriocins, offering insights into their potential antibacterial mechanisms. We also identified putative immunity proteins associated with these bacteriocins. Using this information, along with identification of the putative receptor binding protein we aim to predict the bacteriocin immunity profiles for each isolate. This *in silico* study provides a rapid pathway to targeted, alternative antimicrobial strategies, reducing the time and cost associated with large-scale phenotypic screening.

Identification and Characterisation of *Cryptosporidium* Effector Proteins in Host-Pathogen Interaction

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The apicomplexan parasite *Cryptosporidium* causes severe diarrheal disease, cryptosporidiosis, and is a leading cause of childhood mortality globally. Chronic diarrhoea leads to malnutrition and wasting in children under five, affecting physical and cognitive development. The lack of diagnostic tools, effective treatment and vaccines for cryptosporidiosis exacerbates the global impact of this neglected pathogen. *Cryptosporidium* infects host enterocytes and like its cousins, *Plasmodium* and *Toxoplasma*, secrete proteins into its host cell to establish infection and modulate immunity. These so-called 'effector' proteins of both *T. gondii* and *P. falciparum* are proteolytically processed by aspartic acid proteases which is essential for protein trafficking and function. In recent years, these proteases have been shown to be excellent drug targets, some of which have progressed to human clinical trials. However, little is known about *Cryptosporidium* aspartic acid proteases and their involvement in effector cleavage.

In this study, we used TurboID and APEX- expressing host cell lines to identify 'effector' proteins of Cryptosporidium, which we believe are candidate substrates of a *C. parvum* aspartyl protease, CpASP4. Using newly developed CRISPR-Cas12 technology and mouse models, we have generated transgenic HA-tagged parasites for further study and determined localisation of candidate effectors. With the use of a CpASP4 inhibitor compound, we can investigate protease function in the processing and maturation of substrates along with a better understanding of the inhibitor mechanism of action. This study will expand the knowledge of *Cryptosporidium* host-pathogen interactions and the importance of proteases in this process. Elucidating molecular machinery that is crucial for parasite survival will aid in the discovery of therapeutic targets, allowing development of new anti-cryptosporidial agents.

Legionella pneumophila Dot/Icm effector triggers host heat shock response to facilitate intracellular replication

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Legionella pneumophila is a Gram-negative bacterium that causes Legionnaires' disease, a severe form of pneumonia. *L. pneumophila* primarily infects alveolar macrophages where it is able to establish an intracellular replication niche. An important virulence factor that allows intracellular replication is a type IV secretion system (T4SS) called Dot/Icm, that translocates over 330 bacterial proteins, termed effectors, into the infected host cell. Many Dot/Icm effectors are known to possess eukaryotic-like domains and can manipulate host processes. However, only a minority of effectors have been fully characterized. In this study, we created a library of 14 *L. pneumophila* genomic mutant strains lacking effector-rich regions (85 effector genes deleted in total) and used the mutants to interrogate their roles during infection of THP-1 macrophages.

While all of the mutants replicated efficiently in THP-1 macrophages, infection led to distinct transcriptomic responses from the macrophages. Wild-type (WT) *L. pneumophila* 130b triggered a strong inflammatory and cellular stress responses, including the interferon and the heat shock response (HSR). In contrast, upon infection six mutants displayed a reduced interferon response compared to WT infection, and one mutant displayed a marked decrease in the expression of host HSR genes. Through the creation of further sub deletion mutants, we subsequently identified the Dot/Icm effector protein LegK4 as responsible for the induction of the host HSR genes. We found that the kinase activity of LegK4 induced HSR genes 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 stage of *L. pneumophila* infection. Altogether, these suggest that *L. pneumophila* triggers host HSR to facilitate bacterial replication.

Ecology and Diversity of Avian Paramyxovirus, the Causative Agent of Newcastle Disease, in Australian Wild Birds

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PUBLISH CONSENT WITHHELD.

Characterization of the structure and dynamics of oral polymicrobial biofilms

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Periodontitis is a chronic, inflammatory disease characterised by the destruction of the gums and bone supporting the teeth. It is associated with a build-up of subgingival plaque bacteria, specifically, *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* that correlate strongly with the onset and progression of the disease. A fourth species, *Fusobacterium nucle*atum is considered a bridging species since it acts as an interface between early and late colonizers in dental plaque. The interaction between these bacteria has been documented, but the molecular mechanisms underlying the development of multi-species biofilms are poorly understood.

Our study aims to provide detailed descriptions of the architecture of biofilms containing these species at all stages of their development using high-resolution imaging modalities. We have grown all three bacteria inside the flow cell system for 90 hrs, resulting in microcolonies about 300 µm thick. The biogeography of the system will be explored by analysing it at several time points. Moreover, using cryo-electron tomography, we have recorded the molecular structures of individual bacteria in their native form. Interestingly, T. forsythia is decorated with a 2D crystalline surface (S-) layer, which is composed of two glycoproteins, TfsA and TfsB. We were able to get a high-resolution structure of the S-layer revealing a unique structure consisting of three layers, 13 nm thick. contribute to adhesion and invasion of cells, and they assist in bacterial co-aggregation. Also, T. denticola has been seen to have appendage-like structure decorated by outer membrane protruding out of the cell. Furthermore, using cryo-electron microscopy of biofilm thin sections produced by focussed ion beam (FIB) milling, we have recorded molecular structures that mediate the co-bacterial interactions and thus hold the biofilm together.

This study will aid in the understanding of the structure and ecology of bacterial biofilms in general, and more specifically subgingival plaque. The knowledge gained could be used in the future for designing treatments to interfere with the development of the biofilms that cause periodontitis.

Aztreonam and ciprofloxacin combination therapy yields synergistic results for resistant *Pseudomonas aeruginosa* strains

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Introduction. Antibiotic resistance is an emerging threat throughout the globe. Effective use of antibiotics is crucial for successful treatment of infections and suppression of antibiotic resistance.

Aims. To investigate the efficacy of aztreonam (ATM) and ciprofloxacin (CIP) combination therapy on mutant *Pseudomonas aeruginosa* (Pa) strains with defined resistance mutations and clinical isolates.

Methods. The PAO1 wild-type reference strain, its five isogenic strains (PAΔdacB, PAΔDDh3 and PAΔDDh2Dh3 with AmpC β-lactamase hyperproduction caused by different mutations, and PAΔmexR and PAΔmexZ with MexAB-OprM and MexXY-OprM efflux pump overexpression, respectively), and three multidrug-resistant (MDR) clinical paediatric isolates (ICU-11, FQSE15-0803 and FQSE06-1104) were investigated in 72-h static concentration time kill studies. Clinically relevant concentrations of CIP and ATM were studied. Bacterial viable counts were determined. A mechanism-based mathematical model was developed to estimate the effect of each mutation on intracellular concentrations of the antibiotics, therefore predicting the antibacterial activity of ATM and CIP and the consequent growth or killing of Pa.

Results. For all strains except PAΔmexR, synergy (≥2-$\log_{10}$ difference from most effective monotherapy) was observed with clinically achievable ATM concentrations (ATM 6.4 mg/L for strains FQSE15-0803 and FQSE06-1104 from paediatric patients with CF, and ATM 25 mg/L for all other strains) plus CIP 0.5 mg/L. For PAΔmexR, ATM 25 mg/L plus CIP 1 mg/L resulted in synergy. All monotherapies failed, except for 1 mg/L CIP against PAΔDDh2Dh3 and 25 mg/L ATM against FQSE06-1104 which both successfully suppressed resistance. The mechanism-based mathematical model described the data well.

Discussion. Combination therapy of CIP and ATM successfully killed Pa and inhibited regrowth over 72-h in the wild-type strain, isogenic strains, and MDR clinical isolates when most monotherapies failed to do so. Further investigation of this promising synergistic combination is warranted.

The molecular mechanisms of axon degeneration in flavivirus infection

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Flaviviruses such as Japanese encephalitis and West Nile virus, hijack axonal transport mechanisms to spread throughout the hosts nervous system. They can infect our neurons causing severe and often fatal damage.

During viral infections, neurons activate self-destruction of their long processes called axons. Recently it was identified that this process occurs through the action of Sterile-alpha and TIR motif containing 1 (SARM1). SARM1 has a unique ability to digest the essential energy molecule NAD^+ initiating a cascade of events, committing the axon to degeneration. However, the role of SARM1 during viral neuroinvasion and as an innate immune molecule is not fully understood.

This project uses an ex-vivo model of primary embryonic mouse neural cultures to investigate the role of SARM1 in flavivirus induced axon degeneration. It has been identified that flavivirus infection in neurons activates axonal degeneration partially mediated by SARM1 protein. Further evidence suggests that astrocytes, key neuronal support cells in the nervous system, may also have a role in the activation of this pathway.

This project aims to identify how SARM1 and astrocytes contribute to axon degeneration across multiple strains of flaviviruses. Gaining this knowledge will significantly contribute to understanding the neuropathology induced by flavivirus infection. Furthermore, by using neurotropic viral infections as a tool we can explore the complex role of the SARM1 gene which has implications across other neuroinvasive viral infections and additionally neurodegenerative diseases.

Systemic Inflammation in Solid Tumour Malignancy Patients Impairs Generation of *de novo* SARS-CoV-2 Vaccine Responses

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Patients with solid tumour malignancies are at increased risk of adverse outcomes from COVID-19. In addition, licensed vaccines against SARS-CoV-2 are less effective in immunocompromised patients, suggesting a need for optimised vaccination strategies. However, mechanisms underpinning impaired seroconversion in at-risk populations remain poorly defined. Here, within a cohort of SARS-CoV-2 vaccinated solid tumour malignancy patients, we performed detailed characterisation of vaccine-induced antibody responses to investigate the influence of chronic inflammation upon humoral immunity.

Following two BNT162b2 mRNA vaccine doses, immunoglobulin G (IgG) responses against the antigenically conserved SARS-CoV-2 Spike 2 domain were equivalent between individuals with or without solid tumours, facilitating comparable Spike Trimer-specific Fc effector functions that were maintained across SARS-CoV-2 variants of concern. In contrast, cancer patients generated ~30% reduced IgG against the more novel receptor binding domain (RBD) resulting in ~30% lower neutralisation capacity associated with solid tumour malignancies.

Primary AZD1222 adenoviral vector vaccination of cancer patients, on the other hand, was unable to induce Fc effector functions comparable to those observed in AZD1222 vaccinated healthy controls, underscoring the contribution of distinct vaccination platforms for optimised protection of vulnerable individuals.

Reduced RBD-specific IgG and neutralisation following either BNT162b2 or AZD1222 primary vaccination were further correlated with increased concentrations of inflammatory biomarkers (IL-6, IL-18, and agalactosylated total IgG), implicating the proinflammatory cytokine milieu in suppression of *de novo* antibody responses.

Following a third mRNA vaccine dose, RBD-specific IgG in cancer patients was significantly boosted, matching responses in healthy controls. While cytophilic IgG1 and IgG3 levels were equivalent regardless of cancer diagnosis, IgG4, which exhibits limited Fc functional capacity, remained low or absent in cancer patients (~4-fold lower). Critically, diminished SARS-CoV-2-specific IgG4 was not associated with overall impaired seroconversion, but was negatively correlated with agalactosylated IgG abundance, implicating individualised inflammatory microenvironments in the modulation of IgG subclass distribution.

Our study provides key insights into the influence of the inflammatory microenvironment upon impaired humoral responses to vaccination. We further demonstrate an under-appreciated role for non-neutralising antibody functions central to the development of robust vaccine responses within this vulnerable population. This may inform rational design of vaccines for specific at-risk groups.

Unravelling the Impact of Changing Ionizable Lipids on mRNA-LNP Vaccine Pharmacokinetics and Biodistribution

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In vaccine development, mRNA-based vaccines are valued for their rapid production, cost-effectiveness, and enhanced safety[1]. However, challenges like instability and poor cellular uptake persist[2]. Lipid nanoparticles (LNPs) have emerged as key carriers, yet the influence of different ionizable lipids on pharmacokinetics (PK) and biodistribution remains unclear.

This presentation examines how varying ionizable lipids—MC3, SM102, ALC0315, and 113-O12B—affect PK profiles and biodistribution in mRNA-LNP formulations. We created mRNA-LNP constructs with nLuc-GFP mRNA and administered them to B6 mice via intravenous (IV) and subcutaneous (SC) routes. Plasma and tissue samples were collected at 0-24 hours post-IV and 0-48 hours post-SC to evaluate these effects.

Our study reveals that different ionizable lipids significantly influence mRNA-LNP pharmacokinetics and biodistribution. Notably, SM102 provided the highest mRNA protection and bioavailability, whereas ALC0315 itself presented prolonged plasma circulation (Fig.1). SC injection was associated with lower liver accumulation compared to IV administration. Notably, IV administration showed similar or higher radiance in lymph nodes and spleen compared to SC. After SC injection, biodistribution were significantly influenced by both the type of ionizable lipid and time, with MC3 catching up to other lipids in radiance at 48 hours. For IV injection, ionizable lipids significantly impacted radiance in all organs and tissues, with MC3 showing significant lower initial radiance but similar levels at 24 hours. These results underscore the crucial role of ionizable lipids in determining mRNA-LNP vaccine performance, highlighting key factors for optimizing the effectiveness and safety of vaccines.

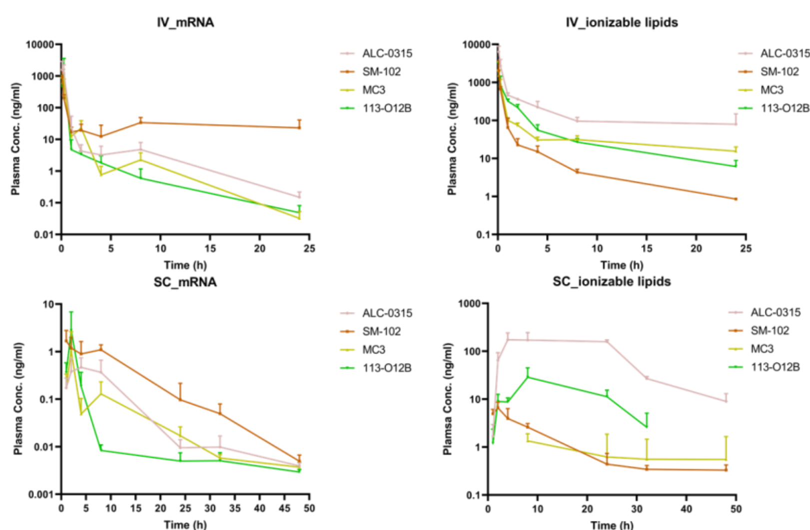

Figure 1. Plasma PK profiles of mRNA and ionizable lipids

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Developing Novel Lipid Nanoparticles To Reprogram Lung Macrophages.

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Respiratory diseases are a major global health issue, causing over 8 million deaths each year. Chronic obstructive pulmonary disease (COPD) and asthma affect more than 500 million people worldwide, while lung cancer, the leading cause of cancer-related deaths, claims 1.8 million lives annually (1, 2). Emerging treatments are focusing on targeting alveolar macrophages (AMs), which play critical roles in immunity, chronic inflammation, and immunopathology. However, targeting these cells has been challenging due to their location within the lung lumen and the low efficiency of current transfection methods. Lipid nanoparticles (LNPs) offer a promising solution as mRNA carriers, potentially overcoming these challenges, however, there is a pressing need for further innovation in developing tissue and cell-specific formulations. We screened a library of LNPs containing ionizable lipids SM-102 and ALC-0315, along with helper lipid monoolein and cholesterol, to find the optimal formulation for delivering mRNA to AMs. Formulations α=50 ALC/MO and α=50 SM/MO exhibited strong binding to the MH-S mouse alveolar macrophage cell line, but only the SM LNP effectively translated GFP mRNA into GFP protein. Adding cholesterol to the SM/MO LNP formulation significantly enhanced translation efficiency, with the 20CHOL LNP showing the highest GFP fluorescence intensity and a transfection efficiency of 70%. Altering the N/P ratio did not significantly impact transfection or fluorescence intensity. This study identified the 50SM/30MO/20CHOL formulation at N/P 6 as a most effective LNP carrier for delivering mRNA to AMs, showing potential for advancing cell-based therapies for lung diseases.

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Using Big Data for Rational Vaccine Design to Elicit Broadly Neutralizing Antibodies Against Hepatitis C Virus

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Prophylactic vaccines are crucial for achieving global elimination of the Hepatitis C Virus (HCV). However, no such vaccine currently exists due to the extreme genetic diversity of HCV and its high mutation rate during replication, which enable the virus to evade host immune responses. Broadly neutralizing antibodies (bNAbs), capable of neutralizing multiple viral strains, have been recognised as an essential component of an effective immune response against HCV. The challenge is how to select optimal vaccine antigens that can elicit bNAbs targeting conserved regions of the virus. To address this, we take advantage of large biological data and advanced biophysics and computational methods to rationally select HCV antigens. We first leverage HCV envelope protein (E2) sequence data from the HCV-GLUE database (http://hcv-glue.cvr.gla.ac.uk/#/home) to infer a statistical fitness landscape model for understanding fitness costs associated with mutations in E2 residues. Residues with high mutational fitness costs generally represent functionally important sites that are difficult to mutate for viral immune evasion, making them ideal vaccine targets. Then, we identify key E2 residues for bNAb recognition using experimental HCV bNAb complex structure data available in Protein Data Bank (https://www.rcsb.org/). We define a novel computational framework integrating fitness costs and bNAb binding information, capable of selecting optimal antigens from a candidate pool for further experimental testing. This data-driven approach for optimal antigen selection can aid in accelerating the development of effective HCV vaccines.

Serological and molecular analyses define the antigenic evolution of the influenza B virus neuraminidase over 81 years

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Abstract

Introduction: Current influenza vaccines are formulated to induce antibodies against the most dominant surface protein, haemagglutinin (HA). The addition of neuraminidase (NA) in vaccines might provide additional protection against IBV isolates that have drifted antigenically in the HA. However, the extent of IBV NA antigenic evolution and the mutations that underpin it are ill-defined.

Methods: An optimised Enzyme-Linked Lectin Assay (ELLA) without HA interference was applied to 15 ferret antisera and 80 IBV isolates from 1940-2021. This NA inhibition (NAI) antigenic dataset was paired with phylogenetic analysis of 319 IBV from the same time period to identify cluster-defining mutations. Fluorescently tagged recombinant NA (rNA) probes were used to identify IBV NA-specific B cells from healthy donors pre- and post-vaccination. Single-cell sorting and BCR sequencing were performed to generate a panel of anti-IBV NA monoclonal antibodies (mAbs). These mAbs were characterized *in vitro* against NAs from 1940-2021 and epitopes were identifying by escape mutant generation. Reverse genetics were used to test the antigenic impact of NA mutations.

Results: Antigenic cartography of NAI data from ferret antisera indicated the existence of IBV NA antigenic clusters, but this was discordant with the antigenic evolution of the IBV HA, without antigenic separation of NA into the B/Victoria and B/Yamagata lineages. These antigenic patterns were also observed in NAI data using human monoclonal antibodies. Escape mutation mapping and reverse genetics identified various mutations on the NA underpinning antigenic evolution of the IBV NA between 1940 and 2021.

Conclusion: We demonstrate a discordant antigenic evolution of IBV NA and IBV HA over the last 81 years, at both serological level and molecular level. Our analyses provide novel insights into the antigenicity of IBV NA, which can inform the development of next-generation NA-based influenza vaccine.

Nanospike Surfaces: A New Frontier in Viral Infection Control

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Abstract

The COVID-19 pandemic and the recent waves of other respiratory viruses has highlighted the urgent need for innovative solutions to prevent viral transmission via contaminated surfaces. Leveraging inductively coupled plasma-assisted reactive ion etching, we nanofabricated silicon surfaces with precise nanospikes parameter designed to target viral particles. Human parainfluenza virus type 3 (hPIV-3) was incubated on both nanospike-enhanced and smooth silicon surfaces across different time intervals. The nanospike silicon surface achieving a significant 1.5 log reduction in the number of infectious viral particles within just 6 hours. This is in stark contrast to the smooth silicon control surface, which only achieved a minimal 0.5 log reduction in the same time frame. Viral inactivation mechanisms were explored through electron microscopy and revealed extensive and irreversible structural damage to the viral particles exposed to the nanospike surface. Theoretical modelling using COMSOL simulations, supported our hypothesis that the nanospikes effectively penetrate the viral envelope, compromising its structural integrity and thereby rendering the virus non-infectious. This study underscores the transformative potential of nanostructured surfaces in preventing the spread of viruses via contaminated surfaces.

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1. Samson W. L. Mah, Denver P. Linklater, Vassil Tzanov, Phuc H. Le, Chaitali Dekiwadia, Edwin Mayes, Ranya Simons, Daniel J. Eyckens, Graeme Moad, Soichiro Saita, Saulius Joudkazis, David A. Jans, Vladimir A. Baulin, Natalie A. Borg, and Elena P. Ivanova ACS Nano 2024 18 (2), 1404-1419 DOI: 10.1021/acsnano.3c07099

iNKT cells develop through a 4-stage pathway in human thymus

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Abstract

Invariant NKT (iNKT) cells, a key subset of unconventional T cells, play important roles in cancer, inflammation, infection, and autoimmunity. Although many studies have examined the iNKT cell development pathway in mice, the low frequency of these cells in human blood and tissues has hampered similar studies in humans. To overcome this issue, we used MACS-enrichment to isolate large numbers of iNKT cells from the human thymus. We defined a thymic development pathway for human iNKT cells, identifying a four-stage pathway that involves the progressive increase of the transcription factor PLZF. PLZF is absolutely critical for the development of functional iNKT cells and other subsets of unconventional T cells such as MAIT cells and gamma delta T cells in mice. Development of iNKT cells in the human thymus involves the expression of transcription factors required for the production of TNF, IFNγ and IL-2. Our work identifies important differences in the thymic development of mouse and human iNKT cells, with human iNKT cells displaying a TH1 phenotype. A comparison of the development pathways of human iNKT cells with MAIT cells and Vδ2+ gamma delta T cells highlights important differences with how these unconventional T cells develop. Taken together, our findings may lead to new opportunities to better manipulate iNKT cells to treat human diseases such as cancer.

The Influence of Repeated Influenza Exposure on the CD8$^+$ T cell Response

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Influenza remains a global health concern as seasonal influenza infections result in 3-5 million cases of severe illness and 290,000-650,000 deaths annually, increasing during influenza pandemic outbreaks. Our current defence is an annual vaccine that evokes an antibody response against rapidly mutating surface glycoproteins that provides protection against seasonal infections only and not against subsequent mutated or pandemic strains. Conversely, internal influenza proteins evoke large CD8$^+$ T cell responses and are relatively conserved among seasonal and pandemic strains, hence developing a single vaccine targeting these proteins could offer immunity to both seasonal and pandemic influenza strains simultaneously.

In humans, it is known adults with prior exposure to influenza or with higher frequencies of pre-existing CD8$^+$ T cells to conserved influenza epitopes demonstrate decreased disease severity. However, there is little knowledge on whether initial priming of CD8$^+$ T cells or repeated exposure to influenza infections dictates the immunodominant CD8$^+$ T cell response in humans repeatedly exposed to influenza. To investigate this, PBMC isolated from a young healthy Victorian donor (under 20 years of age) and influenza strains that circulated in Australia during the individual's lifetime were used to establish influenza strain-specific CD8$^+$ T cells. These CD8$^+$ T cell cultures were used to assess the donor's CD8$^+$ T cell response-pattern to rVVs expressing individual influenza proteins and more specifically to overlapping 18mer peptides covering the entire-length of the immunodominant protein of each strain.

Based on the CD8$^+$ T cell cultures cross-reactivity to the peptides from the stimulating strain and other influenza strains, it was determined immunodominant epitopes that primed the original CD8$^+$ T cell population only elicited an immunodominant response when repeated exposure to the epitope occurred and elicited a subdominant response when repeated exposure did not occur. Moreover, epitopes that did not prime the original CD8$^+$ T cell population but were conserved in subsequent strains elicited large subdominant responses.

These findings provide knowledge on human CD8$^+$ T cell priming and repeated antigen exposure, whilst highlighting the importance of memory CD8$^+$ T cells for protection against subsequent influenza infections. This data also provides insights into epitope selection for T cell-evoking vaccine design.

Age-related changes in T cell early activation events

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With increasing age, our T cells become less functional, more difficult to activate and their proliferative capacity decreases. Recent work from our team has also shown that samples from older patients yield fewer, more differentiated chimeric antigen receptor T cells. It is well-established that T cell signaling pathways are perturbed with increasing age, which may contribute to poorer activation. However, it is unclear how these early activation events change with age in individual T cell subsets and whether these changes are predictive of poorer CAR T cell yield and quality. To evaluate this, we will isolate PBMCs from younger (20-30 years old (yo)) and older (60+ yo) healthy donors and stimulate them with anti-CD3 and anti-CD28 monoclonal antibodies and IL-2. Key steps of T cell activation will be assessed, including Lck, PLCγ, p38 and Akt phosphorylation and calcium flux at baseline and various timepoints after activation. We will also assess expression levels of key costimulatory receptors and common gamma chain cytokine receptors at baseline and various timepoints after activation, to determine if age-related differences in kinetics could impact T cell activation. We will corelate these early activation events with outcomes of a CAR T cell protocol (yield, cytokine production, killing capacity and differentiation profile) to test whether early events are predictive of these manufacturing outcomes. We hope to define the differences in early activation events that may predict CAR T cell manufacturing outcomes. This new knowledge could aid the design of CAR T protocols specifically for older patients.

Categories: Clinical (including vaccines, therapeutics, diagnostics), Tissue-specific or cancer immunology

2’-O-Methyl-guanosine 3-base RNA fragments mediate essential natural TLR7/8 antagonism

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“**PUBLISH CONSENT WITHHELD**”

Mechanisms of mRNA Vaccine Adjuvancy in Aged Human Dendritic Cells

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Ageing impairs our ability to mount robust primary T and B cell immune responses, which can reduce mRNA vaccine efficacy in older individuals. It is clear that T cell-intrinsic defects accumulate with age and impair T cell responses, but T cell-extrinsic defects are not as well-defined. Recent data from our lab demonstrates that ageing reduces dendritic cell (DC) number and may limit antigen processing in a mouse model of mRNA vaccination. This study aims to evaluate whether ageing causes similar defects in an *in vitro* human DC model of mRNA vaccination.

To test for age-related defects in DCs, we first validated a protocol to generate monocyte-derived DCs (mo-DCs) using buffy pack samples from young (20-30 years) and older (65+ years) human donors (n = 10 per group). Mo-DCs will be co-incubated with mRNA vaccines that were either i) loaded with a DiD lipid dye to track vaccine uptake or ii) loaded with mRNA expressing mScarlet to track antigen expression. Using flow cytometry, we will measure DC numbers, evaluate DC activation status via CD80 expression, track vaccine uptake and track antigen expression. Given that we have observed reduced numbers and elevated antigen expression in DCs from aged mice that were administered an mRNA vaccine, we speculate that similar deficits will be seen in aged human DCs.

Our study will provide new insights into age-related T cell-extrinsic defects that could limit T cell responses and reduce vaccine efficacy in older adults. If the age-related defects observed in mice *in vivo* are also seen in human cells *in vitro*, this will aid in the design of mRNA vaccine strategies specifically for older people.

Lipidation of Kv1.3 blocking peptide HsTX1[R14A] alters its pharmacokinetics and biodistribution to target tissues

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Autoimmune diseases impact over 250 million people globally. Current therapeutics typically provide symptomatic relief or halt progression temporarily, but significant side effects and relapses are still major challenges for autoimmune patients. Previous studies have shown that upregulation of the voltage-gated potassium channel Kv1.3 in effector memory T cells is associated with the pathogenesis of several autoimmune diseases (1). HsTX1[R14A] is a 34-residue (MW 3741 Da) disulfide-stabilised peptide that has picomolar potency and high selectivity for Kv1.3, and was effective in a rodent model of rheumatoid arthritis (2). However, like most peptides, HsTX1[R14A] is limited by rapid clearance, a short plasma half-life and non-targeted distribution, which necessitate more frequent dosing. Here we explored how lipid conjugation on HsTX1[R14A] affected its circulating half-life and its distribution in organs. We also investigated the distribution of the peptide to draining lymph nodes, as the pathological T cells that upregulate Kv1.3 are found predominantly in lymph nodes. HsTX1[R14A] was conjugated to lipids with various acyl chain lengths for pharmacokinetic studies, and Cy5 was conjugated to native and lipidated peptides for biodistribution studies (3). LCMS/MS assays were developed to quantify the lipidated peptides in mouse plasma. Conjugation of acyl chains to HsTX1[R14A] was found to extend its exposure in plasma and tissues after subcutaneous injection in mice at 2 mg/kg. Biodistribution studies using Cy5-labelled lipidated HsTX1[R14A] demonstrated enhanced exposure in disease-relevant organs and at the injection site at 4 h after subcutaneous injection (2 mg/kg). Cy5-labelled lipidated HsTX1[R14A] was also found to have increased accumulation in the liver compared to HsTX1[R14A] while having significantly less exposure in the kidneys at 4 h after subcutaneous administration. Thus, lipidation of HsTX1[R14A] is a promising approach to optimising its therapeutic potential by extending systemic exposure.

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Assessing Immune Competence to SARS-CoV-2 Vaccination in Patients with Inflammatory Bowel Disease receiving anti-TNF Treatment

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Background: Booster vaccinations are recommended to improve protection against SARS-CoV-2 (COVID-19) infection, particularly for immunocompromised individuals due to underlying disease or treatment. These patients may generate poor antibody responses to vaccination, however the memory B cell response is not well defined. We aimed to assess immune competence to SARS-CoV-2 booster vaccination. Evaluating the capacity and durability of antibody and memory B cell response in a cohort receiving anti-TNF treatment.

Methodology: Peripheral blood was sampled before, 1 month and 6 months post booster vaccination in 20 patients with inflammatory bowel disease (IBD) receiving intravenous Infliximab. SARS-CoV-2 recombinant spike receptor binding domain (RBD) proteins from ancestral, Omicron BA.1, BA.5, XBB.1.5 and JN.1 variants were produced for ELISA-based serology to determine RBD-specific IgG antibody levels in plasma.

Results: The majority of IBD patients recruited (n=16) received bivalent BA.5 mRNA booster vaccination. Median RBD-specific IgG levels to ancestral strain were significantly increased from pre to 1-month post-vaccination but declined after 6 months, similar to pre-vaccination levels. Variant-specific IgG as a proportion of total ancestral-specific IgG increased from pre to 1-month post-booster vaccination. Comparing boosting capacity across variants, the proportion of BA.5-specific IgG was highest whilst the JN.1 proportion was lowest 1-month post-vaccination. The proportion of variant-specific IgG declined after 6 months, similar to pre-vaccination across all variants tested. Furthermore, patients additionally receiving immunomodulators had lower median ancestral-specific IgG after 6 months compared to those on Infliximab alone.

Conclusion: Preliminary results indicate Infliximab treated IBD patients generate antibodies in response to SARS-CoV-2 booster vaccination, with limited durability and are diminished against recent variants. Immunophenotyping of RBD-specific memory B cells using spectral flow cytometry is ongoing to assess immune competency, particularly in patients on combination therapy.

Evaluating Immune Response Against SARS-CoV-2 In Immunocompromised Children

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Uncovering the design principles of CRISPR/Cas13d as an effective antiviral strategy

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The CRISPR/Cas13d is a programmable RNA endonuclease system that has been harnessed for facile and efficient targeting of cellular and exogenous RNAs, including RNA viruses such as influenza virus. Given the robust catalytic activity and specificity of Cas13d, coupled with the ease to design crRNAs, CRISPR/Cas13d has the potential to offer an advantage over conventional antiviral strategies by rapidly designing antiviral effectors. However, there is a considerable knowledge gap in our current understanding of the principles governing the effectiveness of crRNAs in the context of mutation-driven influenza virus evolution and emerging strains. In this study, we delineated the principles for the development of effective crRNAs by targeting a DsRed fluorescence reporter gene in chicken fibroblast DF1 cells. To systematically determine the optimal design for crRNAs, we designed multiple versions of crRNAs to investigate the minimum length of the crRNA, protospacer flanking sequence, degree of mismatch tolerance, and collateral effects. Our data revealed variable knockdown levels between crRNAs, in which a few crRNAs achieved over 95% DsRed knockdown. Other crRNAs exhibited moderate to no effects, although they targeted adjacent RNA locations. crRNAs showed a preference for length requirements and sequences with fewer than 21-nt failed to knockdown the reporter gene. We demonstrated that crRNAs exhibit a high degree of tolerance to single-nucleotide mismatches, regardless of the position at which the single-nt mismatch was introduced. However, 4-nt mismatches within crRNA significantly reduced targeting efficacy, and eight nucleotide mismatches completely diminished targeting efficacy. Finally, Cas13d induced collateral degradation of bystander RNA, suggesting that additional studies are necessary to understand its pleotropic effects. This study extends our understanding of the Cas13d targeting mechanism through multiple iterations of crRNAs, providing a roadmap to design crRNAs for improved effectiveness, which will be important against rapidly evolving viruses.

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

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Metabolic profiling has emerged as a pivotal tool in understanding the biochemical pathways in Plasmodium, the causative agent of malaria, which remains a major public health challenge. This study employs stable isotope labeling (SIL) with 13C_6-glucose to dissect metabolic changes in infected red blood cells (iRBCs) versus uninfected red blood cells (uRBCs). By integrating highresolution mass spectrometry (HRMS) via Orbitrap analysis, we aim to unravel the metabolic alterations induced by Plasmodium infection and glucose metabolism, contributing to the development of novel therapeutic strategies.

Method: Plasmodium cultures were incubated with ^13C_6-glucose for varying periods during different hours post-infection of the intraerythrocytic cycle to obtain temporal information. Control groups were treated with ^12C_6-glucose in both infected red blood cells (iRBC) and uninfected red blood cells (uRBC). After incubation, metabolites were extracted from the Plasmodium culture and analyzed using Orbitrap high-resolution mass spectrometry (HRMS) for untargeted metabolomics profiling. The IDEOM tool was employed for feature identification, and the average intensity ratios of ^13C to ^12C labeled metabolites in iRBC samples were calculated. This approach was used to identify metabolites derived from ^13C-glucose, enabling the investigation of Plasmodium's metabolic.

Preliminary Data: The application of 13C_6-glucose in Plasmodium culture followed by Orbitrap HRMS analysis facilitated the identification of metabolites in iRBC and uRBC. Preliminary results demonstrate distinct metabolic profiles between infected and uninfected cells. In the 13C-glucose iRBC group, multiple metabolites exhibited significant labeling, indicating active glucose-related metabolism and utilization by Plasmodium. Comparative analysis with control groups revealed specific metabolic pathways altered by the parasite, including glycolysis, pentose phosphate pathway, and amino acid metabolism. These findings highlight the parasite's metabolic flexibility and potential vulnerabilities. Moreover, the study underscores the utility of SIL coupled with HRMS in elucidating host-parasite interactions and metabolic networks, providing a comprehensive overview of Plasmodium metabolism and identifying potential targets for therapeutic intervention.

Dual Plasmepsin IX and X Inhibitors Are Refractory to Resistance

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The emergence of drug-resistant *Plasmodium falciparum* strains poses a significant challenge to malaria control and eradication efforts. In collaboration with MSD, we have discovered a novel class of potent inhibitors of *P. falciparum* growth that (i) are potent inhibitors of the essential aspartic proteases plasmepsin IX and X (PMIX and PMX), (ii) block multiple stages of the parasite lifecycle and (iii) are active against multiple *Plasmodium* spp.

Importantly, *in vitro* experiments using dual PMIX/X inhibitors to select resistant *P. falciparum* parasites were not possible: both classical resistance selection, involving a gradual increase in drug concentration, and standardized *in vitro* studies with a range of parasite inocula (10^7−10^9) and constant high drug pressure, failed to select for resistant parasites. These findings suggest a high barrier to resistance development.

More recently, we investigated the resistance profile in *in vivo* studies using NOD-*scid IL2Rγ*^{null} mice engrafted with human erythrocytes and infected with *P. falciparum Pf3D7*$^{0087/N9}$.

Dual PMIX/X inhibitors were orally administered to mice at various doses, all of which demonstrated significant therapeutic efficacy. Notably, recrudescent parasites were observed only in the lowest dose group. Upon parasite recrudescence, blood samples were collected for phenotyping and genotyping resistance characterization. Samples were adapted to *in vitro* culture, and 72-hour growth inhibition assays were conducted to evaluate potential resistance acquisition: the IC_{50} and IC_{90} values remained unchanged when compared to untreated samples, indicating no acquisition of resistance.

Whole-genome sequencing (WGS) analysis is underway to assess whether single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) were acquired in recrudesced parasites. The results of this analysis will be presented at the Symposium.

With the inability to select resistant parasites *in vitro* and no evidence of resistance emergence *in vivo*, dual PMIX/X inhibitors demonstrate a robust resistance-refractory profile.

Our findings highlight and further support dual PMIX/X inhibitors as a promising antimalarial therapy and a promising tool in the fight against malaria.

Association of novel IgG3 allele with malaria infections in children from Sepik region of Papua New Guinea

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Malaria causes death and severe illness in children under five years of age. Recent work has established the importance of malaria-specific IgG3 in malaria immunity. Antibody allotypes due to single nucleotide polymorphisms (SNPs) in IgG3-Fc regions can modulate IgG3 Fc-mediated functions. A novel IgG3 allele, G3m29, was recently reported in pregnant women from Sepik, Papua New Guinea (PNG), and was shown to have enhanced affinity to FcγRIIa. We hypothesized that the prevalence of G3m29 in this population was associated with protection from *Plasmodium* species infections in children. In a longitudinal study cohort of children aged 1-3 years (N=203), with multiple *Plasmodium* species infections from the Sepik region in PNG, we amplified the Fc region of IgG3 by polymerase chain reaction using C_H2 and C_H3 specific primers. We then used Sanger sequencing to identify SNPs and compared to the reference alleles in immunogenetics (IMGT) database. We identified that 78% of the cohort were either heterozygous (n=82, 40%) or homozygous (n=77, 38%) for G3m29. We found a decrease in total number of *Plasmodium spp.* infections in children with potential G3m29 allele compared to non-G3m29 allele carriers (β= -1.736, 95% CI [-3.39, -0.079], $p < 0.05$). This effect was most pronounced for *P. vivax* asymptomatic infections (β=-1.06, 95% CI [-2.01, -0.12], $p < 0.05$). G3m29 carriers had significantly lower levels of total IgG and IgG1 to *P. vivax* vaccine candidate proteins than non-G3m29 carriers. In conclusion, the G3m29 allele is highly prevalent in the Sepik region of PNG and might be involved in protection against *Plasmodium vivax* infections.

Antibody responses in children given the RTS,S malaria vaccine with and without drug chemoprevention

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Plasmodium falciparum malaria remains a major global health burden with nearly 250 million estimated cases in 2022. In Africa alone, there were 580,000 estimated deaths, with 78.1% occurring in children under 5 years of age. This highlights the need for effective malaria interventions to reduce disease prevalence and mortality in this at-risk population. In October 2021, the first malaria vaccine, RTS,S/AS01 (RTS,S), was recommended for use in children from 5 months of age. However, vaccination confers only modest protection against clinical malaria in the first year and this wanes after 18 months. Recently, it was shown that combining RTS,S with seasonal anti-malarial chemoprevention enhanced efficacy in children by ~72% compared to either intervention alone. This enhanced efficacy was also evident following repeated annual booster doses over 5 years. These finding are especially relevant as malaria vaccines are likely to be given to children receiving seasonal chemoprevention. The immunological mechanisms that underpin enhanced vaccine efficacy with chemoprevention remain unclear but knowledge is needed to inform optimal vaccination strategies and guide the next generation of malaria vaccines.

RTS,S induces antibodies, such as IgG, in vaccinated children. However, IgG magnitude has inconsistently correlated with protection against malaria. It was recently found that RTS,S also induces IgA, which was a stronger correlate of protection than IgG in children. There are currently no data on how vaccine-induced IgG and IgA responses and their functional activity and durability over time may be impacted by drug chemoprevention, or the effect of booster doses on vaccine responses.

To address these knowledge gaps, we will investigate the induction and boosting of RTS,S-induced IgG and IgA in an endemic population with highly seasonal malaria transmission. I will perform high-throughput immunoassays to quantify antigen-specific antibodies in over 1,700 plasma samples from children aged 5-17 months who received RTS,S and subsequent booster doses with or without seasonal chemoprevention as part of a phase III clinical trial conducted across Burkina Faso and Mali. This work will contribute new knowledge to our understanding of antibody induction and decay after RTS,S vaccination, thereby informing vaccine implementation and the design of future vaccines.

Investigating Antibodies against Cerebral Malaria in Children

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Among the severe manifestations of malaria, cerebral malaria has a mortality rate of 15-20% despite treatment. The pathogenesis of cerebral malaria involves the sequestration of *Plasmodium falciparum*-infected erythrocytes (IEs) in the brain microvasculature, mediated by *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) proteins. PfEMP1 is encoded by 60 *var* genes, which can undergo clonal antigenic variation, enabling the parasite to evade the immune systems. Within PfEMP1, DBL and CIDR domains are associated with sequestration in the vasculature by interacting with multiple endothelial receptors. Additionally, PfEMP1 plays a key role in rosetting, where IEs aggregate with uninfected erythrocytes, further contributing to microvascular obstruction and severe disease outcomes. Antibodies targeting specific PfEMP1 domains or variants, including those associated with rosetting parasite lines, have been associated with reduced cerebral malaria severity, highlighting the importance of acquiring antibodies against PfEMP1 variants for developing protective immunity in children. To better understand and assess the targets and features of the antibody response, multiplex immunoassay can be employed. This approach provides a serological approach to assessing specific PfEMP1 domains and antibody features associated with cerebral malaria.

This study investigates the antibody responses in Kenyan children with cerebral and uncomplicated malaria at the time of hospital presentation, six weeks, and six months post-infection. A multiplex immunoassay will be used to characterise the antigen-specific antibody responses and features against 30 selected recombinant proteins or full-length PfEMP1 variants known to be associated with severe malaria in children. Additionally, a variant surface antigen binding assay will measure the level of antibodies binding to PfEMP1 on rosetting parasite lines in cerebral and uncomplicated malaria children's samples. Lastly, by comparing two groups at enrolment and analysing antibody kinetics over convalescence, we aim to identify antigen-specific antibody features that differentiate the two groups and examine immunity development over time.

The findings of this research could contribute to a better understanding of antibody-mediated immunity of cerebral malaria in children. This knowledge will be fundamental in the development of vaccines and therapeutic interventions, which could accelerate the reduction of malaria morbidity and mortality in children.

Dissecting germinal centre B cells induced by infection and vaccination during malaria.

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Background: Malaria remains a global health priority, causing over 600,000 deaths in 2022. Protection from malaria is mediated by antibodies that can be induced by infection or vaccination. Infection-induced antibodies are slow to develop in previously exposed individuals, is in part due to malaria-driven immunoregulation. In children under the age of five residing in malaria-endemic regions, responses to vaccination are less robust, and booster doses fail to provide adequate protection.

Infection-induced and vaccine-induced antibodies are dependent on the formation of memory B cells (MBCs). To date, no studies have characterised malaria specific B cells within secondary lymphoid tissues (SLOs) nor following vaccination in children.

Here we used tonsil cells from Ugandan children with prior malaria exposure and/or current asymptomatic infection to investigate the phenotype of malaria specific B cells within the germinal centre (GC) and SLOs.

Methods: Samples comprise of tonsils and matched PBMCs from Ugandan children aged 2-11 (n=50, 25% infected at collection), with collection ongoing. The control malaria-naïve cohort comprise of tonsils from age matched Australian children.

Expression of malaria antigens CSP, MSP1, AMA1, MSP2 was conducted in Expi293F mammalian cells with BirA enzyme. Plasmid constructs include a leader sequence, 6x His tag for purification and an AviTag for biotinylation. In vivo biotinylation allowed for site-specific tagging with fluorescent streptavidins to form tetramers. Tetramers will bind to antigen specific B cells with high affinity and be identified using high-dimensional spectral flow cytometry.

Results: We have successfully generated site-specific biotinylated C-terminus CSP, NANP10 repeat region, MSP1-19, AMA-1 and SARS-CoV-2 proteins for tetramerisation. We have identified B cell populations of interest in both PBMCs and tonsils using a 25-colour B cell panel. Of particular interest are various malaria-specific MBCs, Ig isotypes and subclasses, atypical B cells, GC plasmablasts and marginal zone B cells. Initial findings of malaria-specific B cells will become available by the date of the symposium.

Data will inform our understanding of B cell development and diversity in the GC compared to periphery in human malaria. This knowledge may inform the impact of prior infections on vaccination responses and provide insights into antigen inclusion in future malaria vaccines.

PLASMODIUM FALCIPARUM-INFECTED ERYTHROCYTES INHIBIT NEUTROPHIL EXTRACELLULAR TRAP FORMATION

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Introduction: Malaria is a deadly illness caused by *Plasmodium* parasites, which are spread to humans through the bites of infected female *Anopheles* mosquitoes. *Plasmodium falciparum* has been documented to be the most infectious *Plasmodium* parasite. Neutrophils are the first line of defense against pathogens and play an important role in the innate immune response, but their role in clearing *Plasmodium* from humans remains largely unknown. They produce neutrophil extracellular traps (NETs), web-like structures composed of decondensed chromatin (DNA and histones) and antimicrobial proteins that trap and prevent the spread of infection. Many pathogenic microorganisms have been reported to induce NET formation, while the effect of *P. falciparum* on NET formation has been little studied.

Methods: SYTOX green-stained neutrophils stimulated with phorbol 12-myristate 13-acetate (PMA), lipopolysaccharide (LPS), and calcium ionophore (CaI) were cocultured in the presence and absence of *P. falciparum*-infected erythrocytes at neutrophils to infected erythrocyte ratios of 1:5, 1:10, 1:20, and 1:40 for 5 hours, and the released DNA was fluorometrically quantified.

Results: Results showed that *P. falciparum*-infected erythrocytes significantly ($p < 0.05$) inhibited NET stimulation by PMA, LPS, and CaI in a dose-dependent manner. Interestingly, this inhibition was not parasite-specific, as uninfected erythrocytes also inhibited NET stimulation induced by PMA, LPS, and CaI in a dose-dependent manner.

Discussion: This study highlights a potential novel immune evasion strategy of *P. falciparum* and suggests that erythrocytes regardless of infection status, can modulate neutrophil responses.

Conclusion: *P. falciparum* inhibits neutrophil extracellular trap formation. The mechanism underlying this inhibition is currently being explored.

Blood Biomarker Discovery: High-Dimensional Blood Immune-Profiling in Children with Different Disease Settings Showed Major Age-Related Changes in Proportion of Immune Cells

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The immune response against disease requires cells from both the innate and adaptive arms. The impact of disease is often significantly influenced by age. In humans, newborns have immature immune system, which typically renders them more susceptible to some infectious diseases, whereas they are more likely to tolerate organ transplantation. However, it is still unclear that how immune cell populations play roles in infants who receive organ transplantation. In contrast how immune cells play roles in severity of genetic muscle diseases, such as Facioscapulohumeral muscular dystrophy (FSHD), where an immune cell infiltrate proceeds the replacement of muscle with fat. Therefore, understanding the composition of human immune system throughout life is crucial if we are to endeavour to find biomarkers of diseases and ultimately manipulate the immune response for treating disease. We used high-dimensional flow-cytometry to analyse PBMC samples from healthy individuals ranging from cord blood samples to 80 years old. Next, we analysed two paediatric patient cohorts include heart transplant recipients (N=35) and FSHD patients (N=23). Finally, we compared the patients' samples with the healthy age-matched controls (N=40). We found that the immune landscape in healthy controls undergoes major changes early on in life, and this likely influences how the body responds to certain diseases. Our results showed that naïve immune cells (innate and adaptive) are replaced with different proportions of memory cells. Moreover, while the proportion of MAIT cells and $\gamma\delta$ T cells were low during the first few years after birth, they were drastically increased in frequency in older children, before waning in number in older adults. We also observed that the proportion of several immune cell subsets such as innate cells significantly changed in the blood of FSHD patients compared to healthy controls. Furthermore, we found that several subsets of cells from innate and adaptive immunity changed comparing pre- and post-HTx which were age-related changes. This work provides the first comprehensive study of the immune system throughout ontogeny and the foundation for understanding how the immune system changes in the context of HTx and FSHD muscular dystrophy.

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