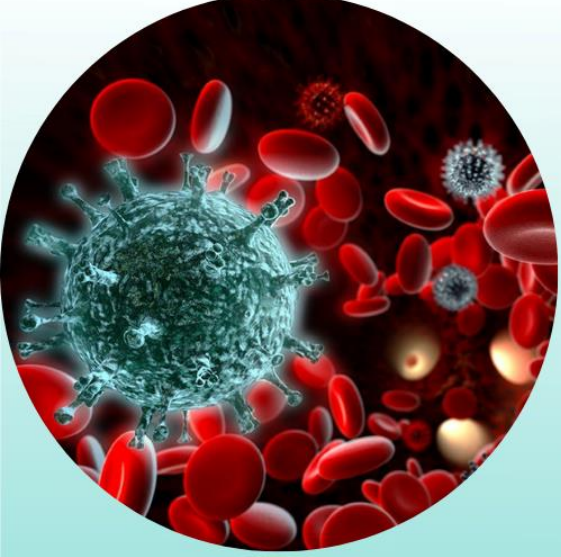


14TH LORNE INFECTION & IMMUNITY CONFERENCE

MANTRA LORNE

14 - 16 FEBRUARY 2024

The Annual Meeting of the

AID | Australian Infectious Diseases Research Centre

Victorian Infection & Immunity Network

www.lorneinfectionimmunity.org

Contents

WELCOME FROM THE CONFERENCE CO-CONVENORS

Dear Colleagues and Friends,

On behalf of the organising committee, we welcome you to our 14th Lorne Infection & Immunity conference. We are delighted by 14 years of support this meeting has received from all of you - delegates, presenters, our invited guests, conference committees, support teams, sponsors and exhibitors. You have all been crucial in establishing this conference as a fixture in the scientific calendar, joining other Lorne Conferences, some of which have been running 40+ years.

The aim of this conference has always been to bring together basic, clinical and translational researchers who examine microbes and their impact on immunity and who apply this knowledge to the prevention and treatment of infections and inflammatory diseases. We have endeavoured to do this while being responsive to emerging trends in our field. The Lorne Infection and Immunity Conference has been a celebration of the highest-quality Australian and international research, and we are particularly pleased to continue fostering new collaborations and promoting emerging scientists in our discipline. Thank you all for supporting the Lorne Infection and Immunity conferences; it has been a pleasure and honour to get to know so many of you at Lorne.

We are also indebted to our sponsors and exhibitors whose support enables us to hold this meeting each year. In the current difficult economic climate, we are especially appreciative of your investment in this conference. Your support enables us to keep prices down, to heavily subsidise student registrations and offer Career Development awards to ECRs. Please reciprocate the support of our sponsors by visiting their exhibition booths, engaging with their posters and by buying their quality products and services for your research. We especially thank our major sponsors, CSL Ltd and Moderna. CSL Ltd has loyally supported this conference for all 14 years and we are delighted to have the support of Moderna as both Silver Sponsor and Student and ECR Networking Sponsor.

Finally, we thank each of the conference's subcommittees, detailed on the following pages of this program. They have worked tirelessly to bring this meeting to fruition. The Program Committee, co-chaired by Begoña Heras and Makrina Totsika, have led the development of the conference's program, selection of invited speakers, presentations from abstracts, and the meeting's overall structure. The Business Development Committee takes responsibility for its strategic direction, budget and sponsorship and registration targets. The Student and ECR Committee, chaired by Andrew Currie and Deputy Chair, Danny Wilson, have developed the program of activities for the conference's students and ECRs. Thanks go to Meg Manohar and Emma Ledger, the chair and deputy chair of the student and ECR sub-committee, and all members of this team, who have organised the student/ECR networking lunch and other activities. Each of these committees continues to be supported by our Program Manager, Rebecca Smith and ASN Event's team comprising Zoe Bailey-Drake, Lianna-Danielle Rizk, Atit Shah and Kara Barker. Thanks to you all.

With best wishes,

Richard Ferrero and Gilda Tachedjian, Lorne Infection and Immunity Conference Co-Convenors

WELCOME FROM THE PROGRAM CHAIRS

Dear Colleagues and Friends,

We are pleased to welcome you to our 14th meeting and hope you will enjoy this year's conference.

Our meeting is being held on the lands of the Eastern Marr people and we wish to acknowledge them as Traditional Owners. We would also like to pay our respects to their Elders, past and present, and Aboriginal Elders of other communities who may be here at the conference. We would also like to acknowledge all the committee members who have worked so hard to develop the program: thanks to all of you for the excellent speaker suggestions and insights that helped us to create an exciting and diverse program.

In developing the program for 2024 we built on the tradition of the Lorne Infection and Immunity conference in bringing together researchers working at the interface of microbiology and immunology. We have also developed sessions around themes such as global health, antimicrobial resistance and advances in immunotherapy, vaccines and clinical translation. We hope you enjoy these topics and invite your feedback about other themes to highlight in future meetings.

We extend a special welcome to our international speakers and thank them for contributing to the high calibre of this conference, particularly those visitors who have travelled along way to be with us. We encourage all attendees to engage with our national and international invited speakers in each session. Students and ECRs – come and meet our invited speakers at Thursday's networking lunch. This is your opportunity to interact with some of the best Infection and Immunity researchers in Australia and internationally.

It has been a pleasure to chair the Program Committee this year and contribute to what we hope will be another great Lorne Infection and Immunity conference.

We are pleased to announce that Antje Blumenthal and Daniel Utzschneider will co-chair the Program Committee for the 2025 conference and look forward to supporting their efforts over the coming year.

Kind regards,

Begoña Heras and Makrina Totsika

ORGANISING COMMITTEE 2024

CO-CONVENORS AND CHAIRS OF THE BUSINESS DEVELOPMENT COMMITTEE	
Gilda Tachedjian Burnet Institute	**Richard Ferrero** Hudson Institute of Medical Research

PROGRAM COMMITTEE

PROGRAM CHAIRS	COMMITTEE MEMBERS
Begoña Heras, La Trobe Institute for Molecular Science, La Trobe University **Makrina Totsika,** Queensland University of Technology	**Antje Blumenthal,** Frazer Institute, University of Queensland **Michelle Boyle,** Burnet Institute **Darren Creek,** Monash Institute of Pharmaceutical Sciences **Matt Johnansen,** Centenary Institute **Philip Hansbro**, Centenary Institute, University of Technology Sydney, Hunter Medical Research Institute and The University of Newcastle **Eugene Maraskovsky,** CSL Ltd **Nicole Moreland,** The University of Auckland **Greg Moseley,** Monash University **Wai-Hong Tam,** Walter and Eliza Hall Institute **Ana Traven, Monash University**

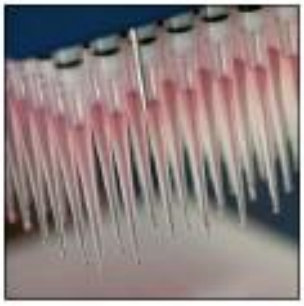
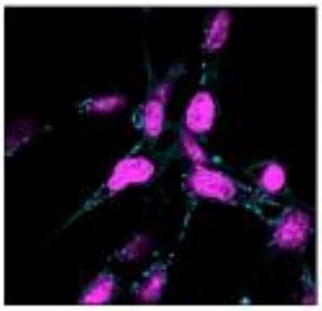
STUDENT & ECR COMMITTEE

CHAIR	COMMITTEE MEMBERS
Andrew Currie, Murdoch University **DEPUTY CHAIR** **Danny Wilson,** University of Adelaide	**Kristie Jenkins, CSIRO Australian Centre for Disease Preparedness** **Si Ming Man,** Australian National University **Daniel Utzschneider,** Peter Doherty Institute for Infection and Immunity, University of Melbourne **ECR COMMITTEE MEMBER** **Meg Manohar, CSIRO Australian Centre for Disease Preparedness** **STUDENT COMMITTEE MEMBER** **Emma Ledger**, University of Queensland **SUB-COMMITTEE OF STUDENTS AND ECRS** **Meg Manohar, CSIRO Australian Centre for Disease Preparedness (Chair)** **Emma Ledger**, University of Queensland **(Deputy Chair)** **Emma Yuxin Mao,** University of Adelaide **Jan Schafer,** Walter and Eliza Hall Institute

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

Hudson Institute of Medical Research

murdoch children's research institute

La Trobe Institute for Molecular Science

WEHI brighter together

The University of Melbourne

La Trobe University

CSIRO

Deakin University

RMIT University

For enquiries contact: info@viin.org.au

INVITED SPEAKERS

INTERNATIONAL INVITED SPEAKERS

PROF JEAN-FRANCOIS COLLET

Jean-François Collet made significant discoveries during his PhD (discovery of one of the largest families of phosphotransferases) at UCLouvain and postdoctoral work at Michigan (engineering a novel pathway for disulfide bond formation in the bacterial periplasm). In 2005, he established his research group at the de Duve Institute (Brussels), focusing on envelope protein folding and protection from stress-induced damage. His group's contributions include identifying a periplasmic reducing system, a methionine sulfoxide reductase system, and the function of CnoX as a quality control device for the Hsp60 protein GroEL. Collet's research also uncovered the mechanism of the stress sensor RcsF and identified the BAM complex as a lipoprotein export machinery across the outer membrane. He is the co-director of the de Duve Institute, a full professor at UCLouvain, and a member of the Royal Academy of Belgium and of the Royal Academy of Medicine of Belgium. With over 110 publications, including several in top journals like Nature, Cell, and Science, Collet has received numerous awards for his achievements.

PROF GABRIEL WAKSMAN

Gabriel Waksman obtained his PhD in 1982 from the University of Paris. He joined the faculty of Washington University School of Medicine (St Louis, USA) in 1993. In 2003, he moved to London (UK) to establish the Institute of Structural and Molecular Biology at UCL/Birkbeck. He held this position until 2019. He was elected to EMBO in 2007, a Fellow of the Academy of Medical Sciences in 2008, a Fellow of the Royal Society in 2012, a member of the German National Academy of Sciences Leopoldina in 2013, and a member of Academia Europaea in 2014. He maintains an active research programme in the Structural and Molecular Biology of Bacterial Conjugation funded by awards from the Wellcome Trust and from MRC.

PROF ANDREAS MEINHARDT

Prof. Andreas Meinhardt studied Human Biology at Philipps University in Marburg. After his postdoctoral stay at Monash University in Melbourne, Australia, he returned in 1995 to Marburg. He was appointed as professor in the Department of Anatomy and Cell Biology at Justus-Liebig-University Giessen, Germany in 2001. His research interest includes reproductive immunology with a focus on the understanding of infection and inflammation of the epididymis and testis as a cause of infertility. Andreas holds two Honorary/Adjunct Professorships with Monash University and the Hudson Institute of Medical Research, Melbourne and is Visiting Professor at the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences at Beijing Union Medical College in China. He is a recipient of the Fuller W Bazer International Scientist Award of the Society for the Study of Reproduction (2019) and the American Journal of Reproductive Immunology Award of the American Society of Reproductive Immunology (2022).

PROF CLARE LLOYD

Clare Lloyd is Head of the Respiratory Division at the National Heart & Lung Institute in London. She trained in immunology at Kings College London and undertook Postdoctoral research at Guys Hospital London and Harvard Medical School, Boston. She worked in a Biotech company in Cambridge USA, investigating the functions of type2 molecules in different disease models. She returned to the UK to start her own group, and has been funded by a serial Wellcome Trust Senior Fellowships. Clare's research the mechanisms underlying the immune response to inhaled

allergens pathogens and pollutants, trying to understand how the immune system senses the inhaled environment, examining how these stimuli influences development of lung inflammation, particularly in very young children. She is Vice Dean for Institutional Affairs in the Faculty of Medicine at Imperial College, taking leading on EDI and career development. Clare is an elected fellow of the Academy of Medical Sciences.

PROF FEDERICA SALLUSTO

Federica Sallusto is Professor of Medical Immunology at the ETH Zurich and USI Lugano and group leader at the Institute for Research in Biomedicine in Bellinzona, Switzerland. She is an expert in the field of human cellular immunology. Her research has focused on dendritic cell and T cell traffic, mechanisms of T cell differentiation and immunological memory. Among her contributions are the definition of "central memory" and "effector memory" T cells as memory subsets with distinct migratory capacity and effector function, the discovery of Th22 cells as a distinct subset of skin-homing T cells, the characterization of non-classic Th1 cells induced by bacteria and of two distinct types of Th17 cells with pro-inflammatory and regulatory properties. She also developed methods for the analysis of human naïve and memory T cell repertoires based on high throughput cellular screenings of expanded T cell libraries that has been instrumental to study T cell responses in patients with immunodeficiencies and to identify autoreactive T cells in patients with narcolepsy. She is member of the German National Academy of Science Leopoldina, of EMBO and of the Henry Kunkel Society, international member of the U.S. National Academy of Sciences and honorary member of the Swiss Society for Allergology and Immunology. In 2022 she was honored with the title of Doctor honoris causa of the Faculty of Science and Medicine of the University of Fribourg. She is currently president of EFIS, the European Federation of Immunological Societies.

DR ERIN DUFFY

Erin Duffy is the Chief of Research & Development at CARB-X. CARB-X is a global biopharmaceutical accelerator for the discovery and early development of products to prevent, diagnose and treat bacterial infections. Most of her professional growth was with Melinta Therapeutics (founded as Rib-X Pharmaceuticals) where ultimately she became EVP, Chief Scientific Officer and R&D site head. Her entry into the pharmaceutical sector began with Pfizer Central Research. Erin's formal training was at Yale University, where she completed a PhD in physical-organic chemistry and an HHMI postdoctoral fellowship in computational structural biology.

A/PROF JOEN LUIRINK

The research focus of my academic group is on protein trafficking pathways in bacteria. After my PhD research on secretion of bacteriocins I worked as a visiting scientist at the EMBL (Heidelberg, Germany) on a novel pathway for targeting of membrane proteins in bacteria, a topic that was continued upon return to Amsterdam. This pathway partly overlaps with the type V secretion system that is used to secrete virulence factors across the cell envelope of Gram-negative bacteria. We have studied the mechanics of this system but also use it as a tool and target in the development of antimicrobials. For instance, we use it to display antigens on the surface of Outer Membrane Vesicles to serve as vaccine nanocarriers. This technology is exploited by the spin-off company Abera Bioscience.

PROF PHOTINI SINNIS

Photini Sinnis MD is Professor of Molecular Microbiology and Immunology at the Johns Hopkins Bloomberg School of Public Health and one of the Deputy Directors of the Johns Hopkins Malaria Research Institute. Her research interests focus on malaria and in particular, on sporozoites, the infective stage of the parasite. She currently serves on the editorial board of Science Advances and is a member of the NIH grant review panel focused on parasitic infections. She is passionate about

mentoring students and junior faculty and served as course director of the Biology of Parasitism course offered at Marine Biological Laboratory in Woods Hole from 2015-2019.

NATIONAL INVITED SPEAKERS

PROF DENA LYRAS

Professor Dena Lyras is the Deputy Director of the Biomedicine Discovery Institute at Monash University. Her laboratory is focussed on enteric pathogens, particularly the clostridia and those involved in antibiotic-associated diarrhoea in humans and animals, and they use genetic approaches to understand how these microbes harness regulatory and virulence factors to interact with the host and cause disease. Antibiotic resistance and DNA mobility are also research areas of focus, in the context of gut pathogens and antibiotic-associated diarrhoeal disease. In collaboration with industry and academic partners, her laboratory is developing immunotherapeutics and small molecules to prevent and treat these infections. She was awarded an Australian Research Council Future Fellowship from 2012-2016 and began an Australian Research Council Laureate Fellowship in 2022.

PROF JEFF ERRINGTON

Prof Jeff Errington FRS has focused his research career on resolving fundamental questions about the structure and function of bacterial cells, especially endospore formation, chromosome replication and segregation, cell division and cell morphogenesis. After 25 years at the University of Oxford, he moved to Newcastle University (UK) as scientific founder and Director of the Centre for Bacterial Cell Biology, the world's first major research centre focused specifically on the molecular and cellular biology of bacteria. Last year he moved to the University of Sydney with an ARC Australian Laureate Fellowship, to continue and expand his work on L-form bacteria.

Errington's contributions to basic science have been recognized by election to various learned societies, including Fellowship of the Royal Society, EMBO, and both the European and American Academies of Microbiology. He has also founded two successful spin-out companies, Prolysis Ltd, and Demuris Ltd, which was recently acquired by US-based Odyssey Therapeutics.

DR TIM WELLS

Dr Timothy Wells is a Senior Research Fellow at the Frazer Institute, The University of Queensland. Dr Wells is a molecular microbiologist whose research focuses on the interactions between the host immune system and Pseudomonas aeruginosa during chronic lung infections.

Dr Wells' research investigates antibody-dependent enhancement of bacterial infection. Dr Wells research identified patients that produced a specific antibody that protected their infecting P. aeruginosa from killing by the immune system. These 'cloaking antibodies' target lipopolysaccharide on the bacterial surface. Importantly, patients with these antibodies have worse lung function and outcomes then those with normal immune killing. His research uses a mixture of molecular microbiology, immunology and genomic approaches and has directly led to novel treatment of critically ill patients with multi-drug resistant bacteria.

A/PROF JOANNA GROOM

Associate Professor Joanna Groom is an NHMRC L1 Investigator fellow and laboratory head in the Immunology division of the Walter and Eliza Hall Institute (WEHI). Dr Groom's research focuses on deciphering how cellular positioning and communication control immune responses, and how this can be leveraged to optimise clearance and protection from infection and cancer. This interest was piqued during her PhD, at the Garvan Institute, investigating the cellular signaling critical to lupus autoimmunity. Her research revealed a novel mechanism of autoantibody production. She performed her postdoctoral research with Prof. Andrew Luster, a leader in chemokine biology at Harvard Medical School/Massachusetts General Hospital (HMS/MGH). Here, she found that directed cell migration into lymphoid niches is intertwined with cell fate and function. Dr Groom's team has made a series of advancements that enable 3D imaging of intact lymphoid organs and used this to discover the distinct niches that support the differentiation of effector and memory T cells. Her current research combines imaging and transcriptional analysis to dissect the cellular interactions that mediate protection against diverse pathogenic infections and cancer.

PROF KATE SEIB

Professor Kate Seib is a NHMRC Leadership Fellow and the Associate Director (Research) at the Institute for Glycomics, Griffith University. Prof Seib's expertise is in the field of molecular microbiology, with a focus on characterising virulence mechanisms and developing vaccines for human mucosal pathogens including Neisseria gonorrhoeae.

Prof Seib's work includes the discovery, pre-clinical characterisation and clinical evaluation of gonococcal vaccine candidates, as well as the use of mathematical modelling to assess the potential impact of vaccines on gonococcal prevalence. While at Novartis Vaccines she was part of the team that developed the outer membrane vesicle-based Neisseria meningitidis vaccine, 4CMenB. She is now leading two clinical trials to evaluate the ability of 4CMenB to prevent gonorrhoea.

PROF TREVOR LITHGOW

Professor Lithgow is a biologist who studies drug-resistant bacteria, at both the single cell and population level. He led the NHMRC Program in Cellular Microbiology that used the fundamental knowledge of bacterial cell biology to better understand mechanisms of antimicrobial resistance (AMR), as well as the mechanics driving entry of bacteriophage (phage) into bacteria and the mechanisms by which phages then control bacterial populations. He now serves as Director of the inter-faculty Centre to Impact AMR located at Monash University. The Centre brings together engineers, social scientists and anthropologists, molecular and microbiologists, chemists, computational and evolutionary scientists, and clinicians, in order to find sustainable solutions to the growing and global problem of AMR. Professor Lithgow was an ARC Australian Laureate Fellow and is a Fellow of the Australian Academy of Science.

PROF HEIDI DRUMMER

Professor Heidi Drummer is Program Director for Disease Elimination at the Burnet Institute in Melbourne, Australia. She oversees a cross-disciplinary response to eliminate public health threats using diverse approaches across treatment and prevention, harm reduction in key populations at risk, vaccine and drug development, and diagnostics. For the last 20 years, her work has focussed on developing candidate vaccines for HIV, Hepatitis C and SARS-CoV-2 and has investigated the use of protein, virus-like particle, viral vectored vaccines and more recently mRNA platforms. She is an inventor on seven patent families

and has worked extensively with Industry partners in Australia and Internationally. Since 2021, she has been Scientific Director of the Burnet Diagnostics Initiative where she leads a research and development program to develop new diagnostics for human health.

PROF PAUL HERTZOG

Professor Paul Hertzog leads a multidisciplinary research team investigating the Regulation of Innate immune and Interferon Signalling in infection, inflammation, and cancer at the Centre for Innate Immunity & Infectious Diseases at the Hudson Institute of Medical Research. The program includes: structure-function of type I IFNs and their receptors. IFN responses are examined by a systems biology approach. They discovered and are characterising the function of the unique type I IFN epsilon, a constitutive, epithelial regulator of mucosal immunity against infection and cancer in the female reproductive, gastrointestinal and respiratory tracts. His group made seminal discoveries about IFN's mechanism of action, published in Nature, Science, Cell and will use their molecular insights for clinical impact. Prof Hertzog was awarded the prestigious Milstein Award for Excellence in cytokine research by the International Cytokine and Interferon Society. He is a keen supporter of open, collaborative science, proud to have co-founded the VIIN network and the Lorne I&I conference which he co-convened from 2010 until 2021.

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SILVER SPONSORS

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ECR/ STUDENT NETWORKING SPONSOR

CONFERENCE SUPPORTER

DELEGATE INFORMATION

THE ORGANISERS – ASN EVENTS

ASN Events Pty Ltd
9/397 Smith St, Fitzroy 3065
P: +61 3 8658 9530
Web: www.asnevents.com.au

Conference Managers

Lianna-Danielle Rizk
ASN Events
Email: lianna-danielle.r@asnevents.net.au
Phone: 61 3 8692 2034

Zoe Bailey-Drake
ASN Events
Email: zoe.b-d@asnevents.net.au
Phone: 6 13 8560 4398

WHAT YOUR REGISTRATION INCLUDES

All face-to-face Delegate and Student registrations include:

- All sessions
- Exhibition Hall
- Conference App
- Catering during conference breaks (morning tea, lunch and afternoon tea)
- Drinks at the Poster Sessions
- Evening Social Functions (at an additional cost – Tickets must be purchased as there is limited availability)

WATCHING SESSIONS ONLINE?

Sessions will be available online via a supplied zoom link to delegates' emails. To support attendance in Lorne, virtual viewing has been limited this year:

- Virtual viewing is limited to slides and audio only.
- No questions can be submitted online.
- Sessions will not be recorded.

CONFERENCE APP

Lorne I&I 2024 has a conference app that includes all information contained in this book and live updates of the program.

To view this app, go to https://infection-and-immunity-2024.m.asnevents.com.au/

DISPLAYING YOUR POSTER

Science Bite Presentations

These presenters will have their posters available for the full duration of the conference and have been advised to be available for discussion during Poster Session II on Thursday, 15th February at 8:00pm.

Poster Presentations

All posters are to be displayed for the duration of the conference. Please see presentation details below:

- Posters with odd numbers are required to present in the first poster session (Wednesday, 14th February at 8:00pm).

- Posters with even numbers are required to present in the second poster session (Thursday, 15th February at 8:00pm).

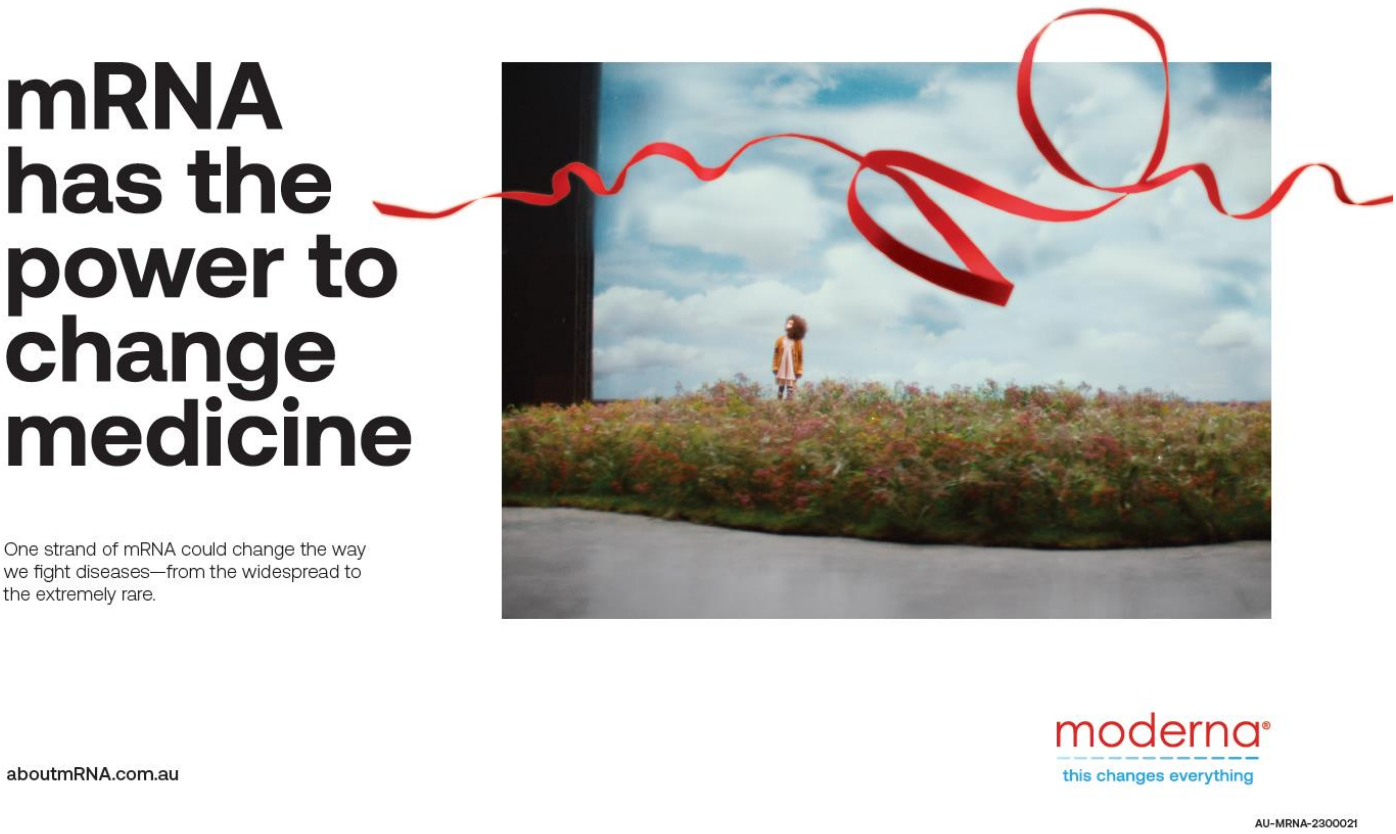
When presenters arrive at the Exhibition Hall to display their poster, search for your poster number on the poster boards provided. Velcro will be supplied at the specific poster location. Please be available during your designated poster session to discuss your research with fellow delegates and speakers. Judges for Student and ECR awards will be using this time to score eligible presenters.

SOCIAL FUNCTIONS

STUDENT AND ECR DINNER Venue: The Peak Lorne Country Club Date: Wednesday 14th February 2024 Time: 6:30 PM - 8:00 PM Cost: $30 - Additional ticket $40	The ticket includes one drink. Additional drinks are available for purchase separately at the venue. All special dietary requirements will be accommodated. Tickets must be purchased as spaces are limited. Please be aware the walk to the The Peak is steep so the conference has made a shuttle bus available from 6:15pm leaving from the Mantra lobby. Return shuttle will begin at 7:45pm back to Mantra Lorne for the poster session.
General Delegate Dinner Venue: The Peak Lorne Country Club Date: Wednesday 14th February 2024 Time: 6:30 PM - 8:00 PM Cost: $50 - Additional ticket $65	This ticket includes one drink. Additional drinks are available for purchase separately at the venue. All special dietary requirements will be accommodated. Tickets must be purchased as spaces are limited. Please be aware the walk to the The Peak is steep so the conference has made a shuttle bus available from 6:15pm leaving from the Mantra lobby. Return shuttle will begin at 7:45pm back to Mantra Lorne for the poster session.
Student and ECR Networking Lunch Seagrass Lawn, Mantra Lorne *Thursday 15th February, 12:35PM - 1:45PM*	This lunch is a networking opportunity for students and early career researchers. The ticket includes a fully catered lunch with non-alcoholic beverages provided on the Seagrass lawn of Mantra Lorne.
Conference Dinner Venue: The Common at Lorne Oval, 45 Otway St, Lorne Date: Thursday 15th February 2024 Time: 6:00 PM - 8:00 PM Cost: General delegates - $70, Students - $40 - Additional ticket $70	This ticket includes one drink. Additional drinks are available for purchase separately at the venue. All special dietary requirements will be accommodated. Tickets must be purchased as spaces are limited. Morning and afternoon teas, the general delegate lunch on Thursday and Poster Sessions will be held in the Exhibition Centre.

EXHIBITOR PRIZES

Interested in winning a prize? At the conclusion of the conference, lucky delegates who maximise their engagement with our exhibitors will win one of many prizes! To enter, check out the exhibition booths and interact with all of our exhibitors. The exhibitors will stamp a card specific to this competition. Once your card is complete, drop it off at the registration desk to go into the draw.



SPONSOR AND EXHIBITOR LISTING

CSL Limited **Silver Sponsor**
Website: www.csl.com

CSL is a leading global biotechnology company, headquartered in Melbourne, Australia, with a portfolio of life-saving medicines, including those that treat haemophilia and immune deficiencies, as well as vaccines to prevent influenza. Our therapies are also used in cardiac surgery, organ transplantation and burn treatment. CSL focuses its world-class research and development, high-quality manufacturing, and patient-centered management to develop and deliver innovations to treat people with life-threatening medical conditions and help them live full lives. Over the last five years CSL has invested more than USD$4 billion in research and development and employs more than 1,700 R&D experts globally.

Moderna **Silver Sponsor**

Moderna's Mission is to deliver the greatest possible impact to people through mRNA medicines. The company was founded in 2010, focused on building the leading mRNA technology platform, infrastructure to accelerate drug discovery and early development, a rapidly expanding pipeline, and a world-class team. Our pipeline includes development candidates for mRNA-based vaccines and therapies spanning several therapeutic areas, with multiple clinical trials underway and other development candidates progressing toward the clinic. In addition, there are numerous discovery programs advancing toward development.

BMG LABTECH **Exhibitor**
Website: www.bmglabtech.com
BMG LABTECH is a leading developer and manufacturer of microplate reader instrumentation with a wide range of measurement methods. Microplate readers are used in the pharmaceutical and biotech industries, as well as in academic research establishments, for both basic research analysis and High Throughput Screening.
We focus solely on microplate readers and offer the most diverse selection of optical detection systems in conjunction with integrated liquid handling equipment.
Since our establishment in Offenburg Germany in 1989, we have been committed to developing and producing high quality microplate readers and microplate handling systems.
Our exceptional worldwide sales and support network is committed to bringing you innovative microplate reader technology with the reliability and quality that you expect from BMG LABTECH.
The highest priority at BMG LABTECH is to have satisfied microplate reader customers! We succeed at this goal by not only offering excellent microplate readers but also providing personal customer support that not found at larger companies. Our support centres have teams of microplate reader development specialists ready to work with you and your applications to get the most out of your microplate reader. This close working relationship allows BMG LABTECH to completely understand your needs and to help develop our instruments to meet the changing needs of the microplate reader market.

Genesearch **Exhibitor**
Website: www.genesearch.com.au
We are committed to delivering high quality scientific consumables, services and support, quickly so our customers can focus on their results. That's why we developed the e•Freezer, an innovative reagent delivery system that gives researchers immediate or next day access to the majority of reagents they need 24 hours a day, 7 days a week.

Lonza **Exhibitor**
Website: www.lonzabioscience.com.au/
At Lonza, we deliver technologies to enable a healthier world. We provide life science researchers with the tools they need to develop and test therapeutics, from basic research to final product release.

Lonza's Bioscience products and services range from cell culture and discovery technologies for research, to quality-control tests and software for biomanufacturing. We supply:-

- Primary cells and media
- Fetal Bovine Serum (Australian sourced)
- Transfection products
- 4D-Nucelofector System using electroporation for difficult to transfect cells.
- Techno Plastic Products TPP® dispossible tissue culture products
- Biochemicals from Central Drug House CDH®.

Mimotopes **Exhibitor**

Website: www.mimotopes.com
Suite1/Unit 9, 677 Springvale Rd,
Mulgrave Vic 3170 Aus
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Fax: +61 3 9565 1199
Email: australia@mimotopes.com
Mimotopes has been a innovator in the Peptide Synthesis Industry for over 30 years, providing critical tools for researchers worldwide.
Our one-stop-shop online offers: Peptide Library Synthesis, Peptide Pools, Custom Peptides, Antibody and Assay Services, Catalogue Peptides, and Reagents.
Call or email us to propel your research to new heights!

Promega **Exhibitor**

Website: www.promega.com.au/
Promega has been a leading global provider of innovative solutions and technical support to the life sciences industry for 40 years. Our portfolio of ~2000 products enables the advancement of knowledge in molecular and cellular research broadly encompassing molecular diagnostics, drug discovery and genetic identity. Manual and high throughput formats are available.

Revvity **Exhibitor**

Website: https://www.revvity.com/
At Revvity, "impossible" is inspiration, and "can't be done" is a call to action. Revvity provides health science solutions, technologies, expertise and services that deliver complete workflows from discovery to development, and diagnosis to cure. Revvity is revolutionizing what's possible in healthcare, with specialized focus areas in translational multi-omics technologies, biomarker identification, imaging, prediction, screening, detection and diagnosis, informatics and more.

With 2022 revenue of more than $3 billion and over 11,000 employees, Revvity serves customers across pharmaceutical and biotech, diagnostic labs, academia and governments. It is part of the S&P 500 index and has customers in more than 190 countries.

Sapphire Bioscience **Exhibitor**

Website: https://www.sapphirebioscience.com/
Ph: +61 2 9698 2022
Ph: 1800 062 088 (AU Toll-Free)
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PROGRAM

WEDNESDAY 14TH FEBRUARY 2024

REGISTRATION OPEN
11:30 AM - 5:00 PM Mantra Lorne Lobby

SESSION 1: WELCOME AND PLENARY 1
1:00 PM - 1:55 PM Heritage Ballroom
Chairs: Makrina Totsika (Queensland University of Technology) & Begoña Heras (La Trobe University)

1:10 PM **Jean Francois Collett** (UCLouvain)
How do Gram-negative bacteria deal with hypo-osmotic shocks? abs# 1

SESSION 2: MOLECULAR BASIS OF INFECTIOUS DISEASES
1:55 PM - 3:30 PM Heritage Ballroom
Chairs: Ana Traven (Monash University) & Emily Furlong (Australian National University)

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1:55 PM **Gabriel Waksman** (Institute of Structural and Molecular Biology)
Structural and Molecular Biology of Bacterial Conjugation abs# 2

2:20 PM **Jeff Errington** (University of Sydney)
How cell wall targeting antibiotics kill bacteria abs# 3

2:45 PM **Jilong Qin** (Centre for Immunology and Infection Control)
Cysteine-dependent antigenic heterogeneity of Shigella flexneri autotransporter IcsA and implications in host immunity evasion abs# 4

3:00 PM **Megan Steain** (The University of Sydney)
Primate Simplexvirus UL39 proteins inhibit RHIM signalling abs# 5

3:15 PM **Emily Furlong** (University of Oxford)
Molecular structure of the intact bacterial flagellar basal body abs# 6

Afternoon Tea and Check In
3:30 PM - 3:55 PM Convention Centre

SESSION 3: ADAPTIVE IMMUNITY
3:55 PM - 5:45 PM Heritage Ballroom
Chairs: Daniel Utzschneider (University of Melbourne) & Dimitra Chatzileontiadou (La Trobe University)

3:55 PM **Federica Sallusto** (ETH Zurich)
T lymphocyte differentiation and function: Lessons learned from patients with inborn errors of immunity abs# 7

4:20 PM **Joanna Groom** (WEHI)

Divergent cytokine and transcriptional signatures control functional Tfh heterogeneity abs# 8

4:45 PM **Dimitra SM Chatzileontiadou** (La Trobe University)
A common allele of HLA is associated with asymptomatic SARS-CoV-2 infection abs# 9

5:00 PM **Praveena Thirunavukkarasu** (Monash University)
Understanding T cell-mediated immunity of Bacteroides fragilis glycosphingolipids by Natural Killer T cell receptor abs# 10

5:15 PM **Pirooz Zareie** (Monash University)
Covalent TCR-peptide-MHC interactions: A new mechanism for T cell antigen recognition and T cell activation
abs# 11

5:30 PM **Robert Anthony** (Harvard University)
Molecular Determinants of IgG Anti-Inflammatory Activity abs# 64

SESSION 4: HERTZOG ORATION
5:45 PM - 6:10 PM Heritage Ballroom
Chair: Gilda Tachedjian (Burnet Institute)

5:45 PM **Andreas Meinhardt** (Justus-Liebig University)
Unveiling the immune shield – a dive into epididymal immunology abs# 12

DINNER AT THE PEAK - STUDENT & ECR DINNER
6:30 PM - 8:00 PM The Peak Lorne Country Club - Outdoor

DINNER AT THE PEAK - GENERAL DELEGATE DINNER
6:30 PM - 8:00 PM The Peak Lorne Country Club - Indoor

POSTERS I
8:00PM - 9:30PM Convention Centre

THURSDAY 15TH FEBRUARY 2024

REGISTRATION OPEN
8:00 AM - 5:00 PM Mantra Lorne Lobby

SESSION 5: HOST-PATHOGEN INTERACTIONS
9:00 AM - 11:05 AM Heritage Ballroom
Chairs: Danny Wilson (University of Adelaide) & Maria Kaparakis Liaskos (University of Melbourne)

9:00 AM **Photini Sinnis** (Johns Hopkins Bloomberg School of Public Health)
Malaria sporozoite biology at the dermal inoculation site abs# 13

9:25 AM **Dena Lyras** (Monash University)
Non-antibiotic strategies to mitigate enteric infections: insights from discovery research abs# 14

9:50 AM **Shankar Devkota** (Monash University)
Utilising the tick's trick for advancing anti-inflammatory therapy abs# 15

10:05 AM **Jason J Paxman** (La Trobe University)
New molecular mechanisms for bacterial toxins abs# 16

10:20 AM **Kitty McCaffrey** (Hudson Institute)
Targeted degradation of host messenger RNA by a bacterial effector modulates macrophage metabolism abs# 17

10:35 AM **Tim Barnett** (Wesfarmers Centre for Vaccines and Infectious Diseases)
Group A Streptococcus associated with recurrent tonsillitis form antibiotic-resistant intracellular communities abs# 18

10:50 AM **Katryn Stacey** (The University of Queensland)
Gut inflammation and barrier breakdown in severe dengue disease abs# 19

MORNING TEA
11:05 AM - 11:35 AM Convention Centre

SESSION 6: PLENARY 2 AND HARTLAND ORATION
11:35 AM - 12:35 PM Heritage Ballroom
Chair: Richard Ferrero (Hudson Institute of Medical Research)

11:35 AM **Paul Hertzog** (Hudson Institute of Medical Research)
Understanding the distinct roles of IFN epsilon in protecting the mucosa from disease abs# 20

12:20 PM **Paula M Cevaal** (The University of Melbourne at The Peter Doherty Institute for Infection and Immunity)
Potent, HIV-specific latency-reversal through CRISPR activation delivered by lipid nanoparticles abs# 21

LUNCH
12:35 PM - 1:45 PM Convention Centre

ECR LUNCH (Career development event)
12:35 PM - 1:45 PM Seagrass Lawn

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AFTERNOON TEA
2:55 PM - 3:25 PM Convention Centre

SESSION 7: ADVANCES IN IMMUNOTHERAPY, VACCINES AND CLINICAL TRANSLATION
3:25PM - 5:15PM Heritage Ballroom
Chairs: Eugene Maraskovsky (CSL Limited) & Jason Paxman (La Trobe University)

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3:25 PM **Marcel Doerflinger** (Walter and Eliza Hall Institute for Medical Research)
Developing a functional cure for HTLV1 in a humanized mouse model abs# 24

3:40 PM **Meredith O'Keeffe** (Monash University)
Resistance to last line antibiotics impairs STING-dependent sensing of MRSA in dendritic cells abs# 25

3:55 PM **Nicole L Messina** (Murdoch Children's Research Institute)
Specific and off-target immune responses following COVID-19 vaccination with ChAdOx1-S and BNT162b2 COVID-19 vaccine abs# 26

4:10 PM **Stephany Sanchez-Ovando** (Doherty Institute)
Influenza vaccine responses to A(H1N1)pdm09 antigens in 2020 and 2021 among repeatedly vaccinated healthcare workers abs# 27

4:25 PM **Kate L Seib** (Griffith University)
Can a meningococcal vaccine prevent gonorrhoea? abs# 23

4:50 PM **Joen Luirink** (Vrije Universiteit)
Trafficking towards antimicrobials abs# 22

SESSION 8: INNATE IMMUNITY
3:25 PM - 5:15 PM Heritage Dining Room
Chairs: Antje Blumenthal (The University of Queensland) & Natalia Sampaio (Hudson Institute of Medical Research)

3:25 PM **Timothy J Wells** (The University of Queensland)
Antibody-mediated enhancement of Pseudomonas aeruginosa acute and chronic infections abs# 32

3:50 PM **Clare Lloyd** (Imperial College)

Stromal-Immune interactions regulating pulmonary immunity abs# 33

4:15 PM **Gesa J Albers** (Imperial College London)
Glycolysis of airway macrophages controls lung homeostasis and responses to aeroallergen abs# 28

4:30 PM **Larisa Labzin** (The University of Queensland)
Defining pro-inflammatory antibody effector functions during viral infection abs# 29

4:45 PM **Kate E Lawlor** (Hudson Institute of Medical Research)
A1 is induced by pathogen ligands to limit myeloid cell death and NLRP3 inflammasome activation abs# 30

5:00 PM **Rukmali Wijayarathna** (Hudson Institute of Medical Research)
Interferon epsilon as a novel anti-viral agent in the testis abs# 31

SESSION 9: SCIENCE BITES
5:15PM - 5:45PM Heritage Ballroom
Chairs: Mark Blaskovich (University of Queensland) & Thomas Schultz (University of Queensland)

5:15 PM **William Clow** (Walter & Eliza Hall Institute of Medical Research)
Modelling & Targeting Cytokine Storm in Dengue Virus-infected Mice abs# 34

5:18 PM **Xavier B Montin** (University of Adelaide)
Understanding the AhR and innate immunity axis in RNA virus infection abs# 35

5:21 PM **Bianca Briscas** (UNSW)
Developing a microbial therapeutic for the prevention of systemic Candida albicans infections arising from the gastrointestinal tract abs# 36

5:24 PM **Karoline D Raven** (The University of Queensland)
Exploring connections between mitochondrial fission and lipid droplets in macrophage inflammatory and antimicrobial pathways abs# 37

5:27 PM **Risa Takahashi** (The University of Auckland)
Exploration of the Immunostimulatory Effects of Group A Streptococcus Pili abs# 38

5:30 PM **Claudia J Stocks** (Nanyang Technological University)
Enterococcus faecalis exploit neutrophils as an intracellular niche for replication and persistence abs# 39

5:33 PM **Li Chen Cheah** (Australian Centre for Disease Preparedness)
Serological profiling of viral infection using peptide phage display abs# 40

5:36 PM **Anurag Adhikari** (La Trobe University)
Human Immunodeficiecny Virus-1 (HIV-1) Neutralisation Profiles in HIV-1 Viremia Suppressed Nepalese Individuals abs# 41

5:39 PM **Nicholas Yuen** (The University of Queensland)
Molecular and cellular pathogenesis of Ross River virus abs# 42

SESSION 10: SCIENCE BITES

5:15PM - 5:45PM Heritage Dining Room

Chairs: Philip Hansbro (University of Technology Sydney) & Nicole Campbell (Hudson Institute)

5:15 PM **Emma Mao** (The University of Adelaide)
Characterising the quick-killing mechanism of action of azithromycin analogues against malaria parasites abs# 43

5:18 PM **Stefanie M. Bader** (The Walter and Eliza Hall Institute)
Dead or alive? Unveiling the Molecular Mechanisms of SARS-CoV-2 Pathogenesis in vivo abs# 44

5:21 PM **Zhi Ying Kho** (Monash University)
SpoT-mediated unique metabolic remodelling potentially underlies polymyxin tolerance in Acinetobacter baumannii abs# 45

5:24 PM **Jan Schaefer** (Walter and Eliza Hall Institute)
Developing precision RNA therapeutics for Tuberculosis abs# 46

5:27 PM **Sunil Sapkota** (Monash University)
3-base long 2'O-methyl oligonucleotides are potent TLR7 and TLR8 modulators. abs# 47

5:30 PM **Yadana Zaw** (University of Melbourne)
Development and characterisation of a SARS-CoV-2 RNA vaccine expressing three linked-RBD domains abs# 48

5:33 PM **Christopher McMillan** (The University of Queensland)
Understanding the enhanced immune responses to high-density microarray patch vaccination through spatial transcriptomics abs# 49

5:36 PM **Long Huynh** (University of Melbourne)
Cytostome formation in artemisinin resistant Plasmodium parasites abs# 50

5:39 PM **Jack Emery** (Monash University)
Just the Tipα of the iceberg: nuclear targeting by an Helicobacter pylori extracellular vesicle-associated toxin. abs# 51

CONFERENCE DINNER
6:00 PM - 8:00 PM Lorne Common

POSTERS II
8:00 PM - 9:30 PM Convention Centre

FRIDAY 16TH FEBRUARY 2024

Registration Opens
7:30AM - 1:00PM Mantra Lorne Lobby

SESSION 11: SYSTEMS IMMUNOLOGY AND GLOBAL HEALTH
8:55AM - 10:20AM Heritage Ballroom
Chairs: Joshua Hayward (Burnet Institute) & Stuart Ralph (The University of Melbourne)

8:55 AM **Heidi Drummer** (University of Melbourne)
Prospects for a vaccine for the prevention of Hepatitis C. abs# 53

9:20 AM **Lindi Masson** (University of Cape Town)
Exploring the female genital tract mycobiome in young South African women using metaproteomics abs# 54

9:35 AM **Alyssa E Barry** (Walter and Eliza Hall Institute)
A population genomic model for measuring antigenic escape and predicting serotypes for malaria vaccine candidates abs# 55

9:50 AM **Paige Skoko** (Murdoch Children's Research Institute)
Understanding persistence of Streptococcus pneumoniae serotype 6B in Mongolia following vaccine introduction abs# 56

10:05 AM **Ariel Isaacs** (University of Queensland)
Towards broad-spectrum henipavirus vaccines & therapeutics: rational design of prefusion F glycoproteins abs# 57

MORNING TEA AND CHECK OUT
10:20 AM - 10:50 AM Convention Centre

SESSION 12: HIGHEST SCORING ABSTRACT
10:50 AM - 11:05 AM Heritage Ballroom
Chair: Kristie Jenkins (CSIRO)

10:50 AM **Michelle Chonwerawong** (Hudson Institute of Medical Research)
Specific gut microbiota modulates intestinal epithelial cell signalling and inflammation abs# 58

SESSION 13: NEW SOLUTIONS FOR ANTIMICROBIAL RESISTANCE
11:05 AM - 12:40 PM Heritage Ballroom
Chairs: Kristie Jenkins (CSIRO) & Susan Hawes (CSIRO)

11:05 AM **Erin Duffy** (Boston University)
Building a Pipeline of High-Impact Products to Prevent, Diagnose and Treat Bacterial Infections abs# 59

11:30 AM **Trevor Lithgow** (Monash University)
Antimicrobial resistance (AMR) abs# 60

11:55 AM **Yaoqin Hong** (Queensland University of Technology)
Cracking the Gram-negative cell envelope to revive otherwise ineffective antibiotics abs# 61

12:10 PM **Meiling Han** (Monash University)
Exogenous arginine enhances the diagnosis of 'undetectable' polymyxin-dependent Acinetobacter baumannii abs# 62

12:25 PM **Emily L Gulliver** (Hudson Institute of Medical Research)
Distribution of phenotypic antimicrobial resistance in the gastrointestinal microbiome. abs# 63

CLOSING REMARKS, PRIZES, AWARDS AND PHOTOS
12:40 PM - 1:00 PM Heritage Ballroom
Chairs: Richard Ferrero (Hudson Institute of Medical Research) & Gilda Tachedjian (Burnet Institute)

BUS DEPARTS
1:55 PM - 2:00 PM Mantra Lobby

POSTER ABSTRACTS

- Odd numbered poster presenters will be available during the poster session on Wednesday.
- Even numbered posters and Science Bite presenters will be available during the poster session on Thursday.

Poster Session I (odd numbers) Poster Session II (even numbers)

101

A validated study protocol to compare microbiome and mycobiome profilesof Inflammatory Bowel Disease patients in remission and active flare

Matias Abregu[1]

1. Microbiome Science, DNA Genotek Inc, Ottawa , Ontario , Canada

Several large cohort studies of the gut bacterial composition of patients with Inflammatory Bowel Disease (IBD) have been published in recent years. While these studies have provided intriguing insights into the disease and promising clues for treatment options, they are often challenged by low enrollment and compliance rates. Low rates are largely influenced by donor perception of self-collection of the severe diarrhea samples common for IBD patients in flare. In collaboration with Crohn's and Colitis Canada we launched a research study with two aims: to adapt and improve current practices for stool collection, preservation, processing and analysis and to use these optimized methods to compare bacterial and fungal profile differences in IBD patients in remission to those in active flare. To optimize methods for self-collection of IBD stool samples we adapted OMNIgene®•GUT (OMR-200, DNA Genotek), a validated gut microbiome self-collection kit that provides ambient temperature stabilization, to donors with severe diarrhea by pairing OMR-200 with a sampling spoon (OM-AC2, DNA Genotek). To optimize sample processing, we compared common extraction methodologies, both literature based "home-brew" methods and commercially available extraction kits. 16S and ITS amplicon sequencing was performed on Illumina's MiSeq platform to interrogate diversity and relative abundance differences for bacterial and fungal taxons. We evaluated donor compliance, ease of use, accuracy of the recovered microbial profile, and sample preservation over time, in addition to investigating microbial and fungal profile differences in a cohort of IBD patients who were either in remission or experiencing flare. Donors with severe diarrhea reported that OMR-200 when combined with OM-AC2, provided an easy to use method for sample self-collection, with a sample return rate of 92%, 96% of donors reporting the method as easy to very easy and a 100% sample utilization rate. Comparison of extraction methodologies found significant differences in discovery of diversity, particularly in the Blautia and Granulicatella genera, and total nucleic acid yields. Our preliminary profile analysis suggests trends in diversity and abundance of the bacterial and fungal microbiome, between IBD patients in remission or experiencing flare. Future work will expand on these associations between disease state and taxonomic communities in IBD patients.

102

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], **Meg Manohar**[1], **Jasmina M Luczo**[1]

1. Australian Centre for Disease Preparedness, CSIRO, East Geelong, VICTORIA, Australia

Pathogen interference describes the ability 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 the ability of influenza virus to establish infection in the murine nasal cavity, although to date, the mechanism for this blockade is unknown. 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 distinct populations of respiratory and olfactory epithelial cells. 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. The presence of key epithelial cell populations and production of mucin in the nasal respiratory and olfactory *ex vivo* models 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. Confocal microscopy 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.

103

A microbiome-focused multi-omic assessment of the impact of intermittent fasting on gut bacteria

Matias Abregu[1]

1. Microbiome Science, DNA Genotek Inc, Ottawa , Ontario , Canada

Most humans will go through one or more dietary modifications or interventions during their lifetime (fasting, supplementation or other dietary changes). Several studies have shown that dietary modifications can have beneficial impacts on the host, some of which may be mediated through changes in the gut microbiome and/or its activity. In this research, we employed a longitudinal, multi-omic approach (shotgun metagenomics, metatranscriptomics and metabolomics) to study the impact of intermittent fasting (IF) on the gut microbiome of healthy individuals. In general, metatranscriptomics (MTS) sequencing profiles were much more variable and dynamic than metagenomic (MGS) sequencing profiles, with select individuals showing a strong correlation between diet and MTS functional profiles. Additional metabolic results and analyses indicated pathway enrichment related to fasting. In conclusion, our results indicate that metatranscriptomics can reveal discrete changes in microbial community functional profiles that are not detected by DNA and may be better suited to understand biological responses to dietary modifications such as intermittent fasting.

105

Inflammatory profiles of vaginal *Gardnerella vaginalis* isolates from South African women with and without bacterial vaginosis

Sarah Amir Hamzah[1], Jenna Wilson[1], Monalisa Manhanzva[2], Célia Mehou-Loko[2], Nina Radzey[2], Andrea Abrahams[2], Rushil Harryparsad[2], Bahiah Meyer[2], David Tyssen[1], Brianna Jesaveluk[1], Hilton Humphries[3, 4], Pamela Gumbi[4, 5], Linda-Gail Bekker[2, 6], Heather Jaspan[2, 7], Jo-Anne Passmore[2, 4, 8], Anna Hearps[1, 9], Gilda Tachedjian[1, 10, 11], Lindi Masson[2, 4, 1, 9]

1. Life Sciences Discipline, Burnet Institute, Melbourne, Victoria, Australia

2. Institute of Infectious Disease and Molecular Medicine and Department of Pathology, University of Cape Town, Cape Town, South Africa

3. Centre for Community-Based Research, Human Science Research Council, Pietermaritzburg, South Africa

4. Centre for the AIDS Programme of Research in South Africa, Durban, South Africa

5. Biochemistry Department, School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa

6. Desmond Tutu HIV Centre, Cape Town, South Africa

7. Seattle Children's Research Institute and University of Washington, Seattle, Washington, United States of America

8. National Health Laboratory Service, Cape Town, South Africa

9. Central Clinical School, Monash University, Melbourne, Victoria, Australia

10. Department of Microbiology, Monash University , Clayton, Victoria, Australia

11. Department of Microbiology and Immunology, University of Melbourne, Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia

Bacterial vaginosis (BV) is highly prevalent amongst women residing in sub-Saharan Africa, where HIV is also widespread. BV, characterised by the overgrowth of non-optimal vaginal anaerobic bacteria, most commonly *Gardnerella vaginalis*, is associated with genital inflammation which facilitates HIV acquisition. However, *G. vaginalis* is also found in healthy women with low levels of inflammation, suggesting strain-level differences that may influence inflammatory responses. Hence, this study aims to characterize vaginal *G. vaginalis* isolates from South African women to elucidate their role in inflammation.

Cervicovaginal fluid samples from 10 BV-negative and 10 BV-positive South African women (aged 16 – 25) were cultured on Columbia Blood agar to isolate single strains of *G. vaginalis*, followed by species-level identification via 16S rRNA Sanger sequencing. Isolates were then co-cultured with vaginal epithelial VK2/E6E7 cells and the concentrations of inflammatory cytokines previously associated with HIV risk were measured via Luminex assay. The protein profiles of the isolates were also analysed via liquid chromatography tandem mass spectrometry.

Thirty-nine isolates were acquired from all women, including $n = 15$ *G. vaginalis* and other bacterial taxa. Three and four *G. vaginalis* isolates from different BV-negative and BV-positive women, respectively, were selected for inflammatory profile assessment. Significant increases in interleukin (IL)-1β, IL-6, IL-8 and chemokine ligand (CCL)2, CCL4 and CCL5 were induced by isolates from BV-positive women compared to BV-negative women ($p < 0.05$). Proteomics analyses detected 2,139 proteins and although 125 proteins were significantly differentially abundant between isolates from BV-negative versus BV-positive women, only two remained significant after adjusting for multiple comparisons. This included chaperone protein ClpB and peptidase.

G. vaginalis isolates from BV-positive women elicited higher levels of inflammatory cytokines previously associated with increased HIV acquisition risk compared to those from BV-negative women. This suggests that strain-level differences may play an important role in genital inflammation and resultant HIV acquisition risk. Few proteins differed significantly between isolates from BV-negative versus BV-positive women, largely due to the small sample size and high protein variance between individual strains. Hence, the proteomic and genomic profiles of additional *G. vaginalis* isolates are being investigated to determine differences that may influence inflammation.

106

Genital InFlammation Test (GIFT) for sexual and reproductive health: point-of-care screening tool for sexually transmitted infections and bacterial vaginosis

Lindi Masson[4, 1, 2, 3], Monalisa T Manhanzva[1], Fezile Khumalo[1], Micaela Lurie[1], Eneyi Kpokiri[5], Ramla Fatime Tanko[1], Tanya Pidwell[1], Bahiah Meyer[1], Phumla Radebe[1], Conita Lombard[1], Celia Mehou-Loko[1], Yacoeb Ganief[1], Tania Crucitti[6], Theodora Mayouya Gamana[6], Katherine Gill[7], Nicola Thomas[7], Anda Madikida[7], Karabo Mahlangu[7], Janneke van de Wijgert[8], Bich-Tram Huynh[9], Camille Fortas[9], Chido Dziva Chikwari[5, 10, 11], Katharina Kranzer[5], Tinashe Mwaturu[5], Lyndon Mungur[12], Ashley Uys[12], Darryl Uys[12], Ayako Honda[13], Sarah Bernays[5, 14], Linda-Gail Bekker[7], Edina Sinanovic[15], Elise Smith[15], Suzanna Francis[5, 16], Saberi Marais[17], Constance Mackworth-Young[5], Emma Harding-Esch[5], David Anderson[4], Jo-Ann S Passmore[1, 2, 18]

1. Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa

2. Centre for the AIDS Programme of Research in South Africa, Durban, South Africa

3. Central Clinical School, Monash University, Melbourne, Australia

4. Burnet Institute, Melbourne, VIC, Australia

5. London School of Hygiene & Tropical Medicine, London, UK

6. Institut Pasteur de Madagascar, Antananarivo, Madagascar

7. Desmond Tutu HIV Centre, Cape Town, South Africa

8. University Medical Center Utrecht, Utrecht, Netherlands

9. Institut Pasteur, Paris, France

10. Organization for Public Health Interventions and Development, Harare, Zimbabwe

11. Biomedical Research and Training Institute, Harare, Zimbabwe

12. Medical Diagnostech, Cape Town, South Africa

13. Hitotsubashi University, Kunitachi, Japan

14. School of Public Health, University of Sydney, Sydney, Australia

15. Health Economics Unit, School of Public Health and Family Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

16. International AIDS Vaccine Initiative, Amsterdam, Netherlands

17. Research Contracts and Innovation, University of Cape Town, Cape Town, South Africa

18. National Health Laboratory Service, Cape Town, South Africa

Background: Female genital tract inflammation increases risk of adverse sexual and reproductive outcomes, including HIV acquisition and preterm birth. This inflammation is primarily caused by sexually transmitted infections (STIs) and bacterial vaginosis (BV), which are asymptomatic in most women. Although gold standard nucleic acid amplification diagnostic tests (NAATs) have been developed to detect these infections, their cost and required resources limit their use for routine screening. We have validated biomarkers of vaginal inflammation (IL-1α, IL-1β, IP-10) caused by STIs/BV in five African cohorts. We aim to develop and implement a low-cost rapid point-of-care test, called the Genital InFlammation Test (GIFT), to measure these biomarkers and increase STI/BV case-finding.

Methods: We developed two lateral flow immunochromatographic prototypes measuring both IL-1α and β in a multiplexed assay and IP-10. The devices were evaluated using recombinant cytokines and vaginal swabs collected from South African women and the results were compared to ELISA. Optimal vaginal swab type and equipment-free processing methods were determined. A two-round modified Delphi survey was administered globally to evaluate stakeholder recommendations for implementation.

Results: The prototype devices had analytical sensitivities <50pg/ml for IL-1α and β and <70pg/ml for IP-10. The test line intensities observed by the naked eye correlated with ELISA concentrations ($r=0.78$, $p=0.02$; $r=0.90$, $p=0.001$; $r=0.97$, $p<0.0001$ for IL-1α and β and IP-10, respectively). Flocked swabs were identified as the best swab type and swab compression by hand in phosphate buffered saline preloaded into soft nozzle-cap tubes increased cytokine recovery from vaginal swab secretions compared to vortexing. Sixty-four stakeholders, mainly healthcare professionals, responded to the Delphi survey. The majority (84%) would offer screening with GIFT to sexually active asymptomatic women in low and middle-income regions.

Conclusion: If offered to women attending healthcare clinics or used for self-testing, GIFT could increase STI/BV case-finding. The GIFT device, user experience and cost-effectiveness are currently being evaluated in three settings in Africa. The next step will be to identify use cases for GIFT in other world regions, including the Asia-Pacific.

107

The plight of the metabolite- metabolipidomic changes to the ocular surface in ocular allergy sufferers across seasons in Victoria, Australia

Esrin Aydin[1, 2], Moneisha Gokhale[2], Serap Azizoglu[2], Luke Chong[2], Cenk Suphioglu[1]

1. NeuroAllergy Research Laboratory (NARL), School of Life and Environmental Sciences, Deakin University, Waurn Ponds, Victoria, Australia

2. School of Medicine, Deakin University, Waurn Ponds, Victoria, Australia

Background: Ocular allergy (OA) is a localised subset of allergy characterised by ocular surface itchiness, redness and inflammation. Inflammation and eye-rubbing, due to allergy-associated itchiness, are common in OA sufferers and may trigger changes to the ocular surface biochemistry. The primary aim of this study is to assess differences in human tear metabolome and lipidome between OA sufferers and healthy controls (HC) across peak allergy (Spring-Summer) and off-peak season (Autumn-Winter) in Victoria, Australia.

Methods: 31 participants (22 OA sufferers, 9 healthy controls) aged 18-45 were recruited. Participants were grouped based on allergy symptom assessment questionnaire scoring. Metabolites and lipids were extracted from human tear samples and analysed using Mass Spectrometry. Data was analysed using TraceFinder, Metaboanalyst and IBM SPSS softwares.

Results: Metabolomics analysis showed 12 Differentially Expressed (DE) metabolites between OA and HC during peak allergy season, and 24 DE in off-peak season. Expression of niacinamide was significantly upregulated in OA vs HC across both seasons ($p<0.05$, Log_2 fold-change>1.5). Lipidomics analysis showed 6 DE lipids between OA and HC during peak season, and 24 between OA and HC in off-peak season. In the off-peak season, (O-acyl) ω-hydroxy fatty acids (OAHFA) had a Log_2 fold-changes up to 5.6 times higher in OA

than HC. Comparison of HC across seasons showed 19 DE lipids ($p<0.05$, Log_2 fold-change>1.5), including OAHFA, wax esters, phosphatidylcholines, sphingomyelins and lysophosphatidylethanolamines.

Conclusions: Tear metabolomics showed dysregulation of metabolites involved in cell structure, inflammation, and homeostasis between OA and HC across seasons, suggesting a possible link between OA-associated itch and ocular surface damage via eye-rubbing. Tear lipidome changes were minimal between OA and HC in peak season, but HC data across seasons suggested a protective role of the tear film on the ocular surface that may reduce the effects of airborne allergens on the lipidome during peak allergy season.

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Improving plasmapheresis: Novel avenues for treating cloaking antibodies

Joshua N Monteith[1], Emma L Ledger[1], Carrie C Coggon[2], Ian R Henderson[2], David W Reid[3, 4, 5], Daniel Smith[4], Daniel C Chambers[6], Simon H Apte[6], Timothy J Wells[1, 5]

1. Frazer Institute, University of Queensland, Brisbane, Queensland, Australia

2. Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD, Australia

3. QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia

4. The Adult Cystic Fibrosis Centre and Department of Thoracic Medicine, The Prince Charles Hospital, Brisbane, Queensland, Australia

5. Australian Infectious Diseases Research Centre, University of Queensland, Brisbane, QLD, Australia

6. Lung Transplant Laboratory, The Prince Charles Hospital, Brisbane, QLD, Australia

During chronic *P. aeruginosa* lung infection, a subset of patients develop an antibody response that is able to paradoxically inhibit complement-mediated killing of their infecting strain. These 'cloaking antibodies' were found to be specific to O-antigen and are correlated with worse disease outcomes in patients with bronchiectasis, and lung transplants recipients. In light of this, plasmapheresis was conducted as a salvage therapy for three patients with chronic *P. aeruginosa* infection, which included the removal of patient antibody from circulation. This treatment resulted in decreased bacterial burden and as such, greatly improved patient outcomes (1, 2). However, the use of plasmapheresis to P. aeruginosa infection is imprecise, requiring the total removal of antibody including potentially beneficial immunoglobulin. In addressing this, three affinity purification methods i) O-antigen affixed polymyxin-B column; ii) O-antigen-streptavidin column, and; iii) O-antigen magnetic nanobeads were developed that selectively deplete cloaking antibody when passaged with patient sera *in vitro*. Selective depletion of cloaking antibody in patient serum was not only able to restore, but in certain modalities improve serum-mediated killing of P. aeruginosa in comparison to healthy, normal serum. Depletion of O-antigen-specific antibody occurred across IgG, A and IgM responses whilst maintaining antibody to *P. aeruginosa* outer-membrane proteins. More promisingly, this novel finding demonstrates that patient sera with cloaking antibody still maintains the ability to promote antibody-mediated serum killing of *P. aeruginosa*. This highlights the need to improve plasmapheresis to recirculate healthy, protective antibody to the host and in turn initiate effective and durable clearance of infection.

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Myeloid derived NLRP3 inflammasomes exacerbate disease in a murine model of Silicosis

Kristian Barry[2, 1], Maggie Lam[2], Christopher Hodges[2], Christopher Harpur[2], Ashley Mansell[2], Michelle Tate[2]

1. Translation and Molecular Sciences, Monash University, Melbourne, Victoria, Australia

2. Hudson Institute of Medical Research, Clayton, VIC, Australia

Silicosis is an irreversible occupational respiratory disease caused by the inhalation of respirable silica particles which induce a non-resolving chronic inflammation of the lung that leads to fibrosis. Critically, there are currently no therapeutic treatments for silicosis to halt or reverse fibrosis. Previous studies have identified that silica particles activate the NLRP3 inflammasome and drive pathology in murine models of silicosis, and that NLRP3 deficient mice have reduced pulmonary fibrosis. While NLRP3 has been implicated in driving disease pathology, the cellular compartment of NLRP3-induced inflammation is not well understood. We have generated a novel myeloid-specific knockout of NLRP3 (LysM-Cre/NLRP3$^{fl/fl}$) to elucidate the role of NLRP3 during silicosis. We demonstrate that LysM/NLRP3$^{fl/fl}$ macrophages do not express NLRP3 protein, fail to respond to multiple NLRP3 agonists, and that NLRP3 expression in the lung is significantly reduced in LysM-Cre/NLRP3$^{fl/fl}$ mice. Interestingly, myeloid specific depletion of NLRP3 has no significant effect on inflammatory cytokine production or inflammatory cell infiltrates into the lung 3 days after silica challenge. Critically however, LysM-Cre/NLRP3$^{fl/fl}$ mice display significantly reduced numbers and size of silicotic nodules at day 14, as well as reduced collagen deposition, suggesting a decreased burden of disease compared to control mice. These data suggest that the myeloid derived NLRP3 inflammasome activity plays a role in the progression and pathogenesis of silicosis, however early NLRP3-derived inflammatory response to silica challenge may be myeloid-independent. Further research will be required to identify the mechanism by which NLRP3 induces fibrosis associated with silicosis, but these studies may suggest that therapeutic targeting of myeloid NLRP3 activity may slow or reduce the disease pathology associated with silicosis.

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Investigation of the immune evasion function of interferon antagonists of different Hendra virus genotypes

Melanie Tripp[1, 2], Cassandra T David[1], Stephen M Rawlinson[1], Jasmina Lucso[2], Sarah Edwards[2], Glenn Marsh[2], Kim Halpin[2], Gregory W Moseley[1]

1. Department of Microbiology, Monash University, Clayton, VIC, Australia

2. CSIRO Health and Biosecurity, Australian Animal Health Laboratory, Geelong, Victoria, Australia

Publish consent withheld

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Building a macrophage: promoter, enhancer and chromatin remodelling in ipsc-derived myeloid progenitors and their progeny.

Miguel A Berrocal[1], Sefi Prawer[1], Michael B Clark[1], Christine A Wells[1], Ricardo De Paoli-Iseppi[1], Hazuki Takahashi[2], Piero Carninci[2]

1. University of Melbourne, CARLTON, VIC, Australia

2. Riken, Tsurumi, Japan

Macrophages are specialised cell types resident in most tissues of the body, and with diverse roles in homeostasis, host defence and tissue repair. While transcription factors such as PU1.1 and other master regulators of human macrophage differentiation are well understood, the chromatin changes that these orchestrate to direct macrophage specialisation are superficially mapped. To increase molecular resolution of developing macrophage networks, we undertook multiomics analysis that includes CAGE mapping of enhancers and promoters, long-read transcript sequencing to identify the isoforms that direct macrophage gene expression networks, and proteomics to identify the specific protein isoforms that are generated through macrophage development. Micro-C and RADCL-seq further map the chromatin interactions that coincide with these transcriptional and phenotypic changes. We identified the Neuregulin 1 locus as a region of particular interest, with the discovery of a myeloid class of NRG1-VII, which is dynamically regulated in progenitors and macrophages. The combined dataset provides unprecedented resolution of the molecular networks that underpin human macrophage differentiation.

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Insights into the role of ribose metabolism for the virulence and persistence of *Haemophilus influenzae* in the host

Saurab Kishore Munshi[1], Marufa Nasreen[1], Alastair G. McEwan[1], Ulrike Kappler[1]

1. School of Chemistry and Molecular Biosciences, The Australian Infectious Disease Research Centre, The University of Queensland, Brisbane, Queensland, Australia

The interaction of bacteria with the human respiratory epithelial mucosa is a crucial factor in the pathogenesis and in persistence of infections with non-typeable *H. influenzae* (NTHi). Analysis of NTHi metabolic endproducts during growth on a tissue culture-related medium has demonstrated that the bacteria consume ribose present in nucleosides such as inosine, making ribose a major nutrient. However, whether the ability to grow on pentose sugars affects virulence has not been studied yet. In this investigation, the physiological effects of mutations in *rbsB*, that encodes the substrate binding protein of the NTHi ribose ABC transporter, and *rbsK* and *rpiA*, that encode two enzymes involved in the pentose phosphate pathway, ribokinase (RbsK) and ribose-5-phosphate isomerase (RpiA) were investigated. Mutant strains were constructed, and complemented using both single and multi-gene complementation. Phenotypical validation showed that *rbsB* and *rbsK* mutants were unable to grow on ribose, while the *rpiA* mutant showed an ~2-fold reduction in growth compared to the wildtype. The mutations also reduced growth on ribose from uridine, indicating that ribose, and not uracil is used as a carbon source. Phenotypic microarrays confirmed a reduced ability to grow on ribose and other pentoses for the *rbsK* mutant. The inability to efficiently use ribose as a carbon source created other phenotypic alterations such as a ~10% reduction ($p<0.0001$) in ATP content, but had no effect on hypochlorite resistance. Infection assays using 16HBE14 bronchial epithelial cells, unexpectedly, showed no significant changes relative to the WT. In contrast, in mouse bone marrow macrophage infections, the *rbsB* and *rbsK* mutants showed 2- and 4- fold reductions, respectively, in intracellular survival at 2h post infection. Overall, the findings indicate that ribose utilization supports NTHi virulence, and appears to be particularly important for oxidative stress survival, i.e. in contact with phagocytic immune cells.

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Assessment of growth dynamics over time and dispersal responses to nitric oxide by *P. aeruginosa* biofilms

Xavier Bertran i Forga[1], Jilong Qin[1], Makrina Totsika[1]

1. Queensland University of Technology, South Brisbane, QLD, Australia

The opportunistic pathogen *P. aeruginosa* is a main causative agent of chronic infections in hospital settings due to its readiness to form biofilms in a multitude of tissues and surfaces. In this state, their tolerance to antimicrobials becomes greatly enhanced. To sensitise biofilms to treatment, dispersal agents such as nitric oxide (NO) have been explored to support the action of antimicrobials due to their ability to revert bacteria to their planktonic phenotype. However, studies exploring the activity and molecular mechanisms activated in *P. aeruginosa* during NO-mediated dispersal have been performed utilising a wide variety of study designs. This lack of consensus over study design spans over factors such as the utilised platform, biofilm growth time, type of NO donor, concentration and treatment time, all variables that could induce changes in biofilm responses. Due to their high throughput design, closed systems are widely used platforms to monitor changes in biofilm biomass, but due to the limited quantity of nutrients and aeration, biofilm growth can be severely impacted over time, potentially affecting how NO-mediated dispersal is interpreted. In this study, the biofilm growth of two strains of *Pseudomonas aeruginosa* (PAO1 and PA14) in M9, BM2 and Mueller-Hinton (MH) media was monitored by CFU counting or crystal violet staining over 24h using common plate-based platforms for *in vitro* biofilm assays in either shaking or static conditions to capture the evolution of biofilm biomass over time in closed systems. Prior to reaching a point of innate dispersal, biofilms were treated with different concentrations of NO donors, and biomass reductions were recorded under different sets of conditions over a span of 2 hours. The goal of this ongoing investigation is to illustrate how growth and treatment conditions can compromise the outcomes that have been collectively identified as dispersal to this day.

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The MIF Myth + post-docs at CSIRO

Tim Muusse[1], Sriprada Mahabhashyam[1], Benjamin Cao[1], Jim Harris[1]

1. CSIRO, Clayton, VIC, Australia

Protein-protein interactions are key to inflammatory signaling and a multitude of proteins 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 pathways, including NF-kB and NLRP3 inflammasome activation. MIF is constitutively present in the cytosol and extracellular space, yet its mode of secretion is unclear. MIF is protective and pro-inflammatory in response to a range of bacterial pathogens, but potentially permissive to some viruses. Moreover, deletion or inhibition of MIF has been shown to decrease severity and/or susceptibility to a number of inflammatory and autoimmune diseases, including rheumatoid arthritis, lupus and inflammatory bowel disease. Here, we show that MIF inhibitors have varied effects on cytokine/chemokine release by human macrophage. While monoclonal antibodies and small molecule inhibitors of MIF have helped to decipher its role in mediating cytokine production and signal transduction, their specificity is questionable and there remains no consensus on the primary role of MIF. Thus, we are now utilising CRISPR-cas9 to tag endogenous MIF, either with a fluorescent protein or biotinylation enzyme, providing a pathway to investigate both transient and stable protein-protein interactions and cellular localisation. Covalently tagged MIF holds potential to decrease the number of non-MIF-specific hits currently seen with immune-precipitation and mass spectrometry. This approach will not only help us to uncover the mysteries of MIF but will provide a pipeline for immune protein interactome studies. Additionally, this project leverages CSIROs post-doc program and the Immune Resilience FSP to bridge the gap between traditional academic post-docs and industry projects while providing a personalised career development program.

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Fluorescent probes derived from antimicrobial peptides: tools for discovery and diagnostics

Bing Zhang[1], Wanida Phetsang[1], Sanjaya KC[1], Glen Lamb[1], Anthony Verderosa[1], Alysha G Elliott[1], Mark AT Blaskovich[1]

1. Centre for Superbug Solutions, Institute for Molecular Bioscience, The University of Queensland, St Lucia, QLD, Australia

Antimicrobial resistance threatens the foundations of modern medicine, with a lack of new antibiotics compounding the crisis. Antimicrobial peptides (AMPs) have attracted substantial attention over many decades as a potential source of new therapeutics, but with the exception of natural product-derived cyclic lipopeptides such as the polymyxins (colistin, polymyxin B) and daptomycin, they have failed to achieve clinical approval.

Our group has research program developing derivatised versions of major antibiotics that allow for facile attachment of additional functional groups, including fluorophores [1]. We have prepared fluorescent versions of a range of antimicrobial peptides, including polymyxin B, octapeptin C4, tachyplesin arenicin-3, and daptomycin. These have employed small fluorophores (nitrobenzoxadiazole, NBD: green, and dimethylaminocoumarin-2-acetic acid, DMACA: blue). Notably, the derivatised antibiotics to retain similar activity and resistance profiles as the parent antibiotic, making them useful probes to investigate antibiotic-bacteria interactions.

The octapeptins are cyclic octamer lipopeptides structurally similar to the polymyxins, but which intriguingly retain activity against polymyxin-resistant Gram-negative bacteria [2]. In contrast, the arenicins are 21-residue amphipathic β-hairpin peptides closely related to tachyplesin and protegrin-1, with arenicin-3 analogs progressed into advanced preclinical testing by Adenium Biotech [3].

High resolution microscopy of fluorescently-labelled bacteria demonstrated strikingly different localisation patterns between the four antibiotics, despite all nominally working via membrane disruption. Further work has found that the polymyxin and daptomycin probes can rapidly distinguish between bacteria with varying levels of resistance to the parent antibiotic, potentially leading to a useful rapid assay for resistance via flow cytometry or plate-based analysis. The data to date suggests that the probes are valuable tools to aid in understanding antibiotic-bacteria interactions, supporting the fight against antimicrobial resistance.

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Exploring targeted immunomodulation as a treatment for legionella induced pneumonia

Joshua M Nickson[1, 2], Thomas Naderer[1, 2]

1. Monash University, Clayton, VIC, Australia

2. Centre to Impact AMR, Clayton, VIC, Australia

Sensing of microbes by the immune system depends on dedicated receptors (pathogen recognition receptors) that bind pathogen-associated molecular patterns (PAMPs). Activation of these receptors triggers immune responses, including inflammation, aimed at eliminating microbial infections. Pathogens, however, have evolved mechanisms that prevent activation of host receptors and downstream signalling pathways to promote their survival. Understanding how pathogens prevent effective immunity will enable the development of alternative strategies that rely on activating the immune system to treat infectious diseases. So far, targeted immune activation during established infection remains challenging due to the associated risks of immunopathology. Here, we discuss efforts to identify activators of pathogen recognition receptors to clear infections without inducing immunopathology in the Legionella lung infection model. We used established murine infections and tested available agonists of toll-like receptors to determine bacterial burdens, immune responses and cell numbers within the local and distal tissues and serum. We identified that the localized activation via Polyinosinic:polycytidylic acid (PolyIC), a double-stranded RNA analog, but not other agonists affected bacterial lung burdens. Spectral flow cytometry and cytokine arrays have identified that PolyIC alters innate immune responses in Legionella-infected lungs. We will discuss how the activation of host immune receptors affects intracellular pathogens and how this can be harnessed to treat infections.

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Mouse precision-cut lung slices as a novel model of early bacterial infection with Methicillin-resistant *Staphylococcus aureus*

Jane E Bourke[1], Evelyn Zhang[1], Joshua Nickson[1], Wiliam Studley[1], Jennifer AE Payne[1], Max J Cryle[1], Thomas Naderer[1], Chris Greening[1], Thomas Watts[1]

1. Monash University, Mulgrave, VIC, Australia

Introduction/Aim:
Methicillin-resistant Staphylococcus aureus (MRSA) is a feature of both hospital- and community-acquired pneumonia, but translatable models to investigate early pathogenesis and potential treatments are limited. Precision-cut lung slices (PCLS), containing all resident structural and immune cells of the lung, may provide a novel approach for dynamic time-course studies of MRSA infection with higher throughput than *in vivo* mouse models.
Methods:
To establish infection, PCLS from agarose-inflated lungs of male C57BL/6 mice (8 -10 weeks old) were incubated for 2 h with MRSA (dsRED-expressing, 10^5 colony-forming units (CFU)) and transferred to fresh media. Up to 48 h post-infection (hpi), MRSA were visualised by fluorescent microscopy or quantitated as CFU in intact or homogenised PCLS. MRSA association with alveolar macrophages (AMs) and dendritic cells (DCs) were detected by flow cytometry. Media was assayed for lactate dehydrogenase activity (viability measure) or released cytokines by BioLegend LEGENDplex™ (inflammation).
Results:
Viability was similar in uninfected and infected PCLS. Diffuse MRSA colonies were evident across all structures (airways, arteries, parenchyma) in PCLS, increasing CFU >100,000 fold by 48 hpi (n=6, $P<0.001$). The increased proportion of CD45+ immune cells associated with MRSA over time (24hpi 9±2%; 48hpi 74±10%, n=4, $P<0.05$) was evident in AMs and DCs but not non-phagocytic B cells. The inflammatory cytokines TNF-α, GM-CSF and IL-6 were increased in MRSA-infected PCLS within 24 hpi (n=6, $P<0.05$).
Conclusion:
Mouse PCLS provide an *ex vivo* model of MRSA infection involving intact resident immune cells and inflammation. Our findings support the future application of human PCLS for the assessment of drugs to prevent infection or limit the replication of resistant bacterial strains.

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Redox-adapted macrophages evade ferroptosis in tumor microenvironments

Patricia P Ogger[1], Julia Sauer[2], Meshal Ansari[2], Carolin K Watson[2], Eva Griesser[2], Katrin Fundel-Clemens[2], Katja Köppen[3], Alina Nessensohn[2], Matthew J Thomas[2], Karim C El Kasmi[3], Peter J Murray[1]

1. Max-Planck Institute for Biochemistry, Planegg, BAVARIA, Germany

2. Boehringer Ingelheim Pharma GmbH & Co KG, Biberach, Germany

3. Boehringer Ingelheim Pharma GmbH & Co KG, Ridgefield, CT, USA

Publish consent withheld

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Inhibition of interferon induction by P protein isoforms of rabies virus fixed and street strains

Kazuma Okada[1, 2], Junki Hirano[1, 2], Kentaro Uemura[1, 2], Chikako Ono[1, 2], Shuhei Taguwa[1, 2, 3], Yoshiharu Matsuura[1, 2]

1. Laboratory of Virus Control, Center for Infectious Disease Education and Research (CiDER), Osaka University, Suita, Osaka, Japan

2. Research Institute for Microbial Diseases (RIMD), Osaka University, Suita, Osaka, Japan

3. Center for Advanced Modalities and DDS (CAMaD), Osaka University, Suita, Osaka, Japan

The P gene of rabies virus (RABV), which plays a crucial role in immune evasion, encodes five proteins known as P protein isoforms (P1-5). These isoforms are essential for suppressing interferon (IFN) induction in muscle cells and contribute to neuroinvasiveness, the ability of the virus to invade the nervous system from peripheral tissues. However, previous studies in which the role of P2-5 as IFN antagonists was investigated were conducted using only the laboratory RABV (fixed) strain. While the wild RABV (street) strain typically exhibits original neuroinvasiveness, the function of P2-5 of the street strain in inhibiting IFN induction has not yet been studied. Therefore, in this study, we investigated the inhibitory activities of P1-5 on IFN induction using the myoblast cell line C2C12 by expressing the P proteins of both the fixed strain CVS and the street strain 5989. Cells were transfected with pIFNb-luc, which expresses firefly luciferase under the control of the IFN-β promoter, and with pRL-SV40, a control reporter vector expressing Renilla luciferase, along with each P1-5 expression vector from CVS and 5989 strains. At 24 hours after transfection, cells were stimulated by transfecting Poly(I:C) LMW, which is a dsRNA analog ranging from 0.2 kb to 1 kb, and Poly(I:C) HMW ranging from 1.5 kb to 8 kb. After 24 hours of incubation with Poly(I:C), the cells were subjected to dual luciferase assays. Cells expressing CVS-P1, P2 and 5989-P1 to P5 stimulated with Poly(I:C) LMW showed significantly lower IFN-β promoter activities than did cells transfected with an empty plasmid (control cells), but there was no significant difference between the control cells and cells expressing CVS-P3 to P5. In contrast, cells expressing 5989-P1 to P5, but not CVS-P1 to P5, stimulated with Poly(I:C) HMW showed significantly lower IFN-β promoter activities than did the control cells. These findings suggest that P2-5 of the street strain, unlike the fixed strain, retain a robust ability to inhibit IFN induction stimulated by both short and long dsRNAs.

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

Nicole K Campbell[1, 2], Jamie Gearing[1, 2], Paul J Hertzog[1, 2]

1. Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, VIC, Australia

2. Department of Molecular and Translational Sciences, Monash University, Clayton, VIC, Australia

The novel type I interferon, interferon epsilon (IFNε), is a unique cytokine which is constitutively expressed by the female reproductive tract (FRT) epithelium. High grade serous ovarian cancer (HGSOC) is a cancer of the FRT which frequently presents with extensive metastasis throughout the peritoneal cavity, and carries a 5-year survival rate of <40%. We recently published research describing the anti-metastatic properties of IFNε in HGSOC, which was largely mediated through regulation of the anti-tumour immune response[1]. To identify which immune cells and molecular pathways determine the mechanism of action of IFNε in HGSOC we performed single-cell RNA sequencing (scRNAseq) analysis of peritoneal cells from control and IFNε-treated mice.

Peritoneal cells were isolated from healthy female C57BL/6J mice treated with intraperitoneally (i.p.) injected PBS or IFNε for 4 hours, and from mice which were i.p. injected with ID8*Tp53-/-Brca2-/-* ovarian cancer cells, and treated with either i.p. PBS or IFNε for two weeks. Gene expression within different populations of peritoneal immune and tumour cells was analysed via scRNAseq.

Increased expression of interferon stimulated genes (ISGs) was observed in all peritoneal immune cell populations following treatment with IFNε for 4 hours, with the highest levels of ISG expression observed in myeloid cells. In the early metastatic setting, IFNε treatment induced significant remodelling of the peritoneal myeloid cells, particularly macrophages and monocytes. Peritoneal myeloid cells from IFNε-treated mice displayed upregulated expression of gene signatures associated with metabolic pathways (e.g. glycolysis, oxidative phosphorylation, fatty acid oxidation), reduced expression of gene signatures associated with TGFβ and IL6/STAT3 signalling, and reduced expression of genes encoding apolipoproteins, including *Apoe*. Alterations were also observed in the peritoneal T cell and NK cell populations within IFNε-treated mice, which demonstrated increased expression of genes associated with activation and cytotoxicity.

Remodelling of peritoneal macrophages and monocytes, and promotion of anti-tumour immune responses by T cells and NK cells, may determine the anti-metastatic mechanism of action by IFNε in HGSOC. Further studies are required to elucidate the contributions of these key peritoneal immune cell populations and molecular pathways towards the suppression of peritoneal carcinomatosis.

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Molecular basis for neutralization of the bacterial type I restriction-modification system by a phage protein

Suk-Youl Park[1]

1. Pohang Accelerator Laboratory, POSTECH, Pohang, GYEONGBUK, South Korea

Type I restriction-modification (R-M) enzymes are oligomeric proteins composed of methylation (M), DNA sequence-recognition (S), and restriction (R) subunits. They recognize bipartite DNA sequences of 2-4 consecutive bases. The two target recognition domains (TRDs) within a single S-subunit provide a background for this recognition. Two M-subunits and a single S-subunit can form a separate oligomeric protein from the holo R-M complex and function as a methyltransferase (MTase). Here, we show the crystal structure of the intact MTase from *Vibrio vulnificus* in complex with the DNA-mimicking Ocr protein. This intact MTase includes regions for an M-subunit and a half S ($S_{1/2}$)-subunit in a single polypeptide. The canonical M-subunit domain structure in the N-terminus is followed by the $S_{1/2}$-subunit of a long a-helix, a TRD, and another long a-helix. Unexpectedly, the two a-helices of the $S_{1/2}$-subunit associate to form a coiled-coil structure that further ensembles with those of the neighboring molecule in the crystalline state and in solution. The TRD cleft of the $S_{1/2}$-subunit and the M-domain of the neighboring molecule form a cleft binding for the Ocr protein. Structure and interpretation of the biophysical data indicate the dynamic character of the protein to form the DNA-binding sites by two molecules with the four-helical bundle as an assembling point. The revealed molecular features of the intact $MS_{1/2}$ structure provides how the phage evades its killing system in bacteria.

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Differential kinetics of splenic CD169+ macrophage death is one underlying cause of virus infection fate regulation

Valentina Casella[1, 2], Eva Domenjo-Vila[2], Anna Esteve-Codina[3], Mireia Pedragosa[1], Paula Cebollada Rica[2], Enric Vidal[4, 5], Iván de la Rubia[1, 6], Cristina López-Rodríguez[7], Gennady Bocharov[8, 9], Jordi Argilaguet [4, 5, 2], Andreas Meyerhans[10, 2]

1. Universitat Pompeu Fabra, Barcelona, SPAIN, Spain

2. Infection Biology Laboratory, Department of Medicine and Life Sciences (MELIS), Universitat Pompeu Fabra (UPF), Barcelona, Spain

3. CNAG-CRG Centre for Genomic Regulation (CRG), Barcelona, Spain

4. Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Bellaterra, Spain

5. IRTA, Programa de Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Bellaterra, Spain

6. EMBL Australia Partner Laboratory Network at the Australian National University, Acton, Australia

7. Immunology Unit, Department of Medicine and Life Sciences (MELIS), Universitat Pompeu Fabra (UPF), Barcelona, Spain

8. Marchuk Institute of Numerical Mathematics, Russian Academy of Sciences, Moskow, Russia

9. Sechenov First Moskow State Medical University, Moskow, Russia

10. Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain, SPAIN

Acute infection and chronic infection are the two most common fates of pathogenic virus infections. While several factors that contribute to these fates are described, the critical control points and the mechanisms that underlie infection fate regulation are incompletely understood. Using the acute and chronic lymphocytic choriomeningitis virus (LCMV) infection model of mice, we find that the early dynamic pattern of the IFN-I response is a differentiating trait between both infection fates. Acute-infected mice generate a 2-wave IFN-I response while chronic-infected mice generate only a 1-wave response. The underlying cause is a temporal difference in CD8 T cell-mediated killing of splenic marginal zone CD169+ macrophages. It occurs later in acute infection and thus enables CD169+ marginal zone macrophages to produce the 2nd IFN-I wave. This is required for subsequent immune events including induction of inflammatory macrophages, generation of effector CD8+ T cells and virus clearance. Importantly, these benefits come at a cost for the host in the form of spleen fibrosis. Due to an earlier marginal zone destruction, these ordered immune events are deregulated in chronic infection. Our findings demonstrate the critical importance of kinetically well-coordinated sequential immune events for acute infection control and highlights that it may come at a cost for the host organism.

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The effects of megadose sodium ascorbate (vitamin C) on bacterial clearance and immune function in rodent sepsis

Rachel M Peiris[1, 2], Clive N May[1], Dianna M Hocking[2], Jose A Villadangos[2, 3], Lindsea C Booth[1], Michael J McKinley[1], Robin M McAllen[1], Laura Cook*[2, 3], Yugeesh R Lankadeva*[1]

1. Preclinical Critical Care Unit, The Florey Institute, Parkville, Victoria, Australia

2. The Department of Microbiology and Immunology, The University of Melbourne at The Peter Doherty Institute for Infection and Immunity, Parkville, Victoria, Australia

3. Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Victoria, Australia

Background: Sepsis results from the inability of the host to resolve an infection, leading to systemic infection, multi-organ dysfunction and death. Despite available treatments, sepsis remains the primary cause of death in intensive care units, accounting for ~11 million global deaths annually. Effective treatments are urgently needed. Our data from human and sheep studies show that sodium ascorbate improves clinical outcomes in sepsis, but the mechanisms are still unknown.

Aim: To assess the ability of intravenous mega-dose sodium ascorbate to accelerate resolution of systemic bacterial sepsis in rodents.

Methods: Sprague-Dawley male rats were intravenously administered a live *Escherichia coli* (*E. coli)* bolus (4×10^9 $CFUmL^{-1}$), and treated with either intravenous mega-dose sodium ascorbate (1-3g/kg; N=7) or fluid-matched vehicle-saline (N=7) for 3-h. Blood samples were collected prior to sepsis-induction and at 1.5-h and 3-h following treatment. We determined blood bacterial counts by using a colony-forming assay. Blood was stimulated *in vitro* to assess phagocytosis with *S. aureus*- or *E. coli*-coated beads, and reactive oxygen species production in myeloid cells using spectral cytometry.

Results: 3-h after receiving the *E. coli* bolus, bacterial counts in rats treated with sodium ascorbate (3.76 ± 0.11 $\log_{10}[CFUmL^{-1}]$) were significantly lower than the animals that received vehicle-treatment (4.27 ± 0.12 $\log_{10}$[CFUmL-1]) ($P_{Group} = 0.01$). At these concentrations sodium ascorbate did not have any bacteriostatic or bactericidal effects. Rats treated with ascorbate had a 5-fold increase in the granulocytes (RP-1+) that were phagocytic and producing reactive oxygen species compared to vehicle-treated animals (26.5 ± 6.63 vs 5.55 ± 2.38%).

Conclusion: IV-administered mega-dose sodium ascorbate rapidly enhanced bacterial clearance in a rodent model of sepsis. This was not due to direct bacterial killing or inhibiting bacterial growth, indicating immune-mediated effects. Our data indicates a key mechanism of ascorbate action is stimulating reactive oxygen species production by granulocytes.

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The *Helicobacter pylori* autotransporter ImaA associates with extracellular vesicles to modulate host immune responses

Angus Cramond[1, 2], Jack Emery[1, 2], Nina Colon[2], Dongmei Tong[2], Caroline Skene[2], Richard Ferrero[1, 3, 2]

1. Department of Molecular and Translational Science, Monash University, Clayton, Vic, Australia

2. Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, VIC, Australia

3. Department of Microbiology, Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia

Helicobacter pylori produces four autotransporter proteins, which use a conserved beta barrel to transport passenger domains through the bacterial membrane. Each *H. pylori* autotransporter protein has a distinct passenger domain, but with the exception of the vacuolating cytotoxin, VacA, these autotransporters remain largely unstudied. Previous work on the *H. pylori* autotransporter ImaA found that *imaA* knockout bacteria induce stronger IL-8 responses in gastric epithelial cells (AGS), suggesting ImaA may be an immunomodulatory protein. Proteomic studies showed that *H. pylori* ImaA associates with extracellular vesicles (EVs) which are known to induce inflammatory responses in host cells. Thus, we hypothesised that EVs containing ImaA may be able to modulate host responses. To address this hypothesis, we generated *H. pylori imaA* mutant bacteria then isolated EVs from these and the corresponding WT bacteria. Nanoparticle tracking analysis showed that these *imaA* and WT EVs have similar particle sizes (median sizes 95 and 100 nm, respectively). Next, we stimulated AGS cells with WT and *imaA* EVs. We found *imaA* EVs induced weaker IL-8 responses than WT EVs (3.4- and 2.6-fold change from unstimulated for WT and *imaA* EVs, respectively). To investigate the immunomodulatory effects of ImaA *in vivo*, mice were vaccinated on days 0 and 28 with *H. pylori* WT or *imaA* EVs, or PBS, then euthanised on day 56. Splenocytes from mice that were vaccinated with either *H. pylori* WT or *imaA* EVs *ex vivo* showed reduced IFN-γ and IL-17 responses to ConA stimulation *ex vivo* relative to splenocytes from unvaccinated mice, suggesting both EVs suppress Th1 and Th17 responses. Taken together, we propose that *H. pylori* ImaA contributes to the pro-inflammatory effects of EVs on epithelial cells. Further work is, however, required to understand the role of EV-associated ImaA *in vivo*.

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TREML4 ablated mice develop protection against lethal Candida albicans secondary infection following polymicrobial sepsis

Corey Pollock 1 , Joseph Menassa 1 , Hamsa Puthalakath 1

1. Department of Biochemistry and Chemistry, La Trobe Institute for Molecular Science, Melbourne, VIC, Australia

Sepsis is a biphasic disease characterised by acute inflammation, leading to extensive immune suppression, exposing patients to secondary infections. Many clinical trials sought to modulate sepsis induced inflammation, but this had no effect on patient mortality, primarily due to secondary infections. Recently, focus has shifted to addressing the immunosuppressive phase of sepsis, aiming to return the host's immune system to a functional state, to prevent further deterioration from secondary infections. Previous work by Nedeva et al., (2020) identified TREML4, a triggering receptor expressed on myeloid cells (TREM) receptor, as the key regulator of immune cell death and inflammation, following polymicrobial sepsis. Genetic ablation of TREML4 in mice using CRISPR demonstrated its role in regulating many cellular responses, such as apoptosis of innate immune cells which was reduced in the absence of TREML4, leading to a higher rate of survival in the acute and chronic phase of sepsis during secondary infection with Pseudomonas aeruginosa and most recently against Candida albicans. This improved survival is due, in part, to long lasting epigenetic changes in innate immune progenitor cells of TREML4 ablated mice. Overall, epigenetic changes to progenitor cells during the early stages of polymicrobial sepsis results in an innate immune memory that protects TREML4 ablated mice from secondary infections during the chronic phase of sepsis.

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A fluorescence-based system for screening CRISPR-Cas13 efficacy and collateral effects.

Sudip Dhakal[1], Arjun Challagulla[1], James Wynne[2], Kristie Jenkins[1]

1. CSIRO, East Geelong, VIC, Australia

2. CSIRO, Hobart, Tasmania, Australia

With advancements in the CRISPR-Cas13 technologies, it has been possible to precisely target specific RNA for knocking it down to supress the gene expression or to edit the bases as per the requirement. However, one of the major concerns in using Cas13 as an RNA targeting enzyme has been its collateral effect on other RNAs that come in its close vicinity when activated. In addition, as the metagenomic discovery of various Cas13 proteins have been rapidly identifying hundreds of Cas13 proteins, it is imperative to have a rapid Cas13 screening platform which can assist in comparing the efficiency and off target effects. In order to address such issue, a three-fluorescent protein-based system has been developed and tested for some variants of Cas13 proteins. As the system is based on fluorescent proteins, the fluorescence imaging and quantifying methods have been employed to visualize the data. In this system, red fluorescent protein (miRFP670nano) was used as a marker for Cas13 expression, blue fluorescent protein (mTagBFP2) as gRNA expression marker and the green fluorescent protein (eGFP) as the target for Cas13. The blue and the red fluorescent proteins also provide information on off target or collateral effect of the Cas13 enzyme. The poster will present an example evaluation of the efficacy and collateral effects of a Cas13 variant and shows how this system can be exploited to characterize the Cas13 proteins rapidly and effectively.

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Longitudinal culturing of the human microbiome reveals site-specific colonisation of bacterial isolates in the respiratory and gastrointestinal tracts

Sara K Di Simone[1, 2, 3], Josephine Owen[1, 2], Elys Green[1, 4], Rebecka Atkinson[1, 4], Emily Rutten [3], Tamblyn Thomason[3], Jodee Gould[3], Steven Cho[1], Claudia Nold[1, 2], Samuel Forster[5, 3], Marcel Nold[1, 2, 4]

1. Ritchie Centre, Hudson Institute of Medical Research, Clayton, Victoria, Australia

2. Department of Paediatrics, Monash University, Clayton , Victoria, Australia

3. Centre for Innate Immunity and Infectious Disease, Hudson Institute of Medical Research, Clayton, Victoria, Australia

4. Monash Newborn, Monash Children's Hospital, Clayton, Victoria, Australia

5. Department of Molecular and Translational Sciences, Monash University, Clayton, Victoria, Australia

The first months of life are critical for microbiome acquisition and mark the earliest interactions between the colonising microbiota and maturing immune system. The lung harbours a variety of microorganisms thought to be critical in contributing to physiological homeostasis and protecting against opportunistic pathogens. Although metagenomic sequencing technologies continue to uncover the diverse microbial community in the human respiratory tract, isolate-level resolution through bacterial culturing and functional analysis of the microbiota across body sites remains limited. Through the GLAM&I (Gut, Lung and their Microbiomes & Immunology) clinical study, longitudinal and time-matched respiratory and stool samples have been collected from 108 neonatal and paediatric patients at Monash Children's Hospital. Bacterial culturing protocols have been optimised for culture of diverse respiratory bacteria and used to purify 5,480 bacterial isolates from 208 respiratory samples within the cohort. Equivalent analysis of gastrointestinal microbes using established protocols identified 1,657 bacterial isolates from 62 stools. To evaluate microbial community composition, respiratory and stool samples were subjected to shotgun metagenomic sequencing. Measures of alpha diversity show changes in species richness and evenness in the respiratory tract and gut over the first weeks of life, as well as patient specific compositional differences between these two sites. The combination of culturing and shotgun metagenomic analysis highlights site-specific bacterial isolate colonisation and the dominance of Firmicutes during early life. Furthermore, 30 bacterial species were demonstrated to be shared across the respiratory tract and gut microbiomes in early life. The application of sophisticated culturing technologies to the respiratory microbiome provides the opportunity to advance beyond microbiome associations to isolate-level causative validation. Specific individual bacterial isolates purified through this work will enable validation the site-specific functional roles and immune interaction during early life. A deeper functional understanding of the microbiota and their interactions with the host will be critical in guiding the development of novel therapeutics and microbiome-based medicines.

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Targeting toll-like receptor 9 signalling with a flavonoid-derived novel small molecule

Refaya Rezwan[1, 2], W. Samantha N. Jayasekara[1, 2], Mary Speir[1, 2, 3, 4], Daniel Wenholz[3, 4], Olivier F. Laczka[3, 4], Michael P. Gantier[1, 2]

1. Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Victoria, Australia

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

3. Noxopharm Limited, Chatswood, New South Wales, Australia

4. Pharmorage Pty Limited, Camberwell, Victoria, Australia

Toll-like receptor (TLR) 9 is an endolysosomal sensor of nucleic acids. It detects endosomal single strand deoxyribonucleic acid (ssDNA) leading to the production of proinflammatory cytokines and interferons through myeloid differentiation primary response gene 88 (MyD88) signalling. Excessive activation of TLR9-MyD88 mediated signalling has been implicated in several immune-mediated diseases including acute pancreatitis and systemic lupus erythematosus. Previous studies have shown that immunomodulators that block this pathway may be useful in the management of these conditions.

Our work focuses on a flavonoid derivative we discovered, referred to as 'PHA', which selectively inhibits TLR9 while largely sparing TLR7, when stimulated by their respective agonists, CpG DNA and R848. Inhibition of TLR9 signalling was observed across species in human HEK293 cells over-expressing human TLR9 and mouse bone marrow derived macrophages. PHA showed preferential inhibition of the signalling cascade downstream of TLR9 (IC50 ~100 nM) with only limited activity on TLR7 signalling, despite both receptors being thought to use similar machineries. The observations were validated with RT-qPCR and ELISA assays. This inhibitory effect of PHA was shared among various TLR9 agonists.

We are currently looking at whether PHA is impacting signalling at the levels of the ligands, the receptor, or their downstream effector molecules to unravel the mechanism underlying the selective activity on TLR9 over TLR7 signalling. Based on our observations thus far and a kinome-wide screen of PHA on 468 kinase enzymes, we hypothesize that PHA regulates a kinase activity uniquely contributing to the TLR9 signalling. This study has the potential for contributing to our understanding of TLR-MyD88 signalling pathway as well as showcasing targeted interventions for TLR9-mediated immune disorders.

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Adeno-associated viral vectors encoding nanobodies targeting mouse and human P2X7 reduce graft-versus-host disease in a humanised mouse model

Amal Elhage[1], Janna Hadaya[1], Chloe Sligar[1], Sahil Adriouch[2], Debbie Watson[1], Ronald Sluyter[1]

1. Molecular Horizons and School of Chemistry and Molecular Bioscience - University of Wollongong, Wollongong, NSW, Australia

2. Unit of Pathophysiology and Biotherapy of Inflammatory and Autoimmune Diseases, French Institute of Health and Medical Research, University of Rouen, Rouen, France

Graft-versus-host disease (GVHD) is an often-fatal T cell-mediated inflammatory disorder that arises following donor stem cell transplantation. P2X7 is an extracellular ATP-gated cation channel present on immune cells. P2X7 blockade with small molecule inhibitors or a human-specific monoclonal antibody impairs GVHD in humanised mouse models. Injection of adeno-associated viral vectors (AAVs) encoding nanobodies (Nb) that block human or murine P2X7 are available, but these have not been investigated in GVHD. NSG mice were injected i.m. with 10^{11} viral particles encoding either green florescent protein (GFP), an anti-murine (m) P2X7 Nb or anti-murine/human (m/h) P2X7 Nb (or an equal volume of saline) on Day -21. Mice were then injected i.p. with 10^{7} human peripheral blood mononuclear cells on Day 0 and monitored for GVHD until disease endpoint or Day 70. The anti-m/hP2X7 Nb and to a lesser extent the anti-mP2X7 Nb reduced clinical GVHD and time to disease onset, as well as histological liver and lung GVHD. The anti-m/hP2X7 Nb and less so the anti-mP2X7 Nb reduced proportions of liver human Th17 cells. Sera (collected at Day 0 and endpoint) from mice injected with AAV encoding anti-P2X7 Nbs, but not from control mice, completely blocked P2X7 activity (ATP-induced cation dye uptake) in murine

J774 and/or human RPMI 8226 cells, confirming the presence of circulating anti-P2X7 Nbs at both time points. This study indicates that P2X7 blockade with anti-m/hP2X7 and less so an anti mP2X7 Nb reduces GVHD progression in humanised mice, supporting the future testing of these P2X7 biologics as a prophylactic treatment for GVHD.

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Gasdermin D deficiency limits the severity of pulmonary disease during influenza A virus infection

Sarah Rosli[1, 2], Christopher Harpur[1, 2], Maggie Lam[1, 2], Alison West[1, 2], Christopher Hodges[1, 2], Ashley Mansell[1, 2], Kate Lawlor[1, 2], Michelle Tate[1, 2]

1. Hudson Institute of Medical Research, Clayton, VIC, Australia

2. Molecular and Translational Sciences, Monash University, Monash University, Clayton, Victoria, Australia

Influenza A virus (IAV) has the potential to cause yet another pandemic. We urgently need new therapies for severe IAV infections, as current antiviral drugs have been reported to be ineffective, particularly due to the increase in antiviral drug resistance. Dysregulated host responses to IAV infection contribute to hyperinflammation, which promotes adverse and irreversible lung pathology. IAV infection of host cells activates several pattern recognition receptors, including the NLRP3 inflammasome, which releases proinflammatory cytokines and ultimately cell death. Gasdermin D (GSDMD) is an executioner of a lytic form of cell death called pyroptosis. Cleavage of GSDMD prompts its bioactive N-terminal GSDMD (NT-GSDMD) domain to create a transmembrane pore, which has been shown to promote the cellular release of pro-inflammatory cytokines including IL-1β and IL-18, as well as danger-associated molecular patterns (DAMPs), promoting further inflammation. While GSDMD has been implicated in many inflammatory diseases, its role in IAV infection was not well characterised. In this study, we investigated the role of GSDMD in modulating the severity of IAV-induced lung pathology. Cleaved GSDMD was upregulated *in vivo* in the lung epithelial cells at day 3 post-IAV infection. Mice lacking GSDMD (*Gsdmd*-/-) were less susceptible to HKx31 (H3N2) IAV infection, displaying significantly improved survival in comparison to wild-type controls. *Gsdmd*-/- mice displayed less severe pulmonary pathology, including epithelial damage and cell death. Further, the bronchoalveolar lavage fluid of *Gsdmd*-/- mice presented with a significant reduction in total cellularity, which correlated with fewer numbers of infiltrating neutrophils and neutrophil chemoattractants, MIP-2/CXCL2 and KC/CXCL1, at days 3 and 5 post-infection. This was also accompanied by a significant decrease in pro-inflammatory cytokines, including CCL2/MCP-1, IL-6, and TNF at day 3. Interestingly, the number of infectious viral particles in lung tissue was reduced on day 3. Together, these results suggest that the absence of GSDMD limits the severity of IAV infection. Inhibition of GSDMD may provide a novel host-targeted IAV therapeutic strategy that limits the development of fatal lung disease.

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Immune cell infiltration of testicular germ cell tumors – what is known and which questions are unanswered so far?

Rashidul Islam[1, 2, 3], Miriam Figura[1, 4], Jannis Heyer[1, 4], Florian Dittmar[4], Benedict Nathaniel[2], Katja Hartmann[1], Christiane Pleuger[5], Monika Fijak[5], Sabine Kliesch[6], Adrian Pilatz[4], Florian Wagenlehner[4], Mark Hedger[2], Bruce Loveland[7], Kate Loveland[2, 3], Hans-Christian Schuppe[4], Daniela Fietz[1]

1. Department of Veterinary Anatomy, Histology and Embryology, Justus Liebig University Giessen, Giessen, Germany

2. Centre for Reproductive Health, Hudson Institute for Medical Research, Clayton , Victoria, Australia

3. School of Clinical Sciences, Monash University, Clayton , Victoria, Australia

4. Dept. of Urology, Pediatric Urology and Andrology, Justus Liebig University , Giessen, Germany

5. Institute of Anatomy and Cell Biology, Justus Liebig University, Giessen, Germany

6. Centre of Reproductive Medicine and Andrology, University of Muenster, Muenster, Germany

7. Burnet Institute, Melbourne, Australia

Testicular germ cell tumors (TGCT) are the most common tumors in young men (14-44 years) and mostly present as seminoma (SE) and embryonic carcinoma (EC), both arising from germ cell neoplasia in situ (GCNIS). TGCTs are thought to “hjack” the immune system to favor their development, using the anti-inflammatory environment of the immune-privileged testis. Besides e.g. macrophages, T cells are the major component of tumor infiltrating lymphocytes; subtypes such as regulatory (Treg) or follicular helper T cells (Tfh) had not yet been analysed.

We used retrospective and prospective patient cohorts to categorise immune cells in TGCT compared to controls. Immunohistochemistry (IHC) was performed on SE (n=75), EC (n=26), GCNIS (n=30), preserved spermatogenesis (NSP, n=10), and testicular inflammation (n=12), with flow cytometry (FC) on SE (n=12) and EC (n=6) using markers for macrophages, dendritic cells (DC), T and B cells. We showed that the immune cell environment is shifted from (resident) macrophages in NSP to (newly recruited) T cells in TGCTs. Treg and Tfh were most abundant in central parts of SE. Comparing SE and EC, SE (n=33) contained mostly T cells and macrophages whereas in EC tumor-samples (n=10), mostly T cells and DCs were detected. In SE, follicular like structures contained clusters of B cells and Tfh.

This study describes the complexity of immune cells in TGCTs by characterising SE and EC patterns, and provides first indications of a potential importance of rarer T cell subtypes in the immune environment of TGCT. The prospective clinical database will allow correlations between immune cell patterns and clinical parameters, e.g. localised and metastatic TGCTs. With this comprehensive approach, we aim to decipher the role of "immune editing" during TGCT development, progression and possible metastatic behaviour. Results will help to identify novel prognostic factors and immune-therapeutic concepts in human TGCTs. Funded by DFG GRK1871/2.

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TLR4 endocytosis is dissociable from Type I IFN expression but requires TLR4 activation and ubiquitin ligase activity

Thomas E Schultz[1], Carmen Mathmann[1], Meg L Donovan[1], Timothy W Muusse[2], Makrina Totsika[3], Dennis A Carson[4], Bostjan Kobe[2], Katryn J Stacey[2], Matthew J Sweet[5], Antje Blumenthal[1]

1. Frazer Institute, The University of Queensland, Brisbane, QLD, Australia

2. School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, Australia

3. Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, QLD, Australia

4. Moores Cancer Center, University of California San Diego, La Jolla, CA, USA

5. Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia

Toll-like receptor 4 (TLR4) is a significant mediator of cellular activation and antimicrobial responses through recognition of pathogen-derived and endogenous ligands. Its clinical relevance is underscored by associations with beneficial or deleterious outcomes in diverse disease contexts including infection, chronic inflammation, and cancer. The current paradigm holds that TLR4 signalling occurs sequentially, with cell surface-expressed TLR4 initially driving pro-inflammatory signalling. Subsequently, TLR4 endocytosis curbs this pro-inflammatory signalling and enables internalised TLR4 to signal from endosomes, resulting in type I Interferon (IFN) expression. Using murine primary macrophages and macrophage cell lines, we show that TLR4-induced type I IFN expression does not require TLR4 endocytosis, challenging the current viewpoint that these processes are inextricably linked. Moreover, we demonstrate that the presence and functionality of the TLR4 intracellular signalling domain are both indispensable for TLR4 endocytosis. This contrasts with current thinking that molecular regulators external to TLR4, such as CD14, control TLR4 endocytosis. Our data indicate that TLR4 endocytosis requires ubiquitin ligase activity, whereas components of canonical TLR signalling pathways are dispensable, implicating a thus far unrecognised mode of TLR4 signalling that governs activation-induced TLR4 endocytosis. Collectively, our data suggest that activation of cell surface-expressed TLR4 results in at least two distinct, TLR4-intrinsic signalling modes that independently control pro-inflammatory signalling and receptor endocytosis. Significantly, the latter is disconnected from endosomal TLR4 signalling. This revised understanding of TLR4 signalling as consisting of multiple functionally-distinct, non-sequential pathways may reveal opportunities for selective, disease context-specific amplification or restriction of TLR4 signalling pathways for beneficial therapeutic outcomes.

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Interferon regulatory factors 1 and 2 regulate expression of programmed cell death-ligand 1 in dendritic cells

Ashley M Firth[1], Christophe Macri[1], Annabelle Blum[1], Cassandra Ouslinis[1], Nishma Gupta[2, 3], Justine D Mintern[1]

1. Bio21 Institute of Molecular Science and Biotechnology, Department of Biochemistry and Pharmacology, The University of Melbourne, Parkville, Victoria, Australia

2. Inflammation Division, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

3. ACRF Chemical Biology Division, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

Programmed cell death-ligand 1 (PD-L1) is a regulatory molecule which is overexpressed in many cancers and plays a major role in suppressing the immune system. As well as tumour cells and virus-infected cells, PD-L1 is expressed by all haematopoietic cells. In particular, the upregulation of PD-L1 on dendritic cells (DCs), one of the primary cells which presents antigen to T cells, can lead to T cell inhibition through binding to programmed cell death-1 (PD-1). Repeated and strong interactions between PD-L1 on DCs and PD-1 on T cells can further result in T cell exhaustion. Despite its importance, the regulation of PD-L1 in DCs remains largely uncharacterised.

A genome-wide CRISPR/Cas9 screen searching for regulators of PD-L1 in DCs identified the transcription factor, interferon regulatory factor 2 (IRF2), as a promotor of PD-L1 cell surface expression. Production of a single-gene knockout of IRF2 in the MuTu DC line (*Irf2*$^{-/-}$) allowed confirmation that *Irf2*$^{-/-}$ DCs have lower cell surface expression of PD-L1 when compared to wild-type. Reduced PD-L1 expression was also observed on *Irf2*$^{-/-}$ DCs following stimulation with toll-like receptor agonists, which are potent inducers of PD-L1 upregulation. In contrast, when *Irf2*$^{-/-}$DCs were activated with interferon-γ, another major inducer of PD-L1, there was no discernible difference in PD-L1 expression relative to wild-type cells. Instead, the upregulation of another interferon regulatory factor, IRF1, is observed. Production of a knockout of IRF1 in MuTu DCs confirmed that it is the main transcriptional driver of PD-L1 expression in interferon-γ-activated DCs. This mirrors the role of IRF1 as a known promoter of PD-L1 in tumour cells.

Overall, this study supports that in DCs, PD-L1 expression is regulated by the transcription factors IRF1 and IRF2, which play interchangeable roles dependent on the stimuli encountered in the microenvironment. Further studies aim to reciprocate these results in primary models.

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The innate immune response of human and mosquito cells to sylvatic dengue infection

Caleb J Stica[1], Roberto Barrero[1], Greg Devine[2], Francesca D Frentiu[1]

1. Queensland University of Technology, Herston, QLD, Australia

2. Mosquito Control Lab, QIMR Berghofer, Herston, QLD, Australia

Dengue virus (DENV) transmission occurs in two distinct cycles, the endemic/urban cycle, between human hosts and primarily *Aedes* (*Ae.*) *aegypti* mosquitoes, and the sylvatic cycle in non-human primates and their associated *Aedes* mosquitoes. With rapid urbanization and encroachment into sylvatic environments, there is increasing opportunity for spill-over events to occur. For infection to occur, DENV must evade the primary defences of the innate immune system in both mosquito and human hosts. However, it is poorly understood how the immune system of these two hosts might respond to sylvatic DENV infection. In this study, mosquito and human cell lines were infected with urban or sylvatic strains of DENV and the transcriptomic response investigated at two timepoints. Several common and unique differentially expressed genes (DEGs) were found, the number of which varied between DENV strain infections. The number of DEGs associated with immune response was found to be highly dependent on DENV strain, with no significant differences observed between urban and sylvatic DENV strains. In mosquito cells there was a notable increase in the number and expression level of long non-coding RNAs (lncRNAs) during infection. The immune response to sylvatic DENV in human cells is currently being analysed. Further investigation of sylvatic strains and their interaction with the host immune system will provide invaluable insight into the potential of sylvatic DENV spillover and establishment into endemic/urban human cycles.

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Helicobacter pylori cytotoxin, VacA, modulates conventional dendritic cell maturation and hijacks extracellular vesicles to dysregulate immune cell functions

Ruby Gorman-Batt[1], Meredith O'Keeffe[1], Terry Kwok-Schuelein[1]

1. Biomedical Discovery Institute, Monash University, Clayton, VICTORIA, Australia

Helicobacter pylori (*Hp*) is a carcinogenic bacterium that persistently infects ~50 % of the world's population and is the greatest risk factor for the development of gastric cancer (GC). Accumulating evidence suggests that a key *Hp* virulence factor, the Vacuolating cytotoxin A (VacA), facilitates *Hp* persistence by limiting immune cell functions. Of interest, VacA has been shown to alter the maturation status of dendritic cells (DCs), professional phagocytic cells that are critical for the induction of protective immunity. These cells are key for bacterial and tumour antigen presentation, meaning they likely participate in the progression of *Hp*-induced gastric cancer. However, studies to date have concentrated on *in vitro* generated monocyte-derived DC that likely do not represent *in vivo* cells. Thus, we aimed to characterise the interactions between VacA and the conventional type 1 cDCs (cDC1s). Here, we show that VacA interferes with cDC1 maturation and activation. In addition, we found that VacA interacts with the cell membrane without being phagocytosed by the cDC1s. Instead, the toxin localised to extracellular vesicles (EVs) being shed from the cDC1 surface. Moreover, $VacA^+$-EVs were enriched in key markers of immune cell functions and possessed the ability to deliver VacA to naïve immune cells. This study reveals that VacA specifically binds EV at the cDC1 surface, altering cDC1 and DC-EV immune marker composition. We propose that VacA perturbs cDC1 maturation and hijacks DC-EVs to dysregulate immune cell signalling, assisting in the chronic persistence of *Hp*. Our findings highlight a previously unrecognised role of host EVs in bacterial pathogenesis.

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How does rabies virus prevent the production of interferon?

Hudson A Smith[1], Gregory W Moseley[1], Stephen M Rawlinson[1], Cassandra T David[1]

1. Microbiology, Monash University, Clayton, VIC, Australia

Publish consent withheld

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$CD4^+$ T cell recognition of Haemagglutinin epitopes across different influenza strains

Hannah Sloane[2, 1], Dhilshan Jayasinghe[2, 1], Christopher Szeto[2, 1], Andrea Nguyen[2, 1], Stephanie Gras[2, 1], Emma J Grant[2, 1]

1. Department of Biochemistry and Chemistry, La Trobe Institute for Molecular Science, La Trobe University, Bundoora, 3086, Victoria, Australia

2. Department of Biochemistry and Molecular Biology, Monash University, Clayton 3800, Victoria, Australia

The ongoing SARS-Cov2 pandemic is a modern-day reminder of the impact that novel viral strains can have on a population without pre-existing immunity. The influenza A virus (IAV) has caused five pandemics in the last 150 years and is particularly prone to mutations in the surface glycoprotein haemagglutinin (HA) which is the primary target of the $CD4^+$ T cell and antibody responses.

$CD4^+$ T cells recognise peptide presented by human leukocyte antigen class II (HLA-II) molecules with their T cell receptor (TCR). Although the pHLA-II-TCR interaction is extremely specific, it may cross-recognise similar peptides leading to a cross-reactive response, and providing broad protection against different IAV strains. The ability of $CD4^+$ T cells to cross-react with variants of a HA peptide from different IAV strains, especially those not in circulation, is not well understood. Furthermore, individuals expressing certain HLA-II molecules are known to be more susceptible to severe viral infections. In the context of IAV, HLA-DRB1*07:01 has been associated with poor response to the influenza vaccine whereas HLA-DRB1*11:01 has been associated with protection from viruses such as HIV and HCV. Investigation into the molecular basis underlying this association is warranted.

Using intracellular cytokine staining and HLA-II tetramer staining, we found that HLA-DRB1*11:01^+ individuals have more cross-reactive responses towards HA peptide derived from different IAV strains than HLA-DRB1*07:01^+ individuals, and that the cross-reacting $CD4^+$ T cells generally have lower polyfunctionality than specific $CD4^+$ T cells. Moreover, we provide the first insight into the molecular and functional basis of IAV epitope presentation by different HLA-II molecules.

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Leveraging genome-based phylogenies to associate bacterial clades with host molecular responses in inflammatory bowel disease

Sean Solari[1, 2], Remy Young[1, 2], Vanessa Marcelino[3], Gemma D'Adamo[1, 2], Michelle Chonwerawong[1, 2], Emily Rutten[1, 2], Linden Gearing[1, 2], Jodee Gould[1, 2], Edward Giles[1, 2, 4], Samuel Forster[1, 2]

1. Centre of Innate Immunity and Infectious Disease, Hudson Institute of Medical Research, Clayton, Victoria, Australia

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

3. Melbourne Integrative Genomics, University of Melbourne, Melbourne, Victoria, Australia

4. Department of Paediatrics, Monash University, Clayton, Victoria, Australia

The success of faecal microbiota transplant (FMT) to induce remission in ulcerative colitis cohorts highlights the gastrointestinal microbiome as a key target for therapeutic intervention in inflammatory bowel diseases (IBD). However, FMT is a broad-spectrum treatment, and a detailed model of the molecular mechanisms through which microbiome transplantation confers disease remission remains elusive. This undermines our ability to manipulate the gastrointestinal microbiome in a more targeted fashion. The technology we use to probe these communities is evolving; shotgun metagenomic sequencing enables high-throughput yield of microbial DNA, and increasing cultivation of hitherto 'unculturable' microbiota is revealing previously unappreciated diversity in microbial lifestyle and function. While taxonomy-guided metagenomics has played a crucial role in illuminating the importance of our intestinal microbes, improved characterisation of cultured bacterial isolates increasingly demonstrates incongruence between ecologically relevant microbial phenotypes and their taxonomic denomination. Instead, large databases of high-quality microbial genomes are ushering in a paradigm of genome-resolved metagenomics, that seeks to explain microbial function at a genetic and molecular, rather than taxonomic, level. We developed Expam for genome-guided analysis of shotgun metagenomic datasets, leveraging modern genomic techniques to quantify phylogenetic clades of bacteria in a taxonomy-agnostic manner. Applying Expam to a paediatric IBD cohort who underwent matched shotgun metagenomic sequencing and host transcriptional profiling from intestinal biopsy samples, revealed closely related phylogenetic clades of bacteria with distinct patterns of association to host transcriptional responses. This highlights the potential for genome-guided metagenomics to increase the resolution of compositional microbiome analyses, and foreshadows a mechanistic understanding of host-microbiome interactions in health and disease.

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An insect-specific chimeric vaccine protects saltwater crocodiles (*Crocodylus porosus*) against West Nile virus-induced skin lesions and viremia

Gervais Habarugira[1, 2], Jessica J Harrison[2], Jasmin Moran[3], Willy W Suen[2, 4], Agathe MG Colmant[2, 5, 6], Jody Hobson-Peters[2, 5], Sally R Isberg[3], Helle Bielefeldt-Ohmann[2, 5], Roy A Hall[2, 5]

1. School of Veterinary Science, The University of Queensland, Gatton, Queensland, Australia

2. School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland, Australia

3. Centre for Crocodile Research, Noonamah, The Northern Territory, Australia

4. Australian Centre for Disease Preparedness, The Commonwealth Scientific and Industrial Research Organisation, Geelong, Victoria, Australia

5. Australian Infectious Diseases Research Centre, The University of Queensland, Brisbane, Queensland, Australia

6. Unité des Virus Émergents (UVE) Aix-Marseille Univ-IRD 190-Inserm 1207, Marseille, France

Introduction

West Nile virus (WNV) is a mosquito-borne zoonotic flavivirus causing mild to severe disease in humans, domestic and wildlife animals species. In saltwater crocodiles, WNV causes skin lesions, known as "pix", leading to economic losses of AUD20 million annually due to hide rejection. Currently, no vaccine for WNV is commercially available in Australia. This study aimed to develop a vaccine preventing WNV-induced skin lesions in farmed saltwater crocodiles.

Material and methods

We assessed the efficacy and safety of a flavivirus chimeric vaccine comprising the genome backbone of the insect-specific Binjari virus (BinJV) and genes for the structural prM and envelope (prME) proteins of WNV. The BinJV/WNV-prME chimeric virus vaccine is antigenically similar to wild-type WNV but replication-defective in vertebrates. Crocodiles were vaccinated with two doses of BinJV/WNV-prME administered at a four-week interval and WNV-challenged four weeks after booster vaccination. Serum samples collected at different time points were tested in a pan-flavivirus blocking ELISA and virus neutralisation test (VNT).

Results

Vaccinated crocodiles developed a robust neutralising antibody response, regardless of whether the vaccine was adjuvanted or not. Vaccinated crocodiles showed no adverse effects and were fully protected from viremia and skin lesions when challenged with a Kunjin strain of WNV. Mock-vaccinated crocodiles became viraemic, and 22.2% exhibited WNV-induced skin lesions.

Conclusion

Our findings suggest that the BinJV/WNV-prME chimera is a safe and efficacious vaccine that prevents WNV-induced skin lesions in farmed crocodiles. This is the first vaccine that protects against a viral disease in a reptile.

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Zinc acquisition and its contribution to *Klebsiella pneumoniae* virulence

Eve A Maunders[1], Matthew W Giles[1, 2], Katherine Ganio[1], Bliss A Cunningham[1], Vicki Bennett-Wood[1], Gregory B Cole[2], Dixon Ng[2], Christine C Lai[2], Stephanie L Neville[1], Trevor F Moraes[2], Christopher A McDevitt[1], Aimee Tan[1]

1. Department of Microbiology and Immunology, University of Melbourne, Melbourne, VIC, Australia

2. Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada

Klebsiella pneumoniae is a World Health Organization priority pathogen and a significant clinical concern for infections of the respiratory and urinary tracts due to widespread and increasing resistance to antimicrobials. In the absence of a vaccine, there is an urgent need to identify novel targets for therapeutic development. Bacterial pathogens, including *K. pneumoniae*, require the *d*-block metal ion zinc as an essential micronutrient, which serves as a cofactor for ~6% of the proteome. During infection, zinc acquisition necessitates the use of high affinity uptake systems to overcome niche-specific zinc limitation and host-mediated nutritional immunity. Here, we report the identification of ZnuCBA and ZniCBA, two ATP-binding cassette permeases that are highly conserved in *Klebsiella* species and contribute to *K. pneumoniae* AJ218 zinc homeostasis, and the high-resolution structure of the zinc-recruiting solute-binding protein ZniA. The Znu and Zni permeases appear functionally redundant with abrogation of both systems required to reduce *K. pneumoniae* zinc accumulation. Disruption of both systems also exerted pleiotropic effects on the homeostasis of other *d*-block elements. Zinc limitation perturbed *K. pneumoniae* cell morphology and compromised resistance to stressors, such as salt and oxidative stress. The mutant strain lacking both systems showed significantly impaired virulence in acute lung infection models, highlighting the necessity of zinc acquisition in the virulence and pathogenicity of *K. pneumoniae*.

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Defective interfering RNA induce innate immunity and broad pan-antiviral activity

David Harrich[1, 2], Min-Hsuan Lin[1], Dongsheng Li[1], Bing Tang[1], Pramilla Maniam[1], Li Li[3], Felicity Y Han[3], Andreas Suhrbier[1, 2], Yaman Tayyar[4, 5], Nigel McMillan[4]

1. QIMR Berghofer, Herston, QLD, Australia

2. Global Virus Network (GVN) Center of Excellence, Australian Infectious Disease Research Centre, Brisbane, Queensland, Australia

3. Australian Institute for Bioengineering and Nanotechnology, University of Queensland, Saint Lucia, Queensland, Australia

4. Menzies Health Institute Queensland and School of Pharmacy and Medical Science, Griffith University, Gold Coast, Queensland, Australia

5. Protenata Biotech, Molendinar, Queensland, Australia

RNA viruses such as dengue (DENV), influenza A (IAV), respiratory syncytial virus (RSV), and SARS-CoV-2 (CoV-2) pose significant challenges to health systems worldwide. These viruses have phenotypic plasticity that allows them to evade humoral immunity and small molecule antivirals, which enables endemic viruses to replicate and reinfect populations. The rapid evolution of human viruses and the potential threat from unknown viruses reinforces the need for new broadly acting antiviral medicines.

The retinoic acid-inducible gene I (RIG-I) is an essential cytosolic pattern recognition receptor (PRR) that recognises viral RNA and activates the production of interferons (IFNs) and pro-inflammatory cytokines in response to viral infections. RIG-I agonists have great potential as antivirals for treating viral pandemics.

Our project focuses on designing and applying an efficient delivery system for the novel RIG-I agonist, DI290 RNA and more recently two small RNA derived from SARS-CoV-2 infected cell culture. DI290 RNA is 290 nucleotides long and was identified through *in vivo* and *in vitro* screenings of defective interfering (DI) RNAs made by DENV. DI290 RNA is a folded double-stranded RNA that mimics infectious viral RNA genome. Our results demonstrate the efficacy of DI290 RNA in activating RIG-I-mediated type I IFN response and suggest that DI290 RNA can be used as a novel, effective RIG-I agonist that inhibits viral infections. We also studied the ability of DI290 RNA to provide pan-antiviral protection and defence against different viral infections. To achieve this, we utilised robust tissue culture and synthetic systems to produce different types of nanoparticles containing DI290 RNA and SARS-CoV2-derived RNAs. Our recent experiments show that DI290 has broad-spectrum activity, capable of inhibiting the replication of DENV, RSV, Yellow Fever virus, Zika virus and CoV2. In vitro and in vivo studies will be presented.

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Modelling infection in an *ex vivo* precision-cut lung slice model of chronic obstructive pulmonary disease

Julia G Chitty[1, 2], Philip G Bardin[3, 1], Jane Elizabeth E Bourke[1, 2], Belinda J Thomas[3, 1]

1. Hudson Institute, Clayton, VIC, Australia

2. Pharmacology, Monash University, Clayton, VIC, Australia

3. Monash Lung and Sleep, Monash Medical Centre, Melbourne, Vic, Australia

Introduction & Aims: Patients with respiratory diseases, such as chronic obstructive pulmonary disease (COPD), are highly susceptible to viral infection. Therapy with glucocorticosteroids (GCS) provides some anti-inflammatory benefit, however, GCS suppress immune responses required to combat infection, enhancing viral growth and adversely impacting lung health. Precision-cut lung slices (PCLS) provide a unique platform for integrated assessment of inflammatory and immune responses. The aim of this study was to establish an *ex vivo* mouse elastase-induced model of COPD and to model infection using the viral mimetic poly I:C (pI:C).

Methods: PCLS prepared from C57Bl/6 mice were incubated with elastase for 16 hr, and a further 24 hr without elastase. PCLS were then stimulated with pI:C (10 µg/ml) for 24 hrs before supernatant and PCLS were collected for analysis of COPD phenotype (mean linear intercept (MLI) and alveolar cell destruction) and elastase- and pI:C-induced effects on viability (LDH, MTT assays) and inflammatory mediators (mRNA, protein).

Results: PCLS viability was maintained following elastase treatment. Indicative of the induction of a COPD phenotype, MLI (mean free distance in the air spaces) was increased ($p<0.01$) and gene expression of alveolar type II cell markers, *Aqp5*, *Rage* and *Sftpc* was decreased ($p<0.05$) in the elastase-treated PCLS. Inflammatory gene expression (*Il-6*, *Kc*, *Tnfα*; $p<0.05$) and mediators (IL-6, KC, MCP-1, RANTES; $p<0.01$) were also significantly increased following elastase. pI:C-induced production of MCP-1, IL-6, KC and RANTES was further enhanced in elastase-treated PCLS, without affecting viability.

Conclusion: We have successfully established an elastase-induced COPD phenotype in mouse PCLS with enhanced inflammation in response to pI:C. Future studies will extend this pathogen-free mouse model to compare responses to viral infection in elastase-treated human PCLS and PCLS from COPD patients, to provide insights into immune responses to viral infection in disease context and opportunities for relatively high throughput screening of novel therapies.

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Investigating antimicrobial activity in the human gastrointestinal microbiome

Jamia Hemphill[1, 2], Jodee Gould[1, 3], Marina Yakou[1], Cristina Giogha[1, 2], Rhys Dunstan[2], Sarah Larcombe[2], Heidi Yu[2], Jian Li[2], Dena Lyras[2], Trevor Lithgow[2], Elizabeth Hartland[1, 2], Emily Gulliver[1, 3], Samuel Forster[1, 3]

1. Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Victoria, Australia

2. Department of Microbiology, Infection and Immunity Program, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

3. Department of Molecular and Translational Science, Monash University, Clayton, Victoria, Australia

Antimicrobial resistance is rapidly increasing across the globe, driving the need for novel therapeutics to treat bacterial pathogens. In microbial communities, some bacterial species produce antimicrobial molecules to gain competitive advantage, and thereby present a promising source of new antimicrobial therapeutic agents. Antimicrobials produced by the gut microbiota and the role they play within commensal communities remains poorly understood due to difficulties in culturing the fastidious anaerobic gut microbes.

However, with recent advances in culturing techniques, alongside analysis of metagenomic data, antimicrobial production in the gut microbiome can be further investigated. In this study, we have demonstrated that members of the human gut microbiota have inhibitory effects against multi-drug resistant gastrointestinal pathogens due to the production of antimicrobial molecules. Using high throughput culturing methods, a panel of human gastrointestinal microbiota isolates were screened for antimicrobial activity against eight multi-antimicrobial resistant strains of gastrointestinal pathogens: *Clostridiodes difficile, Escherichia coli, Enterococcus faecium,* and *Klebsiella pneumoniae*. Of the 287 bacterial isolates screened, 148 (52%) exhibited inhibition of at least one of the pathogens. From these isolates, candidates that displayed inhibition of at least three of the four pathogens were selected for genomic screening. Genes with a high similarity (>90%) to known antimicrobial genes were identified in 4 of the 20 candidates. To confirm if the inhibitory phenotype of these candidates was due to antimicrobial production, an Enterolysin A gene from a commensal *Enterococcus* sp. strain was successfully expressed in an *E. coli* BL21 C43 strain, where it demonstrated inhibition of the *E. faecium* pathogens. Further investigation into the action of this antimicrobial when in a community of commensal bacteria may provide valuable insight into how gut microbiome-derived antimicrobial therapies can be leveraged to prevent and treat antimicrobial resistant gastrointestinal infections.

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A new fluorescent tool to investigate a novel KoRV-related bat retrovirus

Joshua A Hayward[1, 2], Shuoshuo Tian[1], Betty Kouskousis[1], Paula Ellenburg[1], Gilda Tachedjian[1, 2, 3]

1. Life Sciences, Burnet Institute, Melbourne, VIC, Australia

2. Department of Microbiology, Monash University, Clayton, VIC, Australia

3. Department of Microbiology and Immunology, University of Melbourne, Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia

Bats are major reservoirs of deadly human viruses. While retroviruses, such as the human immunodeficiency virus, are among the most significant of virus families that have jumped from animals into humans, whether bat retroviruses have the potential to infect and cause disease in humans remains unknown. We reported the discovery of the first infectious bat retrovirus, the Hervey pteropid gammaretrovirus (HPG), circulating in populations of the Australian black flying fox (*Pteropus alecto*) (Hayward J, Tachedjian M *et al.*, 2020, PNAS 177(17)). HPG is closely related to the pathogenic koala retrovirus (KoRV) and gibbon ape leukemia virus (GALV). Given the role of bats in viral transmission networks, HPG may pose a threat to other animals of ecological, economic, or domestic importance.

We have generated HPG-specific antibodies and an in vitro replication competent HPG reporter construct (HPG-iGFP) carrying a green fluorescent protein internally encoded within the HPG *gag* gene. The HPG-iGFP construct alone generates immature, non-infectious, fluorescent viral particles in mammalian cells, and when expressed alongside wild-type HPG, produces infectious, fluorescent hybrid virions (HPG-iGFP-H). Mammalian cells infected with HPG-iGFP-H express Gag-GFP proteins enabling fluorescence-based visualisation and quantification by methods including fluorescence microscopy and high content imaging.

HPG-iGFP is being used to analyse viral protein kinetics and infectivity in diverse mammalian cells. This will enable novel insights into gammaretroviral biology and the range of animal species that may be susceptible to infection by HPG.

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Vaccine development approaches for African Swine fever virus (ASFV)

MD Tanjir Islam[1], Alex McAuley[1]

1. CSIRO, East Geelong, VIC, Australia

African swine fever (ASF) is a lethal and contagious pig disease caused by the African swine fever virus (ASFV), which can result in mortality rates of up to 100% in infected pigs. Clinical signs of ASF in pigs can range from mild to severe depending on the viral isolate. ASFV cannot be transmitted from pigs to humans but is one of the highest biosecurity threats to Australia and the broader Asia Pacific region. There are currently no licenced vaccines available for ASF, and the poor understanding of ASFV pathogenesis and viral innate immune evasion strategies hinder ASF vaccine development. Live and inactivated vaccines are effective against ASFV but cannot be commercialized because of side effects and reversion to pathogenic forms. Subunit, live vector, and DNA vaccines are less effective against ASFV. The challenge is to develop a safe vaccine, effective against all ASFV lineages that can be produced economically, at scale, and can be readily adopted by pig producers. Recent studies reveal that the combination of cellular and humoral responses tend to be more effective against ASFV. An ideal ASF vaccine should be stable, stimulate both humoral and cellular immunity, provide complete protection across ASFV subtypes, have minimal side effects, and be DIVA (Differentiating Infected from Vaccinated Animals) compatible. Using novel adjuvants and delivery systems in conventional vaccine strategies can be promising to elicit higher efficacy. Understanding the knowledge gaps of mechanisms of pig immunity and protective responses to ASFV can pave the way for developing an effective ASFV vaccine.

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Promoting extrinsic apoptosis to diminish latent HIV reservoir in vivo

Le Wang[1, 2], Liana Mackiewicz[1], Merle Dayton[1], William Clow[1, 2], Kathryn Davidson[1, 2], Marc Pellegrini[1, 3], Marcel Doerflinger[1, 2]

1. The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia

2. Department of Medical Biology, The University of Melbonrne, Melbourne, VIC, Australia

3. Centenary Institute, University of Sydney, Sydney, NSW, Australia

Human Immunodeficiency Virus (HIV) poses a significant global health threat. The development of combination antiretroviral therapy (ART) has led to the suppression of viremia in patients and a substantial improvement in the health condition of people living with HIV (PLWH). However, the existence of a latent HIV reservoir (cells harbor replication-competent HIV provirus) leads to HIV rebound following ART interruption. This requires patients to take life-long medication and suffer from side-effects and chronic inflammation. A functional cure for HIV is urgently needed.

Here we hypothesize that the HIV reservoir persists for a long time partly because they resist to cell death, which may serve as a therapeutic target to diminish the HIV reservoir. To study HIV infection in vivo, we injected human hemopoietic CD34+ stem cells in 48 hours-old immunodeficient (NSG) mouse pups to generate a humanized immune system mouse model (HIS mice). In this model, we used Xevinapant (Debio 1143), a SMAC-Mimetic that induces extrinsic apoptosis and has undergone multiple clinical trials as cancer treatment, to treat HIV-infected, viremia-suppressed HIS mice. During the experiment, the percentage of human immune cells, CD4 T cell, and CD8 T cell count was monitored by flow cytometry, and HIV viral load was quantified by qPCR. We evaluated our treatment's efficacy by analytical treatment interruption (ATI), in which we interrupted all treatment including ART, and compared the time to HIV rebound between groups.

Our results indicate that this HIS mice model can successfully recapitulate essential features of HIV infection in humans. Intact proviral DNA assay (IPDA) is also successfully adapted to this mouse model. Notably, during ATI, we witnessed a delay in HIV rebound after 4 weeks of Xevinapant treatment compared to vehicle. These results suggest that targeting the extrinsic apoptosis pathway may be an effective way to purge the latent HIV reservoir and shed light on designing new strategies to cure HIV.

Currently, we are working on using a prolonged treatment to achieve better reservoir clearance and determining Xevinapant's efficacy by IPDA.

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A key *Lactobacillus* metabolite reduces HIV internalisation and migration through the cervicovaginal epithelial barrier

Brianna Jesaveluk[1, 2], David Delgado Diaz[1], Anna Hearps[1, 3], Gilda Tachedjian[1, 2, 4]

1. Disease Elimination Program and Life Sciences Discipline, Burnet Institute, Melbourne, VIC, Australia

2. Department of Microbiology, Monash University, Clayton, VIC, Australia

3. Department of Infectious Diseases, Monash University, Clayton, VIC, Australia

4. Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, VIC, Australia

Introduction: Young women in sub-Saharan Africa are disproportionately affected by HIV. A *Lactobacillus*-dominated cervicovaginal microbiome reduces the risk of HIV acquisition by decreasing genital inflammation, which disrupts the female reproductive tract (FRT) epithelial barrier and enables the virus to infect target cells in the submucosa. We have discovered that lactic acid (LA), a key metabolite of optimal *Lactobacillus* spp. strengthens the cervicovaginal epithelial barrier. However, LA's ability to inhibit passage of cell-free virus in between epithelial cells (transmigration) or HIV uptake (internalisation) and transcellular migration through epithelial cells (transcytosis) via LFA/ICAM-1 interactions are unknown.

Methods: Immortalised ectocervical (Ect1) and vaginal (VK2) cell lines were cultured in a transwell system, treated apically for 1h with 0.3% LA (pH 3.9), lactate (pH 7.0), or acidified media (pH 3.9, HCl adjusted). At 24h post-treatment, cells were thoroughly washed, and HIV (HIV_{Ba-L}, 10ng p24) was added apically for 24h, after which p24 was quantified in basolateral supernatant and cell lysates. Antibodies against ICAM-1 and LFA-1 were added prior to HIV addition to block binding.

Results: LA treatment (pH 3.9), but not HCl or lactate (neutral pH) reduced HIV migration to the basolateral supernatant by 72±5.8% in Ect1 (mean ± SEM) and 89±6.8% in VK2 cells relative to untreated cells and reduced internalised virus in cell lysates by 49±7.3% in Ect1 and 67±10% in VK2 cells ($p<0.05$, n=5-11). This reduction was abrogated in the presence of antibodies to ICAM-1 and LFA-1. LA treatment reduced ICAM-1 expression in the presence of inflammatory mediators, indicating a potential protective mechanism for LA against HIV.

Conclusions: This study is the first to demonstrate a direct effect of LA on HIV migration through epithelial cells and provides novel insights into its potential mechanism of action. These findings have implications for developing novel strategies to prevent HIV transmission in women.

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CRISPR diagnostics: The versatile, rapid & accurate detection of pathogens

Lewis Williams[1]

1. WEHI, Parkville, VICTORIA, Australia

PCR has been long been considered the diagnostic gold-standard. However, its technical requirements restrict PCR's utility to a laboratory setting and with high costs for setup and operation. Whilst inconvenient in metropolitan areas, it becomes critically limiting for remote communities of Australia and smaller health clinics. CRISPR-based diagnostics have become increasingly promising as a screening technique or even replacement for PCR. We have established a *k-mer* and pan-genome bioinformatic pipeline to generate novel primer and CRISPR guide combinations for subsequent use in the RPA isothermal amplification. Enhancements to the RPA assay has yielded the ability to amplify single copy numbers whilst being able to transfer this reaction directly into the CRISPR assay without further purification. Finally, subsequent CRISPR detection has also been substantially improved, with a 10-fold improvement on the sensitivity published in the literature through extensive optimisation of assay conditions. In combination, we have developed an assay can that operate continuously at a single temperature, is at least 30 minutes faster than PCR, can detect as few as 5 copies of DNA all whilst retaining complete specificity. We have adapted our platform for the detection of Human T-cell Lymphotropic Virus-1c (HTLV-1c) but the detection of many other pathogens is possible. We aim to transform our research into a diagnostic platform that is more appealing to communities and hospitals, encourages rates of testing and community awareness of HTLV-1.

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Influenza virus internal proteins as vaccine targets

Zhirong Jia[1], **Asmaa Hachim**[1], **Carolyn A Cohen**[1], **Niloufar Kavian**[1], **Nancy Leung**[1], **Benjamin J Cowling**[1], **Sophie Valkenburg**[1]

1. The University of Hong Kong, Hong Kong Island, China

Influenza viruses are a constant threat to public health and significant measures have been taken to prepare for the potential emergence of a novel pandemic strain. However, seasonal vaccination has partial efficacy against drifted and novel strains, with the continued need for annual vaccination due to immune waning and antigenic drift for the vaccine to be updated to representative strains. Thus, there is a need for a universal influenza vaccine to more conserved antigen targets. Whilst the Influenza A viruses (IAV) haemagglutinin (HA) is highly variable between strains and subtypes, other proteins like the nucleoprotein (NP), non-structural proteins (NS) and polymerase acidic (PA) are more conserved and may provide cross-reactive immune mediated protection by vaccination. These alternate targets are currently under appreciated and may provide universal vaccine targets.

We utilized single IAV recombinant A/Puerto Rico/8/1934 (*H1N1*) proteins to immunize mice to decipher the contribution to protection of each influenza protein. Protective targets identified to enhance reduced viral load including the nucleoprotein, that could be incorporated to new vaccines due to high conservation with new influenza viruses to generate universal vaccines. Whilst the surface haemagglutinin (HA) provided the highest level of protection both from lethal infection, increased viral clearance, and remains our best target compared to others tested. To complement mouse studies, H1N1-specific antibody levels from pre- and post-infection human serum samples are being assessed to determine antigen specificity and viral loads and symptom severity.

Our study support the possibility of the existence of novel vaccine target in addition to the canonical HA proteins. It is worth to explore the other potential internal targets in other influenza viruses that can be used for the design of more effective and long-lasting vaccines.

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Seroprevalence and risk factors of flavivirus infections in horses in South-East Queensland post Japanese encephalitis virus incursion

Nicholas Yuen[1], **Althea Wang**[1], **Jessica Harrison**[2], **Gervais Habarugira**[2], **Mitchell Coyle**[3], **Helle Bielefeldt-Ohmann**[2, 4]

1. School of Veterinary Science, The University of Queensland, Gatton, QLD, Australia

2. School of Chemistry and Molecular Biosciences, The University of Queensland, St. Lucia, QLD, Australia

3. Equine Unit, Office of the Director Gatton Campus, The University of Queensland, Gatton, QLD, Australia

4. Australian Infectious Diseases Research Centre, The University of Queensland, St. Lucia, QLD, Australia

Introduction

Mosquito-borne flaviviruses, including Japanese encephalitis virus (JEV), Murray Valley encephalitis virus (MVEV) and West Nile virus (WNV), present a growing threat to animal and human health in Australia. The recent emergence of JEV in Australia following extended periods of substantial rainfall due to the La Niña weather event prompted an update into the seroprevalence of flaviviruses and better understanding of the influence of meteorological conditions on flavivirus circulation.

Methods

Serum samples collected from a South-East Queensland mare and foal populations between 2020 – 2023 were tested in a pan-flavivirus blocking-ELISA. Positive samples were further subjected to testing for JEV, MVEV and WNV-specific antibodies. Cox proportional hazards analysis was applied using two approaches to determine if the meteorological variables, daily and cumulative rainfall, relative humidity and air temperature are associated with flavivirus seropositivity.

Results

Serological testing revealed JEV was the most prevalent of the three flaviviruses tested for in both mares (63.7%) and foals (61.5%), followed by MVEV (9.1% and 26.9%, respectively) and WNV (0% and 11.5%, respectively). The highest number of seroconversions were observed in 2022 in foals, peaking between March and May. Significant hazard ratios ($p < 0.05$) were identified for air temperature, relative humidity, and cumulative rainfall. In addition, air temperature with time-lag effect was significantly associated with reduced hazard ratio.

Conclusion

Our findings indicate the significance of meteorological conditions on flavivirus seroconversions in horses and demonstrate seroprevalence to JEV for the first time in South-East Queensland horses.

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Integrating Artificial Intelligence into Stemformatics: Identifying Influential Variables in Stem Cell Biology through Enhanced Data Curation and Deep Learning

Chen Zhan[1], Jarny Choi[1], Jamie Gearing[2], Suzanne Butcher[1], Christine Wells[1]

1. Department of Anatomy and Physiology, The University of Melbourne, North Melbourne, VIC, Australia

2. Regulation of Interferon and Innate Signalling, Hudson Institute of Medical Research, Clayton, VIC, Australia

Stemformatics is a pivotal data portal and visualisation platform for stem cell research. Initially established to host curated gene expression datasets, it now becomes a central repository for transcriptional profiles of both pluripotent and adult stem cells, with a particular focus on haematopoiesis and myeloid subsets. Stemformatics hosts an extensive data collection derived from multiple platforms, offering an in-depth view of stem cell biology. In recent developments, Stemformatics has expanded its focus beyond simple curation to collating and integrating public datasets with shared phenotypes. This strategic shift has led to the creation of several integrated expression atlases, with a notable emphasis on human myeloid, blood and dendritic cells. These atlases are critical in cross-dataset comparisons, enabling researchers to annotate myeloid subsets in external single-cell data and identify tissue-specific or activation properties of laboratory models or primary cells.

The Stemformatics team has a long-term vision to develop Artificial Intelligence models to identify experimental or environmental drivers of complex gene expression phenotypes. Specifically, we are implementing Deep Neural Networks to identify influential variables such as cell type, tissue origin, or time-dependent/dose-dependent ligand responses. This requires deep curation of the metadata accompanying a transcriptional dataset. We are assessing the usefulness of Large Language Models, such as ChatGPT, for method extraction from academic papers to assist with rapid curation and integration of public data. Stemformatics atlases are freely available at www.stemformatics.org and updates are provided via the Git repository https://github.com/wellslab/s4m-api.

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Does a leopard change its spots? *Pseudomonas aeruginosa* in cystic fibrosis after CFTR modulation

Emma L Ledger[1], Daniel J Smith[2, 3], Jing Jie Teh[1], Michelle E Wood[2], Page E Whibley[2], Mark Morrison[1, 4], Joanna B Goldberg[5], David W Reid[6, 3, 7], Timothy J Wells[1, 7]

1. Frazer Institute, the University of Queensland, Brisbane, Queensland, Australia

2. Adult Cystic Fibrosis Centre, the Prince Charles Hospital, Brisbane, Queensland, Australia

3. Northside Clinical Unit, the University of Queensland, Brisbane, Queensland, Australia

4. Australian Infectious Diseases Research Centre, Brisbane, Queensland, Australia

5. Emory University, Atlanta, Georgia, United States

6. QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia

7. Australian Infectious Diseases Research Centre, Brisbane, Queensland, Australia

Background. *Pseudomonas aeruginosa* is a multi-drug resistant, opportunistic pathogen causing intractable respiratory infections in people with cystic fibrosis (pwCF). In the past decade there has been considerable improvements in CF precision medicine including the development of CF transmembrane conductance regulator (CFTR) modulators that partially correct the underlying defective chloride channel driving disease[1]. Despite the many clinical benefits, multiple studies have revealed that whilst *P. aeruginosa* sputum load is often reduced, chronic infection persists[2,3]. Here, we investigate how *P. aeruginosa* in pwCF may change and adapt in the face of the altered lung environment post CFTR modulation.

Methods. One hundred and five *P. aeruginosa* strains were isolated from the sputum of 11 pwCF at baseline and up to 21 months post treatment with the latest modulator combinations, Elexacaftor-Tezacaftor-Ivacaftor (ETI, n=8) and Tezacaftor-Ivacaftor (TI, n=3). Isolates were sequenced and comparative genomics and phenotypic characterization were performed before and after CFTR treatment.

Results. Considerable improvements in clinical parameters were observed post CFTR modulation, however, not only did participants remain colonised with *P. aeruginosa*, but the same clonal lineages persisted after modulator therapy with no evidence of displacement by alternative strains. Sustained clonal lineages accumulated between 19 to 3400 modifying mutations after modulator commencement and these mutations were more frequently in previously described pathoadaptive genes but not in antibiotic resistance genes in a large proportion of lineages. Additionally, we identified commonly mutated genes across lineages between participants that may be positively selected for in the CFTR modulated lung environment. Despite the potential impact of these mutations, classic chronic *P.*

aeruginosa phenotypes such as excessive alginate-producing mucoid morphology and lack of O-antigen expression were sustained, and isolates remained just as resistant to clinically relevant antibiotics.

Conclusion. Despite the clinical benefits of CFTR modulators, clonal lineages of *P. aeruginosa* persist with the same chronic phenotypes that may prove just as difficult to manage in the future especially in individuals with advanced lung disease and irreversible lung damage. Therefore, future research and clinical care should remain focused on *P. aeruginosa* as well as other lung infections in this post modulator era.

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The exploration of HLA-B*18:01 influenza A derived peptides identified from lung infected cells

Samuel Liwei Leong[1], Emma Grant[1], Lawton Murdolo[1], Stephanie Gras[1]

1. La Trobe University, South Morang, VICTORIA, Australia

The influenza virus is responsible for seasonal flu epidemics that account for approximately 600,000 deaths worldwide. In addition, influenza pandemics in the past have caused more than 50 million deaths. CD8+ T cells are known to be critical in the control and clearance of viral infections and could be an attractive target for future vaccines. $CD8^+$ T cells generally recognise peptides that are derived from internal influenza proteins that are less likely to be affected by antigenic drift. However, for $CD8^+$ T cells to recognise immunogenic targets, peptides must be presented by the Human Leukocyte Antigen (HLA) molecules. As HLA molecules are highly polymorphic this poses a significant hurdle for global vaccine design. Thus, it is critical to identify and characterise specific new epitopes for highly prevalent HLA molecules in the global population.

Here, we assessed the immunogenicity of six newly identified influenza-derived peptides from Nicholas *et a*l. (2022) that were predicted to bind to the prevalent HLA-B*18:01 molecule (2.3% of global population). Using $CD8^+$ T cell activation assays, we showed that 3/6 of the novel peptides were immunogenic in several HLA-B*18:01^+ individuals, and tetramer staining confirmed their HLA-B*18:01 restriction. We subsequently compared these $CD8^+$ T cell responses to those against the previously identified highly immunogenic HLA-B*18:01-restricted NP_{219} peptide. We saw an immunodominance hierarchy towards these peptides, with responses against the NP_{219} peptide being the strongest, followed by responses towards TEV8, EEI9 and QEI8 by tetramer staining and intracellular cytokine staining assays. Finally, we dissected, the first TCR repertoires specific for any pathogen derived peptides presented by HLA-B*18:01 and revealed a private and restricted repertoire for each of the four epitopes. Overall, we confirmed the immunogenicity and HLA-B*18:01 restriction of three new influenza-derived CD8+ T cell epitopes and showed that immune responses towards the known immunogenic NP_{219} peptide are higher, indicating its potential as a vaccine candidate for HLA-B*18:01^+ individuals.

SCIENCE BITES ABSTRACTS

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Modelling & Targeting Cytokine Storm in Dengue Virus-infected Mice

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

1. Walter & Eliza Hall Institute of Medical Research, Abbotsford, VICTORIA, Australia

2. Centenary Institute, Sydney, New South Wales, Australia

With an estimated 100 million symptomatic cases annually, Dengue is well recognised as the most common human arboviral infection globally. A subpopulation of infections exhibits debilitating symptoms of systemic inflammation and vascular leakage, which may be deadly unless monitored closely in hospital. This severe disease is characterised by a cytokine storm, a dysregulated host immune response in which overabundant immune signalling becomes harmful to the host. Dengue treatment is limited to fluid replacement therapy to replace lost blood volume, and Dengue vaccines have had a troubled development that limits public uptake. Novel therapeutic options are urgently needed.

To address this, I have optimised a Dengue mouse model that recapitulates the inflammatory disease observed in humans, where viremia peaks 1-2 days post symptom onset, before symptoms worsen simultaneously with immune activation and viral clearance. Type 1 interferon (IFN)-deficient mice infected with Dengue virus type 2 (DENV2) develop peak viremia at 2 days post-infection followed by weight loss and immune activation up to 4 days post-infection, at which point viremia reaches low or undetectable levels. 26-plex cytokine analysis of infected plasma and spleen samples demonstrated broad immune activation, including key inflammatory cytokines upregulated in human disease such as IL-6, TNF, and IL-1b.

Interestingly, severe Dengue shares many risk factors, immune signatures and inflammatory symptoms with severe COVID-19. Based on the recent progress in understanding cytokine storm in COVID-19, I am now targeting cytokine signalling with clinical-stage anti-inflammatory drugs to treat Dengue in my established mouse model. Preliminary data suggest that broad immunosuppression with Dexamethasone may improve Dengue symptoms, while TNF blockade with etanercept did not mitigate disease outcomes. I am currently determining the efficacy of GM-CSF blockade, and plan to assess Jak inhibition, IL-6 blockade and IL-1b blockade. Collectively, this work

will provide crucial information for the development of treatment strategies for moderate/severe Dengue, and my discoveries here may also be applied to the significantly deadlier Dengue/COVID-19 co-infection.

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Understanding the AhR and innate immunity axis in RNA virus infection

Xavier B Montin[2, 1], Timothy P Allen[2], Emily N Kirby[2, 1], Murray Whitelaw[2], Michael R Beard[2, 1]

1. Research Centre for Infectious Diseases, University of Adelaide, Adelaide, SA, Australia

2. School of Biological Sciences, University of Adelaide, Adelaide, SA, Australia

Interferon (IFN) signalling is a crucial part of the host response to viral infection, comprising rapid, non-specific and potently antiviral responses via the expression of interferon stimulated genes (ISGs). The antiviral effects of these ISGs are robust enough that nearly all pathogenic viruses impede IFN responses in some manner. While many of the main signalling pathways mediating IFN have been described, there is little characterisation of interactions between IFN signalling and other cellular pathways which may impact innate immune responses. Recently implicated in IFN modulation is the Aryl Hydrocarbon Receptor (AhR), a ligand-activated transcription factor primarily involved in toxicological responses and neuronal development, which was shown to be spontaneously activated upon infection with Zika virus (ZIKV) (1) and some coronaviruses (2). We have demonstrated that AhR activation is triggered by a multitude of RNA virus infections in different cell types, including ZIKV, the Kunjin strain of West Nile Virus (WNV_{KUN}), Dengue Virus (DENV) and human coronavirus 229E, and have shown via RNA sequencing and qPCR that IFN responses and the expression of a variety of antiviral ISGs are significantly enhanced in an AhR knockout context, as well as increases in the replication of RNA viruses ZIKV and WNV when functional AhR is not present. This demonstrates the potential for an AhR-IFN signalling axis that is exploited by RNA viruses to enhance their replication. Ongoing experiments aim to use CRISPR knockout and activation techniques to elucidate the mechanism behind virus-mediated AhR activation, with preliminary results indicating the involvement of cytosolic sensors such as RIG-I. In addition, we aim to investigate the mechanism of the inhibitory effect AhR itself exerts on innate immune responses. Our study demonstrates that AhR is a novel proviral host factor for a variety of RNA viruses, and this work may thus represent potential therapeutic angles for a multitude of RNA virus infections of which there is a scarcity of available treatment, such as ZIKV, via targeting the AhR.

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Developing a microbial therapeutic for the prevention of systemic *Candida albicans* infections arising from the gastrointestinal tract

Bianca Briscas[1], Megan D Lenardon[1]

1. School of Biotechnology and Biomolecular Sciences, UNSW, Sydney

Candida albicans is a commensal fungus found in the gastrointestinal (GI) tract of over 60% of adults. However, it is also one of the WHO’s ‘critical group’ fungal priority pathogens. Despite antifungal intervention, the mortality rate of invasive *C. albicans* infections that most often arise from the GI tract is ~ 47%. Combined with increasing antifungal resistance, an alternative strategy to manage invasive *C. albicans* infections is needed.

We propose that bacteria from the human GI tract with anti-*C. albicans* activity can be used as a microbial therapeutic to clear *C. albicans* from the GI tract of at-risk patients, thereby preventing invasive infections from arising.

To identify bacteria in the human GI tract with anti-*C. albicans* activity, we obtained faecal samples from 27 anonymous, healthy, adult donors. *C. albicans* was co-cultured with faecal homogenates in an *in vitro* colon model. Seventeen faecal homogenates thar reduced *C. albicans* colony forming units (CFUs) (compared to a *C. albicans*-only control) were identified.

16S rRNA gene sequencing was used to determine the bacterial composition of the faecal microbiota(s). Multivariate statistical analyses allowed us to identify bacterial amplicon sequence variants (ASVs) that were statistically significantly associated with the *in vitro* killing of *C. albicans*.

To experimentally validate these associations, gut bacterial isolates that correspond to the ASVs associated with anti-*C. albicans* activity were obtained (n=39). So far, 19 have reduced *C. albicans* CFUs compared to the control, therefore demonstrating their ability to inhibit *C. albicans* growth in colon-simulating conditions.

Future work will include investigating the mechanisms by which these bacterial isolates kill *C. albicans.* Overall, this work supports the premise of using microbiome-based intervention for invasive *C. albicans* infections in a way that will circumvent the threat of rising antimicrobial resistance.

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Exploring connections between mitochondrial fission and lipid droplets in macrophage inflammatory and antimicrobial pathways

Karoline D Raven[1], Syeda Farhana Afroz[2], James E.B Curson[1], Robert Parton[1], Matt Sweet[1]

1. Institute for Molecular Bioscience (IMB), The University of Queensland (UQ), Brisbane, Queensland, Australia

2. Department of Cellular and Molecular Physiology, Penn State University College of Medicine, Hershey, Pennsylvania, United States

Mitochondria and lipid droplets (LDs) have important roles in innate immunity. Mitochondria are dynamic organelles, changing their state from a fused network (fusion) to undergoing fragmentation (fission), with this affecting cellular metabolism. Mitochondria and LDs interact during cell metabolism, demonstrating that these organelles have an intimate relationship. This study aimed to investigate the relationship between mitochondrial fission and LDs in macrophages, since both organelles contribute to antibacterial responses in these cells. I demonstrated that the Toll-like receptor 4 (TLR4) agonist lipopolysaccharide (LPS) induced mitochondrial fission and LD synthesis in both murine and human macrophages. To determine whether there is a functional link between fission and LD formation in bone marrow-derived macrophages (BMM), LPS-inducible

mitochondrial fission was genetically or pharmacologically inhibited. Both regimes abrogated LPS-induced LD formation, suggesting that LPS-inducible fission drives LD formation. Signalling mechanisms driving LPS-inducible LDs are poorly understood. One potential candidate is the lysine deacetylase HDAC7 which is required for TLR-inducible fission. LPS-inducible LD formation was abrogated by either genetic or pharmacological targeting of HDAC7, whereas overexpression of HDAC7 in primary macrophages was sufficient to induce mitochondrial fission and LD formation. In bacterial infection studies, challenge with *Escherichia coli* (*E. coli*) increased both mitochondrial fission and LD formation. Interestingly, infection with *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium) didn't induce mitochondrial fission but induced LD formation, indicating a potential form of pathogen manipulation. Finally, whereas LPS-inducible fission and LD formation required HDAC7, these responses occurred independently of HDAC7 in macrophages responding to bacterial challenge. These data further support a connection between fission and LD formation and suggest that distinct mechanisms can initiate inducible fission and LD formation, depending on the nature of the stimulus. Collectively, my data suggest that the antimicrobial effects of mitochondrial fission in macrophages may be mediated by inducible production of antibacterial LDs.

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Exploration of the Immunostimulatory Effects of Group A Streptococcus Pili

Risa Takahashi[1], Thomas Proft[1], Nikki Moreland[1], Catherine Tsai[1]

1. Molecular Medicine and Pathology and Maurice Wilkins Centre, The University of Auckland, Auckland

Despite the global health burden posed by Group A Streptococcus (GAS) infections and complications, there is currently no available vaccine against this pathogen. One potential vaccine candidate is the GAS pilus (*plural*, pili), a long, hair-like structure expressed on the cell surface which involves in the initiation of infection. While current research mainly focuses on pili's function and their potential as a vaccine target, little is known about the interaction between the pilus and host immune system. Accordingly, the interaction between GAS pili and components of the innate immune system was explored to characterise the implication of pili-based vaccines on the immune system.

Recombinant forms of pilus proteins and *L. lactis* gain-of-function strains expressing GAS pili on the cell surface were generated to investigate the immunomodulatory properties of the structure. Pili mediated interaction with toll-like receptors (TLRs), cytokine production, and immune cell activation was investigated using immunoassays and flow cytometry experiments. Pili induced inflammation was also studied in moth larvae and a mice model was utilised to study pilus based vaccine.

Pili and pilus proteins induced upregulation of proteins and pro-inflammatory cytokines in innate immune cells associated with adaptive immune modulation. Assays using TLR reporter cell lines indicated pilus specificity to TLR2 and binding assays confirmed physical interactions with the receptor. This was confirmed by cytokine measurement, where production of downstream cytokine was inhibited in the presence of a TLR2 antagonist. Additionally, the TLR2/6 heterodimer was pinpointed as the TLR2 heterodimer recognising pili. Further experiments indicated that pili mediated inflammation stimulated antibody production in mice and did not correlate with disease severity in wax worms.

The GAS pilus proteins were shown to be highly immunostimulatory ligands of TLR2, with the ability to prime the immune system for enhanced antibody production. The insight gained into the immunomodulatory characteristics of GAS pili emphasise the pilus proteins' potential as a GAS vaccine candidate and as an adjuvant in other vaccine formulations

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Enterococcus faecalis exploit neutrophils as an intracellular niche for replication and persistence

Claudia J Stocks[1], Ronni AG Da Silva[1, 2], Navin S/O Jeyabalan[1], Siu Ling Wong[3, 1], Kimberly Kline[1, 2, 4]

1. Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University , Singapore

2. Antimicrobial Drug Resistance Interdisciplinary Research Group, Singapore-MIT Alliance for Research and Technology, Singapore

3. Lee Kong Chian School of Medicine , Nanyang Technological University , Singapore

4. Department of Microbiology, Faculty of Medicine, University of Geneva , Geneva, Switzerland

Chronic wound infection is a major global public health issue, with diabetic foot ulcers (DFU) of particular concern. *Enterococcus faecalis* is one of the most commonly isolated pathogens from infected wounds, including DFU. Neutrophils are highly reactive, inherently short-lived immune cells that play a critical role in bacterial defence. Currently, *E. faecalis* – neutrophil interactions are very poorly understood. Unexpectedly, extended *in vitro* infection of neutrophils with *E. faecalis* did not induce host cell death. Rather, *E. faecalis* infection significantly prolonged both murine and human neutrophil lifespan even compared to uninfected controls. Quantification of intracellular CFU and assessment of infected cells via confocal microscopy demonstrated that bacteria were phagocytosed by neutrophils regardless of opsonisation and persisted intracellularly out to 24 h p.i. Using RADA, which incorporates into newly synthesised bacterial cell walls to monitor intracellular *E. faecalis* replication, we observed that *E. faecalis* actively replicates within murine neutrophils between 6-18 h p.i., followed by a predominately persistent phase between 18-24 h p.i. Examination of murine wound beds and extracted ex vivo cells revealed intracellular *E. faecalis* within infiltrating neutrophils at 24 h p.i. Investigations are currently underway to host and bacteria cell mechanisms mediating this cell death suppression and intracellular occupation. Such mechanistic insights offer the potential of targeted pharmacological intervention to undermine intra-neutrophil reservoirs of *E. faecalis* during chronic wound infection.

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Serological profiling of viral infection using peptide phage display

Li Chen Cheah[1], Nagendrakumar Singanallur[1], Wilna Vosloo[1], Petrus Jansen van Vuren[1]

1. Health & Biosecurity, Australian Centre for Disease Preparedness, East Geelong, VIC, Australia

Serology is an important tool for the diagnosis of viral infection and biosecurity surveillance. Unlike PCR and antigen tests, which only work in a narrow time window, antibodies serve as a long-lasting 'immunological record' that remain detectable for months or years after exposure. However, conventional serology tests are difficult to multiplex and are usually run as one test per virus, per sample. Additionally, antibodies tend to cross-react between related and sometimes unrelated viruses, which can make test results difficult to interpret.

An emerging serology technique called phage immunoprecipitation-sequencing (PhIP-Seq) [1][2] combines phage display and next-generation sequencing to enable one-pot screening of thousands of antigens. This method uses a library of overlapping peptides that span viral proteins, which could offer better resolution than whole viruses or protein antigens. PhIP-Seq also uses a standardised 'plug & play' workflow which can be rapidly adapted to a new target panel.

Here, we present the first use of PhIP-Seq in a veterinary application. Using foot-and-mouth disease virus as a model, we used PhIP-Seq to simultaneously profile antibody responses against a selection of structural and non-structural viral proteins. Even with a limited set of samples, we were able to identify candidate regions that were highly targeted by antibodies and exhibited partial serotype-specific reactivity. Additionally, different antibody profiles towards the non-structural proteins were observed between vaccinated and infected animals, indicating the potential utility of PhIP-Seq for DIVA (Differentiating Infected from Vaccinated Animals). This work demonstrates the capability of PhIP-Seq as a powerful technique for epitope discovery and virus serotype discrimination.

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Human Immunodeficiecny Virus-1 (HIV-1) Neutralisation Profiles in HIV-1 Viremia Suppressed Nepalese Individuals

Anurag Adhikari[1, 2], Sauhardra Manandhar[2], Gopiram (Shulav) Syangtan[2], Manjula Bhattarai[2], Purnima Shrestha[2], Lloyd D'Orsogna[3], Stephanie Gras[1]

1. La Trobe Institute for Molecular Sciences, La Trobe University, Bundoora, VIC, Australia

2. Department of Infection and Immunology, Kathmandu Research Institute for Biological Sciences, Lalitpur, Bagmati, Nepal

3. Pathology & Laboratory Medicine, University of Western Australia, Perth, WA, Australia

Publish consent withheld

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Molecular and cellular pathogenesis of Ross River virus

Nicholas Yuen[1], Melodie Eng[1], Nicholas Hudson[2], Albert Sole-Guitart[1], Mitchell Coyle[3], Helle Bielefeldt-Ohmann[4, 5]

1. School of Veterinary Science, The University of Queensland, Gatton, QLD, Australia

2. School of Agriculture and Food Sustainability, The University of Queensland, Gatton, QLD, Australia

3. Equine Unit, Office of the Director Gatton Campus, The University of Queensland, Gatton, QLD, Australia

4. School of Chemistry and Molecular Biosciences, The University of Queensland, St. Lucia, QLD, Australia

5. Australian Infectious Diseases Research Centre, The University of Queensland, St. Lucia, QLD, Australia

Introduction

Ross River virus (RRV) has for decades been recognized as the most prevalent arbovirus infection in Australia and identified as an emerging infectious disease worldwide. Infected humans and horses experience acute febrile illness with joint pain being the most common clinical sign. Around 50% of symptomatic individuals experience chronic debilitating symptoms which can last for > 12 months. However, our understanding of RRV pathogenesis in human and horses remains unclear.

Methods

We established a horse model for the study of RRV pathogenesis in a naturally susceptible host. Peripheral blood mononuclear cells (PBMC), primary synovial fibroblasts and epidermal keratinocytes were infected *in vitro* with RRV at a MOI (multiplicity of infection) of 1, 3, and 800, respectively. RNA sequencing, metabolic flux analysis, virus growth kinetics and immunohistochemistry were performed to elucidate the molecular and cellular functional immune responses of these cell types upon RRV infection.

Results

Transcriptomic analysis revealed that transcription of *mxra8*, a recently discovered molecule responsible for RRV cell entry, was significantly downregulated in all cell types following infection with RRV, while potent antiviral and inflammatory responses were generated by these cells upon RRV infection. Significant upregulation of *tlr3*, but not *tlr7,8,9*, was seen in all infected cells suggestive of RRV abortive replication or immune evasion. Cells infected with RRV had reduced mitochondrial functions as evident by insignificant expression of *mavs* despite transcriptional upregulation of *rig1* and *mda5*, and a reduction in oxygen consumption capacity in infected PBMC and fibroblasts. RRV antigens were undetectable by immunohistochemistry in monocytes-derived macrophages and keratinocytes infected with RRV. Virus growth kinetic studies revealed inefficient virus replication in fibroblasts and keratinocytes.

Conclusion

By using multi-modal analyses using a relevant host model system, this study revealed previously unexplored molecular and functional mechanisms of RRV immuno-pathogenesis.

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Characterising the quick-killing mechanism of action of azithromycin analogues against malaria parasites

Emma Mao[1], Maria Gancheva[1], Brad Sleebs[2], Danny Wilson[1]

1. The University of Adelaide, Adelaide

2. WEHI - Walter and Eliza Hall Institute of Medical Research, Melbourne

Malaria is a mosquito-borne disease caused by *Plasmodium* spp. parasites. In 2021, malaria was responsible for approximately 247 million cases and 619 thousand deaths worldwide. While antimalarials have contributed significantly to the decline in global mortality, drug resistance is a looming threat. Azithromycin is a safe and long-acting antibiotic known to target the parasite's essential plastid organelle, the apicoplast, resulting in a delayed-death phenotype whereby parasite death is manifested only in the lifecycle after treatment initiation. At higher treatment concentrations, azithromycin also has quick-killing activity independent of apicoplast targeting, leading to parasite death within the first replication cycle. Chemical modification of azithromycin can greatly enhance this quick-killing activity, however, the mechanism by which this occurs remains elusive.

We investigated the antimalarial activity of five azithromycin analogues. All analogues rapidly killed multidrug sensitive and resistant *Plasmodium* parasites within one blood-stage lifecycle at IC_{50}s < 500 nM. Two of our compounds possess chloroquinoline moieties, raising the possibility that they act like chloroquine, an antimalarial that targets haemoglobin digestion and is now associated with widespread drug resistance. Using synergy assays with both inhibitors of haemoglobin digestion and reversers of chloroquine resistance, alongside beta-haematin formation assays, we show that chloroquinoline-modified analogues, but not non-chloroquinoline ones, exhibit a chloroquine-like mechanism of quick-killing. However, non-chloroquinoline analogues are still able to improve quick-killing potency by up to 40-fold over azithromycin independently of chloroquine-like activity, with the addition of a chloroquinoline moiety seen to further enhance this by up to 17-fold through a chloroquine-like mechanism. These data support that the bulk of quick-killing improvement is likely driven by other non-chloroquine related mechanisms, allowing for improved late-stage gametocytocidal activity over chloroquine and maintenance of quick-killing potency in multiple chloroquine-resistant *Plasmodium* lines. Further elucidation of these non-chloroquine related mechanisms may identify novel drug targets suitable for future drug development efforts.

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Dead or alive? Unveiling the Molecular Mechanisms of SARS-CoV-2 Pathogenesis *in vivo*

Stefanie M. Bader[1, 2], Reet Bhandari[1, 2], James P. Cooney[1, 2], Dylan Sheerin[1, 2], Liana Mackiewicz[1], Merle Dayton[1], Marc Pellegrini[1, 2], Marcel Doerflinger[1, 2]

1. The Walter and Eliza Hall Institute of Medical Research (WEHI), Melbourne, VIC, Australia

2. Department of Medical Biology, University of Melbourne, Melbourne, VIC, Australia

During the course from pandemic to endemic, SARS-CoV-2 transformed our lives in many ways. However, this persistent yet not entirely novel coronavirus still has surprises up its sleeve. While the precise mechanisms leading to acute disease are not fully understood, the cumulative damage of infection is now giving rise to a second pandemic of its long-term effects. Regardless of vaccination status, a resolved infection correlates with higher risk of hospitalization, multiorgan complications and death. Therefore, much still needs to be learned about this complex disease.

We developed unique pre-clinical *in vivo* models that reproduce aspects of mild, severe and fatal COVID-19. By serially passaging a clinical SARS-CoV-2 isolate in mice, we generated a mouse adapted strain that causes weight loss, inflammation and lung pathology in adult mice and is deadly in aged animals, reflecting key aspects of human disease. Interestingly, months after the virus is cleared, animals still display pronounced lung inflammation, heart abnormalities and cognitive impairment, collective hallmarks of Long COVID.

COVID-19 and its long-term sequelae have been associated with a dysregulated hyperinflammatory immune response. Depending on the cytokine profile, cell dead or survival pathways are activated, and these opposing outcomes can both lead to inflammation. Our existing knowledge of SARS-CoV-2 predominantly relies on correlative or *in vitro* studies, and it is yet unclear which pathways are causative of severe inflammation *in vivo*. Here, we utilise our pre-clinical models to conduct a detailed gene-targeted investigation to understand and systematically dissect the pathways underlying SARS-CoV-2 pathogenesis.

Using proteomic, transcriptomic and genetic approaches, we show that TNF and IL-1b drive pathogenesis. Interestingly, inflammasome pathways upstream of canonical IL-1b release do not influence disease outcomes *in vivo*. Similarly, the lytic process of necroptosis, which lies downstream of TNF, does not contribute to SARS-CoV-2 driven disease. Instead, the central determinant of severe disease outcome is Caspase-8, a protein essential for the activation of apoptosis. Remarkably, instead of triggering cell death, SARS-CoV-2 infection drives Caspase-8 to activate survival/inflammatory pathways. In this study, we shed light on the molecular decisions that Caspase-8 makes in response to SARS-CoV-2: the fine balance between inflammation and cell death.

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SpoT-mediated unique metabolic remodelling potentially underlies polymyxin tolerance in *Acinetobacter baumannii*

Zhi Ying Kho[1], Jinxin Zhao[1], Meiling Han[1], Mohammad Azad[1], Christopher Barlow[1], Ralf Schittenhelm[1], Yan Zhu[1, 2], Tony Velkov[1], Jian Li[1]

1. Monash University, Clayton, VICTORIA, Australia

2. Tianjin Institute of Industrial Biotechnology, Tianjin, China

Background: The (p)ppGpp synthetase/guanosine-3',5'-bis(diphosphate) 3'-pyrophosphohydrolase SpoT is a bifunctional bacterial enzyme that globally regulates physiological adaptation to various environmental stressors including antibiotics. We have previously demonstrated that multidrug-resistant (MDR) *Acinetobacter baumannii* upregulated SpoT in response to polymyxin treatment and disruption of the *spoT* gene resulted in enhanced polymyxin B susceptibility.

Aim: To elucidate the role of SpoT in rewiring the *A. baumannii* metabolome in response to polymyxin B treatment.

Methods: We performed comparative untargeted metabolomics of a *spoT*-disrupted *A. baumannii* AB5075 mutant versus the wild-type and compared the metabolome differences at 1 and 4 h post 2 mg/L polymyxin B treatment.

Results: The comparative metabolomics data revealed that following polymyxin B treatment the metabolome of the *spoT* mutant at 1 h was significantly depleted in guanine-based purines (i.e., guanosine triphosphate, guanosine diphosphate). This is reflective of impaired (p)ppGpp hydrolysis activity, possibly leading to lethal accumulation of the alarmone (p)ppGpp. Moreover, we observed a significant depletion of phosphoenolpyruvate, succinate, coenzyme A, nicotinamide adenine diphosphate, adenosine diphosphate, glutathione, gamma-L-glutamyl-L-cysteine and (R)-*S*-lactoylglutathione in the *spoT* mutant compared to wild-type at 1 h post treatment. Intriguingly, a distinctive time-dependent perturbation of fatty acids was evident following polymyxin B treatment in the *spoT* mutant relative to the wild-type, suggesting that the mutant maybe more reactive to the outer membrane disruption activity of polymyxin B.

Conclusions: Taken together, our findings highlighted the role of SpoT in coordinating nucleotide, carbon, energy, membrane homeostasis and antioxidant metabolism in *A. baumannii* in response to polymyxin B treatment.

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Developing precision RNA therapeutics for Tuberculosis

Jan Schaefer[1], George Ashdown[1], Paula Cevaal[2], Wai-Hong Tham[1], Marc Pellegrini[3], Marcel Doerflinger[1]

1. Walter and Eliza Hall Institute, Melbourne, VIC, Australia

2. The Peter Doherty Institute, Melbourne, VIC, Australia

3. Centenary Institute, Camperdown, 2050, NSW, Australia

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is the single biggest infectious killer worldwide, and current antimicrobial treatment strategies are increasingly ineffective due to skyrocketing multi-drug resistance [1]. Host-directed therapies acting on the host-pathogen interface to induce apoptosis of infected cells offer a promising avenue to clear the pathogen and promote adaptive immune responses while limiting the risk of drug resistance [2]. We have provided proof-of-principle for the potential success of this strategy by using small-molecule IAP antagonists to degrade key regulators of host cell apoptosis, cellular inhibitors of apoptosis (cIAP1/cIAP2), promoting TB clearance *in vivo [3]*. However, the need for systemic administration of these inhibitors increases the risk of unwanted side effects and toxicity in patients.

In its early stages, Mtb is predominantly found in alveolar macrophages, and thus, precise drug delivery is desirable to limit off-target effects [4]. Lipid nanoparticles (LNPs) have revolutionised the targeted delivery of RNA therapeutics. We have developed LNPs that enable improved RNA transfection and preferential targeting of alveolar macrophages *in vivo*. These LNPs allow for direct RNA delivery to the lung, with luciferase mRNA expression shown 16h post intranasal instillation.

Leveraging this versatile delivery platform, we were able to induce cell death in Mtb-infected primary human macrophages using siRNAs against cIAP1 and 2. 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 our siRNA approach. By combining these approaches we aim to generate a multiplexed RNA therapeutic to further boost apoptosis of infected macrophages and promote pathogen clearance. We currently assess the potential of these LNP-delivered RNA therapeutics in our clinically relevant mouse model of TB infection.

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3-base long 2'O-methyl oligonucleotides are potent TLR7 and TLR8 modulators.

Sunil Sapkota[1, 2], Arwaf Alharbi[1, 2], W. Samantha N Jayasekara[1, 2], Ruitao Jin[3], Jasiah Bones[3], Mary Speir[4, 1, 2, 5], Daniel Wenholz[4, 5], Olivier Laczka[4, 5], Julia Ellyard[6], Ben Corry[3], Michael P. Gantier[1, 2]

1. Department of Molecular and Translational Science, Monash University, Clayton, Victoria, Australia

2. Centre for Innate Immunity and Infectious Diseases, HUDSON INSTITUTE OF MEDICAL RESEARCH, Clayton, Vic, Australia

3. Research School of Biology, College of Science, Australian National University, Canberra, ACT, Australia

4. Noxopharm Limited, Sydney, NSW, Australia

5. Pharmorage Pty. Ltd., Sydney, NSW, Australia

6. Department of Immunology and Infectious Diseases, John Curtin School of Medical Research,, Australian National University, Canberra, ACT, Australia

RNA therapeutics all rely on chemical modifications, which help to stabilise these molecules against nucleases and are paramount to their drug-like properties. Critically, such modifications are also essential to blunt activation of innate immune nucleic acid sensors by such RNA therapeutics, but a detailed mechanistic understanding of how this operates remains poorly defined.

We have now made the ground-breaking discovery that degradation fragments as short as three bases (3-mers) from 2'O-methyl-modified phosphorothioate gapmer antisense oligonucleotides can directly bind Toll-Like Receptor (TLR) 7 and TLR8 to impair their RNA sensing activity. Systematic analyses have identified the optimal 3-mer sequences and chemistries impacting TLR7/8 signalling. Functionally, select 3-mer oligonucleotides have divergent effects on TLR8 activity, allowing for both inhibition or potentiation in a sequence-dependent manner, while TLR7 sensing is strictly suppressed by immunoregulatory 3-mers. Mechanistically, molecular dynamics analyses indicate that antagonistic 3-mers do not bind as well as agonistic 3-mers to site 2 of TLR7, potentially underlying their inhibitory function. Critically, TLR7-inhibiting 3-mer oligonucleotides showed significant protection against systemic and topical TLR7-driven inflammation *in vivo*.

Collectively, our findings add to the understanding of TLR7/8 sensing of RNA, indicating a complex interplay between activating and inhibiting fragments according to base and sugar modification of their nucleotides. Our studies suggest that the distinction between self and non-self RNA by TLR7/8 relies on the competitive activities of 3-base long "immune codons", acting as agonists or antagonists. Finally, our work defines a novel class of ultra-short immunomodulatory oligonucleotides with a broad range of potential therapeutic applications.

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Development and characterisation of a SARS-CoV-2 RNA vaccine expressing three linked-RBD domains

Yadana Zaw[1], Marvin Holz[1], Stanislaw Kaczmarczyk[1], Damian Purcell[1]

1. Department of Microbiology and Immunology, University of Melbourne, Melbourne, VIC, Australia

Background:

The emergence of new variants of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has consistently been a challenge for vaccine development progress. While the leading vaccines such as BNT162b2 and mRNA-1273 make use of mRNA-based technology, the unmodified self-amplifying mRNA (SAmRNA) vaccine platform uses low doses and has self-replicative properties conferred by the alphavirus replicase genes. The aim of our study is to increase the neutralisation breadth conferred by our RNA vaccines by expressing three different linked-receptor binding domains (RBDs) using either a typical modified mRNA or an alphavirus SAmRNA.

Methods:

The 3RBD antigen we designed tethered together the RBDs of Beta, Delta and BA.1 variants with a short flexible linker and incorporating a C-terminal transmembrane domain. A plasmid template was optimized for cap, codons, poly(A) and pseudouridine for maximum mRNA expression. We compared the same antigen expressed from native structured RNA in a Venezuelan equine encephalitis virus-derived (VEEV) SAmRNA expression vector. A 2kb mRNA and 9kb SAmRNA were produced by *in vitro* transcription with T7 RNA polymerase using 5' N-1-m7G cap and polyadenylated at 3' end. Our vaccine RNAs were encapsulated into lipid nanoparticles (LNPs) and particle quality controls were assessed by dynamic light scattering.

Results:

The 3RBD vaccine antigen was robustly expressed *in vitro* and readily detected by Western blot from either a typical mRNA or VEEV SAmRNA. The binding of our antigen to human ACE2 receptor was visualised via protein modelling and was confirmed *in vitro* by binding to hACE2 monomers. Flow cytometry-based binding assays demonstrated the recognition of human monoclonal antibodies to neutralising epitopes across the 3RBD antigen surface. Expression of single RBDs from both types of RNA platform were used as a comparison. Encapsulated RNA LNPs were made to optimal size, polydispersity and encapsulation efficiency.

Conclusion:

We were successful in producing two alternate versions of a booster RNA COVID vaccine and have validated the antigenicity of our novel polyvalent 3RBD antigen. Our research shows promising results for the development of a high neutralisation breadth yet RBD focused SARS-CoV-2 vaccine antigen which has now proceeded to preclinical mice study to investigate protective efficacy *in vivo.*

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Understanding the enhanced immune responses to high-density microarray patch vaccination through spatial transcriptomics

Christopher McMillan[1, 2], Jovin Choo[1], Paul Young[1, 2], David Muller[1, 2]

1. The University of Queensland, St Lucia, QUEENSLAND, Australia

2. Australian Infectious Diseases Research Centre, Global Virus Network Centre of Excellence, Brisbane, Queensland, Australia

The ongoing COVID-19 pandemic has highlighted the importance of vaccination as a critical public health tool against current and emerging pathogens. While vaccines have traditionally been delivered via needle-and-syringe injection, much work into alternative delivery systems has been conducted. The immunologically active microenvironment and the high density of antigen-presenting cells make the skin an attractive target for vaccination. The use of microarray patches to deliver vaccines directly these layers of the skin presents a promising alternative to traditional vaccine delivery mechanisms. One such microarray patch is the Vaxxas High-Density Microarray patch (HD-MAP). Delivery of vaccines via the HD-MAP has shown dramatic improvements in immunogenicity in terms of magnitude, breadth and quality of the immune response. We used newly available spatial transcriptomics tools to examine the immunological mechanisms underpinning the immune enhancement phenomena associated with HD-MAP vaccine delivery, including the nanoString GeoMx and 10x Genomics Visium and Xenium (single cell resolution). Using these techniques, we investigated the response to HD-MAPs at the transcriptome level in both mice and humans temporally and spatially in the skin. Analysis revealed the HD-MAP triggers a localized enhanced inflammatory state in the skin within 1 hour, characterized by TNF and IL-17 signaling, resulting in rapid infiltration of multiple immune cells. Distinct cell infiltrates into the epidermis and dermis were observed with the 10x Xenium platform. This work provides unprecedented detail into the precise transcriptional mechanisms following HD-MAP vaccination. These findings have implications for future microarray patch-delivered vaccine development and design.

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Cytostome formation in artemisinin resistant *Plasmodium* parasites

Long Huynh[1], Stuart Ralph[1]

1. Department of Biochemistry and Pharmacology, University of Melbourne, Melbourne, VIC, Australia

Resistance against the frontline antimalarial, artemisinin, is mediated by mutations of the Kelch 13 (K13) gene. K13 is involved in the formation of the cytostome; the apparatus which brings haemoglobin from the host red blood cell into the *Plasmodium* parasite. Haemoglobin digestion supplies essential amino acids for parasite growth and releases haem which is required for the activation of artemisinin. Parasites expressing mutant K13 have a slowed rate of parasite feeding which is characterised by a reduction in haem biosynthesis and delayed growth. This reduced level of haem leads to less artemisinin activation, resulting in less parasite death. However, the mechanisms as to how mutation of K13 causes this slowed feeding phenotype remains unclear. We hypothesise that mutation of K13 reduces its stability and abundance, affecting the rate at which new cytostomes are formed and thus parasite feeding. Using expansion microscopy, we have resolved K13 as ring-shaped structures that localise to the periphery of the parasite. We performed expansion microscopy at various timepoints throughout the asexual life cycle and compared the morphology and number of K13 rings present in the mutant vs WT. We found that K13 mutant parasites formed new K13 rings at a slower rate than the wild type. Some K13 mutants also appeared to form cytostomes that were not regulated by a cytostomal ring. These data provide a potential mechanism linking the artemisinin resistance mutations to reduced rate of haemoglobin uptake.

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Just the Tipα of the iceberg: nuclear targeting by an *Helicobacter pylori* extracellular vesicle-associated toxin.

Jack Emery[1, 2], Variya Nemidkanam[3, 2], Nina Colon[2], Dongmei Tong[2], Caroline Skene[2], Laurent Terradot[4], Richard Ferrero[1, 2, 5]

1. Department of Molecular and Translational Sciences, Monash University, Clayton, VIC, Australia

2. Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, VIC, Australia

3. Clinical Biochemistry and Molecular Medicine, Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand

4. Molecular Microbiology and Structural Biochemistry, Institut de Biologie et Chimie des Protéines, Université de Lyon, Lyon, Cedex, France

5. Biomedicine Discovery Institute, Department of Microbiology, Monash University, Clayton, VIC, Australia

Pathogenic bacteria produce a family of toxins, 'nucleomodulins', that target the nuclear compartment of host cells to cause epigenetic changes or modulate nuclear functions. While studies have examined how nucleomodulins alter nuclear processes, their intracellular trafficking is less defined. Recombinant forms of the *Helicobacter pylori* tumour necrosis factor-a-inducing protein (Tipa) were reported to translocate to the host cell nucleus, leading to tumour necrosis factor gene expression and carcinogenic effects. Furthermore, it was reported recombinant Tipa (rTipa) bound to surface expressed nucleolin as a receptor for internalisation in gastric cancer cells. Although Tipa is secreted by *H. pylori,* we observed in proteomic studies that it is also packaged within bacterial Extracellular Vesicles (EVs); membrane 'blebs' that are highly efficient at entering and subverting host cell functions. We hypothesised that *H. pylori* utilises EVs as a system to deliver Tipa to the nucleus, promoting carcinogenesis. To address this hypothesis, we characterised the secretion of Tipa by immunoblotting and observed that the majority of secreted Tipa was packaged within EVs. Next, we screened a collection of gastric epithelial cells for surface expression of nucleolin and identified MKN28 cells as expressing more nucleolin than other cell lines. Despite this, we observed by confocal microscopy and co-immunoprecipitation that rTipa localised to the perinuclear region of MKN-28 cells without binding nucleolin for internalisation. To determine the intracellular trafficking of Tipa, we cultured AGS gastric epithelial cells with *H. pylori* EVs or rTipa and observed by immunoblotting that Tipa associated with the nuclear compartment within 4 hours post-treatment. Importantly, recombinant and EV-associated Tipa trafficked through the golgi apparatus and endoplasmic reticulum to deliver Tipa to the nuclear compartment as evidenced by confocal microscopy. Taken together, we propose Tipa is an EV-associated nucleomodulin which targets the nucleus to promote gastric carcinogenesis.

ORAL ABSTRACTS

1

How do Gram-negative bacteria deal with hypo-osmotic shocks?

Jean Francois Collett[1]

1. UCLouvain, Hevillers, Belgium

Bacteria are subject to a substantial concentration differential of osmolytes between the interior and exterior of the cell, which results in cytoplasmic turgor pressure. Failure to mechanically balance turgor pressure causes cells to burst. In my talk, I will show that in Gram-negative bacteria, the outer membrane and peptidoglycan layer function together to resist turgor: when attached to each other, these two layers form a robust mechanical unit that allows pressure build-up in the periplasmic compartment, which in turn balances cytoplasmic turgor across the inner membrane, preventing cell death. Thus, the peptidoglycan layer is necessary but not sufficient to maintain turgor, which challenges the general view that protecting cells from bursting is the specific task of the peptidoglycan cell wall.

2

Structural and Molecular Biology of Bacterial Conjugation

Gabriel Waksman[1]

1. Institute of Structural and Molecular Biology , UCL and Birkbeck, London, United Kingdom

Bacterial conjugative type IV secretion systems (T4SS) are nanomachines that transfer DNAs unidirectionally from a donor cell to a recipient cell in a process called conjugation. They are the main means by which antibiotic resistance genes spread among bacterial populations. Before transferring DNA, they elaborate a long extracellular filament, termed conjugative pilus, which serves as conduit for the passage of DNA from donor to recipient cell. Here we will describe recent advances in the field that have provided mechanistic insights on aspects of pilus biogenesis and DNA transfer by these secretion machineries.

3

How cell wall targeting antibiotics kill bacteria

Yoshikazu Kawai[1], **Yousef Dashti**[1], **Jeff Errington**[1]

1. University of Sydney, Eveleigh, Sydney, NSW, Australia

Inhibition of bacterial cell wall synthesis by antibiotics such as β-lactams is traditionally thought to kill cells by explosive lysis, through loss of cell wall integrity. However, Gram positive bacteria generally do not die by this mechanism. Rather, recent studies have suggested that cell wall inhibitors work by perturbing central carbon metabolism, contributing to death via oxidative damage. Genetic dissection of these effects in *Bacillus subtilis*, has identified the key metabolic steps that stimulate the generation of damaging reactive oxygen species through cellular respiration. We screened a collection of natural product extracts for the ability to protect cells from killing under certain cell-wall perturbing conditions. We purified a highly protective factor, which turned out to be a siderophore-like compound, mirubactin C, suggesting a critical role for iron homeostasis in the oxidative damage-mediated lethal effects. We showed that the compound uncouples changes in cell morphology normally associated with cell death, from lysis as usually judged by a phase pale microscopic appearance. The results of biochemical and imaging experiments identify membrane damage by lipid peroxidation as a major factor in killing by cell-wall targeting antibiotics. These findings have important implications for the development of new antibiotics and antibiotic combinations.

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4

Cysteine-dependent antigenic heterogeneity of *Shigella flexneri* autotransporter IcsA and implications in host immunity evasion

Jilong Qin[1], **Yaoqin Hong**[1], **Renato Morona**[2], **Makrina Totsika**[1]

1. Centre for Immunology and Infection Control, School of Biomedical Sciences, Queensland University of Technology, Brisbane, QLD, Australia

2. Department of Molecular and Biomedical Science, The University of Adelaide, Adelaide, SA, Australia

Shigella IcsA is a versatile surface virulence factor required for both early and late pathogenesis stages, extracellularly to intracellularly. Despite IcsA serving as a model Type V secretion system (T5SS) autotransporter to study host pathogen interactions, its detailed molecular architecture is poorly understood. Recently, IcsA was found to switch to a different conformation for its adhesin activity upon sensing of the host stimuli by *Shigella* Type III secretion system (T3SS). Here, we report that the single cysteine residue (C130) near the N-terminus of IcsA passenger has a role in IcsA adhesin activity. We also show that the IcsA passenger (IcsAp) exists in multiple conformations, and the conformation populations are influenced by a central pair of cysteine residues (C375 and C379). Disruption of either or both central cysteine residues alters the exposure of epitopes to polyclonal anti-IcsA antibodies previously shown to block *Shigella* adherence, yet without loss of IcsA intracellular functions in actin-based motility (ABM). Anti-IcsA reactivity was restored when the IcsA paired cysteine substitution mutants were expressed in a *ΔipaD* background with a constitutively active T3SS, highlighting an interplay between T3SS and T5SS. The work here uncovers a unique molecular switch empowered by centrally localised, short-spaced cysteine pairs in a Type V autotransporter that maintains IcsA's conformational landscape to aid host immunity evasion.

5

Primate *Simplexvirus* UL39 proteins inhibit RHIM signalling

Joseph Tannous[2, 1], **Nicholas Shields**[2, 1], **Caroline Ashley**[2, 1], **Olivia Lavidis**[2, 1], **Jamie Triccas**[2, 1], **Margie Sunde**[1], **Megan Steain**[2, 1]

1. School of Medical Sciences, The University of Sydney, Camperdown, NSW, Australia

2. Sydney Infectious Diseases Institute, The University of Sydney, Camperdown, NSW, Australia

Macacine herpesvirus 1 or "Herpes B virus" (BV) naturally infects macaques, and *Papiine herpesvirus 2* (HVP2) infects baboons. BV and HVP2 are closely related to Herpes simplex virus (HSV)-1 and -2 and all four viruses cause a similar self-limiting infection in their natural, immunocompetent hosts. However, these viruses are often associated with extreme neurovirulence following cross-species transmission, for example BV can cause encephalitis in humans with a mortality rate of ~80%. The HSV encephalitis in humans has been associated with genetic defects in innate immune

signalling molecules that contain RIP homotypic interaction motifs (RHIMs). RHIM-containing proteins play key roles in cell death and proinflammatory pathways and HSV-1 and -2 encode viral decoy RHIMs in the UL39 gene that can modulate these signalling pathways in a species-dependent manner. The neurovirulence of HVP2 in mice has been mapped to the UL39 gene and RHIM sequences have been identified in the UL39 genes of BV and HVP2, however their function/s have not yet been characterised. Our recent focus has been on determining if the UL39 genes of BV and HVP2 encode functional viral RHIMs capable of modulating host RHIM signalling pathways. We have shown that the UL39 genes of both BV and HPV2 can significantly impair NF-kB pathway activation in a RHIM-dependent fashion. We are now exploring the ability of these viral RHIM-containing proteins to modulate RHIM-mediated cell death in human and murine cells. This work expands our knowledge of viral RHIMs capable of interfering with human RHIM signalling and may help us better understand the inter-species neurovirulence of herpesviruses.

6

Molecular structure of the intact bacterial flagellar basal body

Steven Johnson[1, 2], Emily Furlong[1, 3], Justin C Deme[1, 2], Ashley L Nord[5, 4], Joseph Casear[1], Fabienne FV Chevance[6], Richard M Berry[5], Kelly T Hughes[6], Susan M Lea[1, 2]

1. Sir William Dunn School of Pathology, University of Oxford, Oxford, UK

2. Center for Cancer Research, National Cancer Institute, Frederick, Maryland, USA

3. Research School of Biology, Australian National University, Canberra, ACT, Australia

4. Centre de Biologie Structurale, INSERM, CNRS, Univerite de Montpellier, Montpellier, France

5. Department of Physics, University of Oxford, Oxford, UK

6. Department of Biology, University of Utah, Salt Lake City, Utah, USA

Bacterial flagella self-assemble a strong, multi-component drive shaft that couples rotation in the inner membrane to the microns-long flagellar filament that powers bacterial swimming in viscous fluids. We solved structures of the intact *Salmonella* flagellar basal body, using cryo-electron microscopy to resolutions between 2.2 and 3.7 Å. The structures reveal molecular details of how 173 protein molecules of 13 different types assemble into a complex spanning two membranes and a cell wall. The helical drive shaft at one end is intricately interwoven with the inner membrane rotor component, and at the other end passes through a molecular bearing that is anchored in the outer membrane via interactions with the lipopolysaccharide. The *in situ* structure of a protein complex capping the drive shaft provides molecular insight into the assembly process of this molecular machine.

7

T lymphocyte differentiation and function: Lessons learned from patients with inborn errors of immunity

Federica Sallusto[1]

1. ETH Zurich, Zürich, Switzerland

We study the human immune system to address fundamental questions in the context of the immune response to different classes of antigens. Our efforts have resulted in the development of high-throughput cell-based screening methods to analyze the repertoire and functional diversity of effector, memory and regulatory T cells in immunocompetent individuals and in immunodeficient patients. These methods, together with high throughput TCRVB sequencing, which can analyze millions of T cell clonotypes in blood or tissues, single-cell gene expression analysis, and cell engineering are used to get insights as to the mechanisms of T cell differentiation and of immune-mediated protection and pathology.

8

Divergent cytokine and transcriptional signatures control functional Tfh heterogeneity

Joanna Groom[1]

1. WEHI, PARKVILLE, VICTORIA, Australia

Flexible immune responses enable clearance and protection against distinct infection types, including viral, bacterial, fungal, and helminth infections. In all these infectious settings, $CD4^+$ T follicular helper (Tfh) cells promote high-affinity class-switched antibodies, long-lived plasma and memory B cells. How Tfh cells tailor B cell responses in a pathogen-specific manner is unclear. Here we identify Tfh transcriptional networks in response to diverse infections. The core Tfh signature, distinct from $CD4^+$ effector and follicular regulatory cells, indicated stem-like potential coordinated by Bcl6. Pathogen-specific signatures highlighted cytokine pathways that establish Tfh heterogeneity and impact B cell output. Cytokine-transcriptional Tfh programing was conserved in human tonsil. This blueprint of Tfh heterogeneity offers new avenues to treat antibody-mediated diseases and inform the development of context-specific vaccines.

9

A common allele of HLA is associated with asymptomatic SARS-CoV-2 infection

Dimitra SM Chatzileontiadou[1, 2], Lawton D Murdolo[1], Danillo G Augusto[3, 4, 5], Joseph J Sabatino Jr[3], Stephanie Gras[1, 2], Jill A. Hollenbach[3, 6]

1. La Trobe University, Bundoora, VIC, Australia

2. Biochemistry and Molecular Biology, Monash University, Clayton, Melbourne, VIC, Australia

3. Department of Neurology, Weill Institute for Neurosciences, University of California, San Francisco, CA, USA

4. Department of Biological Sciences, The University of North Carolina at Charlotte, Charlotte, NC, US

5. Programa de Pós-Graduação em Genética, Universidade Federal do Paraná, Curitiba, Brazil

6. Department of Epidemiology and Biostatistics, University of California, San Francisco, CA, USA

Throughout the COVID-19 pandemic, research has centred on understanding why some people infected with the SARS-CoV-2 virus experience severe disease. However, 10-30% of individuals with the virus do not show any symptoms. Investigating asymptomatic infection could shed light on features of the immune system that help to eliminate SARS-CoV-2. Here, postulating that variation in the human leukocyte antigen (HLA) loci may underly processes mediating asymptomatic infection, we investigated the genetics of almost 30,000 registered bone marrow donors who participated in a voluntary program to track COVID-19 infection and symptoms[1]. Our analysis revealed a strong association between HLA-B*15:01 and asymptomatic infection, observed in two independent cohorts. Suggesting that this genetic association is due to pre-existing T cell immunity, we show that T cells from pre-pandemic samples from individuals carrying HLA-B*15:01 were reactive to the immunodominant SARS-CoV-2 Spike-derived peptide NQKLIANQF. The majority of the reactive T cells displayed a memory phenotype, were highly polyfunctional and were cross-reactive to a peptide derived from seasonal coronaviruses (NQKLIANAF). The crystal structure of HLA-B*15:01–peptide complexes demonstrates that the peptides NQKLIANQF and NQKLIANAF share a similar ability to be stabilized and presented by HLA-B*15:01. Finally, we show that the structural similarity of the peptides underpins T cell cross-reactivity of high-affinity public T cell receptors, providing the molecular basis for HLA-B*15:01-mediated pre-existing immunity[1]. Our findings have major implications for public health because such knowledge could inform the design and development of vaccines and therapies for COVID-19. More broadly, identifying genetic factors that control the course of the disease could begin to explain the wide variation in how people respond to SARS-CoV-2 and other viral infections.

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Understanding T cell-mediated immunity of *Bacteroides fragilis* glycosphingolipids by Natural Killer T cell receptor

Praveena Thirunavukkarasu[1], Sungwhan F.Oh[2, 3], Heebum Song[4], Ji-Sun Yoo[3], Da-Jung Jung[3], Deniz Erturk-Hasdemir [2], Yoon Soo Hwang [4], Changwon C. Lee [2], Jérôme Le Nours[1], Hyunsoo Kim[4], Jesang Lee[4], Richard S. Blumberg [5], Seung Bum Park [4], Dennis L. Kasper [2], Jamie Rossjohn[7, 6]

1. Infection and Immunity program & Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Melbourne, Victoria, Australia

2. Department of Immunology, Blavatnik Institute of Harvard Medical school, Boston, USA

3. Center for Experimental Therapeutics and Reperfusion injury, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Boston, USA

4. CRI center for Chemical Proteomics, Department of Chemistry, Seoul National University, Seoul, Republic of Korea

5. Division of Gastroenterology, Hepatology and Endoscopy, Department of Medicine, Brigham and Women's Hospital, Boston, USA

6. Institute of Infection and Immunity, Cardiff University, Heath Park, Cardiff, UK

7. Australian Research Council Centre of Excellence for Advanced Molecular Imaging, Monash University, Australia, Melbourne, Victoria, Australia

The human gut microbiota comprises more than 50% of *Bacteroides* species that produce small diffusible molecules like sphingolipids that play a key role in modulating the host's immune responses. In particular, *Bacteroides fragilis* produces glycosphingolipids similar to α-galactosylceramides termed as 'BfaGCs' that can activate type I Natural Killer T (NKT) cells. While they share key chemical similarities with the type I NKT cell marker antigen, α-galactosylceramide (KRN7000), they possess distinctive structural features including short sphinganine chains, branching and functional groups, implying a basis for their unique immunomodulatory properties. The co-culture assay performed with bone marrow-derived dendritic cells and NKT cells in the presence of specific BfaGCs indicated that branching in their sphinganine chain is a critical determinant of NKT cell activation. As such, the strong stimulators measured by their IL-2 release were the compounds that contained the branched sphinganine chains. Our structural studies on two such CD1d-presented BfaGCs in complex with the type I NKT TCR revealed the TCR adopted a parallel docking topology atop the F'-pocket of CD1d in recognising the presented BfaGCs. Interestingly, the terminal sphinganine branching of the BfaGCs mediated unique interactions within the F'-pocket of CD1d, providing a mechanism for their differing agonistic properties. The NKT TCR recognised the CD1d presented stimulatory and non-stimulatory BfaGCs with nanomolar affinities. Thus, BfaGCs were demonstrated to be bonafide CD1d ligands that function as immunomodulatory mediators influencing the host's defence in the context of NKT cells. Together, this study highlights the structural and molecular-level paradigm of existing symbiotic relationship between the microbes producing these endogenous lipids and the host.

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11

Covalent TCR-peptide-MHC interactions: A new mechanism for T cell antigen recognition and T cell activation

Pirooz Zareie[2, 1], Christopher Szeto[1, 3], Rushika C Wirasinha[1], Justin Zhang[1], Andrea T Nguyen[2, 3], Alan Tunnicliffe-Riboldi[4], Nicole La Gruta[1], Stephanie Gras[3], Stephen R Daley[5]

1. Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Instititute, Monash University, Clayton, VIC, Australia

2. The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia

3. Department of Biochemistry and Chemistry, La Trobe Institute for Molecular Science, La Trobe University, Bundoora, VIC, Australia

4. Australian Synchrotron, Australian Nuclear Science and Technology Organisation, Clayton, VIC, Australia

5. Centre for Immunology and Infection Control, Queensland University of Technology, Brisbane, QLD, Australia

Interactions between a T cell receptor (TCR) and a peptide-major histocompatibility complex (pMHC) ligand are typically mediated by non-covalent bonds. By studying T cells expressing natural or engineered TCRs, here we describe covalent TCR-pMHC interactions that involve a cysteine-cysteine disulphide bond between the TCR and the peptide. By introducing cysteines into a known TCR-pMHC combination, we demonstrate that disulphide bond formation does not require structural rearrangement of the TCR or the peptide. We further show these disulphide bonds still form even when the initial affinity of the TCR-pMHC interaction is low. Accordingly, TCR-peptide disulphide bonds facilitate T cell activation by pMHC ligands with a wide spectrum of affinities for the TCR. Physiologically, this mechanism induces strong Zap70-dependent TCR signalling, which triggers T cell deletion or agonist selection in the thymus cortex. Covalent TCR-pMHC interactions may thus underlie a physiological T cell activation mechanism that has applications in basic immunology and potentially in immunotherapy.

12

Unveiling the immune shield - a dive into testicular and epididymal immunology

Christiane Pleuger[1], Monika Fijak[1], Sudhanshu Bhushan[1], Andreas Meinhardt[1]

1. Institute of Anatomy and Cell Biology, Justus-Liebig University, Giessen, Germany

Macrophages are the principal leukocytes of the epididymis and testis, but their origins, heterogeneity, development, maintenance and function are not well understood. In a multi-pronged approach, we found that CD64^{hi}MHCII^{lo} and CD64^{lo}MHCII^{hi} macrophage populations of epididymis and testis arise sequentially from yolk sac erythro-myeloid progenitors, embryonic hematopoiesis, and nascent neonatal monocytes. While monocytes were the major developmental source of epididymal and testicular macrophages, both populations self-maintain independent of bone marrow hematopoietic precursors. However, during infection, bone marrow-derived circulating monocytes are recruited to both organs, giving rise to inflammatory macrophages that promote tissue damage. Associated damage of the testis of affected men and in rodent models include leukocytic infiltration, edema formation, fibrosis, germ cell loss and reduced androgen levels. The mouse epididymis comprises four distinct regions: the initial segment (IS) which receives the spermatozoa from the testis, caput, corpus and cauda, where sperm are stored and pass to the vas deferens. The epididymis faces opposing immunological challenges. In the IS and caput, local tolerance is required to avoid autoimmune reactions against immunogenic spermatozoa. Conversely, the cauda is a port of entry for bacteria ascending from the urethra. In both mice and human, the magnitude of immune response in the cauda following bacterial infection is much higher than in the proximal parts of the epididymis, often leading to male infertility due to fibrosis and duct obstruction. We show that immune cell populations are strategically positioned along the epididymis and represent a possible key factor to maintain the immunological equilibrium across the organ.

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Malaria sporozoite biology at the dermal inoculation site

Photini Sinnis[1]

1. Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Malaria infection is initiated when mosquitoes inoculate sporozoites into the skin. Sporozoites are actively motile and must exit the inoculation site to go to the liver where they initiate the next stage of infection. Little is known about how the parasite interacts with the host at the dermal inoculation site. I will briefly discuss the host's innate immune response to the parasite and recent findings on sporozoite motility which is likely critical to escape this response.

14

Non-antibiotic strategies to mitigate enteric infections: insights from discovery research

Dena Lyras[1]

1. Monash University, Clayton, VIC, Australia

One of the biggest barriers to developing new, non-antibiotic strategies for the treatment of enteric infections is that we do not understand well enough how these pathogens function in the gastrointestinal niche, or how these infections impact the host extra-intestinally. This presentation will discuss how fundamental investigations of new disease mechanisms have recalibrated our understanding of enteric infection and disease, and how this new understanding has allowed us to develop effective therapeutic strategies for gut infections that do not involve the use of antibiotics.

15

Utilising the tick's trick for advancing anti-inflammatory therapy

Shankar Devkota[1], Ram Bhusal[1], Pramod Aryal[1], Martin Stone[1]

1. Monash University, Clayton, VIC, Australia

A hallmark of chronic inflammatory diseases is the excessive accumulation of white blood cells in the affected tissues, which is coordinated by pro-inflammatory mediators called chemokines. Humans have ~50 chemokines divided into two major classes – CC and CXC chemokines. Specific groups of chemokines are associated with different inflammatory diseases; for example, the chemokines CCL2, CCL7 and CCL8 are involved in atherosclerosis. As natural chemokine inhibitors, evasin proteins secreted in tick saliva are potential anti-inflammatory therapeutic agents. However, the development of tick evasins as chemokine-targeted anti-inflammatory therapeutics requires an understanding of the factors controlling their chemokine-binding specificity. Structures of the evasins EVA-P974 and EVA-AAM1001 bound to several human CC chemokine ligands (CCL7, CCL11, CCL16 and CCL17) and to a CCL8-CCL7 chimera reveal that the specificity of evasins for chemokines of the CC subfamily is defined by conserved, rigid backbone-backbone interactions. Whereas the preference for a subset of CC chemokines is controlled by side chain interactions at hotspots in flexible structural elements. The substitution of amino acid residues at hotspots provides evasin variants with new chemokine-binding properties. Moreover, we identified a novel class A evasin, EVA-AAM1001 (class A3), which possesses an additional disulfide bond near the chemokine recognition site. This additional bond allows

EVA-AAM1001 to form a critical hydrophobic pocket, allowing it to bind to CC chemokines with high affinity using a different set of hotspots compared to class A1 evasins. These structural insights enabled rational engineering of evasins to tailor their chemokine selectivity. These studies provide a basis for development of evasins with applications in anti-inflammatory therapy.

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New molecular mechanisms for bacterial toxins

Lilian Hor[1], Akila Pilapitiya[1], Kait Clarke[1], James A Mckenna[1], Santosh Panjikar[2], Marilyn A Anderson[1], Mickael Desvaux[3], Jason J Paxman[1], Begoña Heras[1]

1. Department of Biochemistry and Chemistry , La Trobe Institute for Molecular Science, La Trobe University , Melbourne, Victoria , Australia

2. Macromolecular Crystallography, Australian Synchrotron, Clayton, VIC, Australia

3. INRAE UMR454 MEDiS, Université Clermont Auvergne, Clermont-Ferrand, France

Bacterial pathogens utilise surface bound and secreted proteins in order to promote infections and disease, with the largest family of such proteins being the autotransporters. Autotransporters are used by a range of important bacterial pathogens to facilitate colonisation, biofilm formation, spread and tissue destruction. However, the vast majority of autotransporters remain uncharacterised.

Aims: We sought to better define the relationships between autotransporters, in order to find new mechanisms of action that can be applied towards new medical outcomes.

Methods: We employed a comprehensive array of methods to explore autotransporter function including X-ray crystallography, enzyme assays, mutational analysis, cell culture, invasion assays, infection models along with bioinformatics.

Results/Discussion: Partly driven by our own accumulation of autotransporter structural and mechanistic data we decided to perform an extensive phylogenetic study of functionally described autotransporters. This updated analysis allowed us to define new and under-studied autotransporter groups [1].

The subtilase autotransporters were identified as a large structurally unknown autotransporter group. Hence we determined the first crystal structure of a subtilase autotransporter Ssp from the pathogen *Serratia marcescens* [2]. We found that Ssp was a potent toxin, capable of entering human epithelium to cause cytotoxic effects along with being highly lethal in a *Galleria mellonella* infection model. Importantly, the Ssp structure revealed a completely new structural layout, amongst the backdrop of the many well known bacterial toxins. Moreover, many of the unique structural attributes of Ssp were required for its novel mode of cellular entry and toxicity.

This Ssp story is another addition to our growing list of new molecular mechanisms that we have uncovered for this large family of bacterial virulence factors [3-5], and show that the autotransporter family still contains many more unique structures and mechanisms that await to be revealed. Of significance is that we are now using these molecular mechanisms to develop new therapies such as biofilm inhibitors [6] and new cell penetrating drug carriers.

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Targeted degradation of host messenger RNA by a bacterial effector modulates macrophage metabolism

Kitty McCaffrey[1], Hari Sivaraman[1], Joshua von Ameln[1], Raissa Wibawa[1], Garrett Ng[1], Natalio Garbi[2], Ian R van Driel[3], Wilson Wong[1], Elizabeth L Hartland[1]

1. Hudson Institute, Clayton, VICTORIA, Australia

2. University of Bonn, Bonn, Germany

3. University of Melbourne, Melbourne, VICTORIA, Australia

Macrophages are the first line of defence against bacterial infection and, upon activation, undergo a shift to glycolytic metabolism that is essential for the antimicrobial and pro-inflammatory functions of these innate immune cells. Macrophages are also the primary target of many intracellular bacterial pathogens that inject virulence effector proteins into the macrophage intracellular environment to alter cellular responses, thereby enabling the survival and replication of the pathogen. In this study, we observed that infection with the intracellular bacterial pathogen *Legionella pneumophila* depleted host-cellular messenger RNA (mRNA) encoding metabolic enzymes, including the rate-limiting glycolytic enzyme GAPDH. By screening a library of *L. pneumophila* deletion mutant strains, we identified the secreted Dot/Icm effector LegC4 as essential for the degradation of host mRNA and the suppression macrophage glycolysis during infection. Using cross-linking immunoprecipitation (CLIP), we demonstrated that LegC4 directly interacted with GAPDH mRNA during infection. Electrophoretic mobility shift assays (EMSA) also showed that LegC4 bound directly to GAPDH mRNA *in vitro* in the absence of other bacterial or host factors. Analysis of RNA bound to LegC4 showed enrichment for guanine (G)-rich RNA recognition motifs that occurred within the GAPDH transcript and within all mRNA-degradation targets, suggesting that these motifs conferred LegC4-mRNA specificity. Finally, cryo-EM structural analysis of an inactive mutant of LegC4 ($LegC4_{H60A}$) in complex with RNA revealed a non-canonical RNA-binding domain (RBD) and the molecular basis for the putative ribonuclease activity of LegC4. This work is the first description of a secreted bacterial effector that binds to eukaryotic host mRNA and has revealed a novel mechanism by which functionally related mRNAs may be post-transcriptionally regulated to alter cellular metabolism during infection.

18

Group A *Streptococcus* associated with recurrent tonsillitis form antibiotic-resistant intracellular communities

Jua Iwasaki[1], Jasreen Kular[1], Jessica Daw[1], Ruby Goedsir[1], Alisha Wilson[1], Vanessa Tenaglia[1], Kalindu Rodrigo[1], Andrew Hayes[2], Jonathan Carapetis[3], Anthony Kicic[3], Mark Davies[2], Robert Parton[4], Nathan Pavlos[5], Tim Barnett[1]

1. Wesfarmers Centre for Vaccines and Infectious Diseases, Telethon Kids Institute, Nedlands, WA, Australia

2. Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia

3. Telethon Kids Institute, Subiaco, WA, Australia

4. Institute for Molecular Bioscience, University of Queensland, St Lucia, QLD, Australia

5. School of Biomedical Sciences, The University of Western Australia, Nedlands, WA, Australia

Antimicrobial resistance (AMR) is a major threat to global human health. Improved AMR surveillance is one of six priorities identified by the World Health Organization to mitigate the burden of antibiotic-resistant infections. However, existing AMR genes do not explain all antibiotic-resistant infections. One common example is the Group A *Streptococcus* (GAS) infections that fail β-lactam (e.g. penicillin) therapy, despite universal susceptibility of this pathogen in vitro. Here we show that GAS strains isolated from penicillin treatment failures are able to invade into epithelial cells and replicate in the presence of penicillin, forming large intracellular bacterial communities. We further show that this process requires a GAS surface protein that promotes uptake into epithelial cells via an alternative invasion pathway, distinct from that used by most GAS strains. Our study describes a mechanism for phenotypic antibiotic resistance conferred by a bacterial invasin, and explains why penicillin often fails to clear GAS infections despite this pathogen being highly-susceptible to penicillin *in vitro*. Our findings may allow identification of GAS infections at risk of failing penicillin therapy at the point of care, and allow treatment with alternative antibiotics to reduce rates of recurrent GAS infections.

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Gut inflammation and barrier breakdown in severe dengue disease

Adriana Pliego Zamora[1], Jaehyeon Kim[1], Naphak Modhiran[1], Paul Young[1], Kate Bowerman[1], Dan Watterson[1], Helle Bielefeldt-Ohmann[1], Katryn Stacey[1]

1. SCMB, The University of Queensland, Brisbane, Qld, Australia

Dengue virus (DENV) is the most prevalent mosquito-borne flavivirus. Severe disease including dengue shock syndrome has an abrupt onset when fever and viraemia are abating. Serum bacterial lipopolysaccharide levels correlate with disease severity in patients (1,2), and gut-related symptoms are included amongst the warning signs for severe disease. We hypothesised that gut barrier breakdown and influx of inflammatory bacterial products is a key factor promoting severe disease and could account for sudden deteriorations. We examined the time course of appearance of DENV-infected cells in various tissues in mouse models of DENV infection. Following the peak of DENV replication in the spleen of AG129 and IFNAR-/- mice, infected cells appeared in the small intestine and colon, prior to substantial infection in other tissues. Major inflammatory pathology was seen in the gut, accompanied by elevated cytokine and chemokine expression, transient gut barrier breakdown and diarrhoea. However, pathology in other tissues was relatively mild. Gut pathology scores as well as cytokine and DENV RNA levels in the colon and liver were decreased in mice treated with an antibiotic cocktail, suggesting pathogenic roles for gut bacteria. Gut inflammation and leak are frequently associated with dysbiosis, and the faecal microbiome was substantially altered by day 3 of DENV infection. We propose that microbiome change and influx of bacterial products when the gut becomes leaky promotes inflammation and liver infection, and exacerbates dengue disease. Supporting our conclusions, a recent study directly demonstrated the loss of gut epithelial barrier integrity in severe dengue patients (2). Inhibiting innate immune activation by bacterial products, supporting healthy microbiota or maintaining gut barrier integrity may help to limit disease severity.

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Understanding the distinct roles of IFN epsilon in protecting the mucosa from disease

Paul Hertzog[1]

1. Hudson Institute of Medical Research, Clayton, VIC, Australia

Protecting mucosal surfaces from infection requires coordinated molecular and cellular responses aligned with physiological changes such as cell morphology, proliferation, programmed death, and with innate and adaptive immune responses tolerant to commensals but responsive to pathogens. Cytokines such as IFNs are crucial mediators of such responses. Classic type I IFNs, the 14 a subtypes and b, and the type III IFN l are induced by pathogens, produced transiently to control their efficacy:toxicity; but are distinguished by specific type I and III receptors that are respectively ubiquitous or cell specific especially to epithelial cells. By comparison the unique type I IFNe is constitutively expressed by epithelial cells at low levels and has unique properties commensurate with spatiotemporal nature of its expression[1]. We have shown using knock-out mice and neutralizing antibodies, etc that IFNe is critical for optimal responses to viral infections HSV, HIV, Zika and bacterial infection , chlamydia [2-4]. IFNe can directly induce antiviral molecules in cells, and can also control immune responses. We have shown in these different models that IFNe regulates CD8 T cells, Treg or NK cells[5]. We have also shown that these molecular and cellular responses to IFNe equip it as a potent suppressor of tumours in the female reproductive tract[6]. The challenges are to dissect how we can utilize these unique properties of IFNe for clinical benefit.

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Potent, HIV-specific latency-reversal through CRISPR activation delivered by lipid nanoparticles

Paula M Cevaal[1], Stan Kan[1], Abdalla Ali[1], Abigail Tan[1], Marvin Holz[2], Denzil L Furtado[3], Damian Purcell[2], Angus Johnston[4], Colin Pouton[4], Thomas Payne[4], Frank Caruso[3], Jori Symons[1, 5], Michael Roche[1], Sharon R Lewin[1, 6, 7]

1. Department of Infectious Diseases, The University of Melbourne at The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia

2. Department of Microbiology and Immunology, The University of Melbourne at The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia

3. Department of Chemical Engineering, The University of Melbourne, Melbourne, VIC, Australia

4. Monash Institute of Pharmaceutical Sciences, Melbourne, VIC, Australia

5. Department of Medical Microbiology, University Medical Center, Utrecht, The Netherlands

6. Victorian Infectious Diseases Service, Royal Melbourne Hostpital at The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia

7. Department of Infectious Diseases, Alfred Hospital and Monash University, Melbourne, VIC, Australia

Introduction: Activation of HIV transcription through LTR-targeted CRISPRactivation (CRISPRa) provides a promising strategy of reversing HIV latency without affecting host-cell transcription. However, the advancement of this novel latency-reversing agent is hampered by the lack of a delivery vehicle for the CRISPRa machinery to resting $CD4^+$ T cells. We hypothesized that targeted mRNA-lipid nanoparticles (LNPs) could be used to advance CRISPRa as a next-generation latency-reversing agent.

Methods: Fluorescently labelled standard and modified LNPs encapsulating reporter mCherry mRNA (mCherry-LNP) or co-encapsulating the dCas9-SAM CRISPRa system (CRISPRa-LNP) were formulated through microfluidic mixing using two different lipid mixes. T cell-targeting antibodies were captured onto the LNPs following controlled-orientation conjugation of anti-IgG1 nanobodies to the LNP surface. Transfection efficiency and associated toxicity were assessed in non-stimulated or αCD3/αCD28 pre-stimulated primary $CD4^+$ T cells or PBMCs from HIV-negative donors. Reactivation of HIV transcription was assessed in J-Lat LTR-Tat-IRES-GFP reporter cells.

Results: Transfection efficiency of pre-stimulated $CD4^+$ T cells using standard LNPs was moderate (mean±SEM $mCherry^+$ cells 29±5%) but toxic (43±5% viability) after 72h. In non-stimulated cells, few cells were transfected (2±0.3%) with lower toxicity (68±8% viability), which coincided with a 20-fold reduction in LNP association. In contrast, transfection of non-stimulated $CD4^+$ T cells with modified LNPs resulted in a striking 92±2% efficiency at minimal toxicity (88±3% viability) within 72h. Similarly, treatment with modified but not standard CRISPRa-LNPs induced potent HIV transcription with all five targeting guideRNAs, reaching up to 76±13% GFP^+ J-Lat cells compared with 0.89±0.1% using non-targeting guideRNA, both at viabilities >90%. Functionalization of the modified LNPs with T cell-targeting antibodies enhanced the T cell transfection efficiency in the presence of bystander cells by over 20-fold.

Conclusions: We developed a novel LNP formulation capable of delivering nucleic acid-based therapeutics to resting $CD4^+$ T cells. Antibody-functionalization of the modified LNP surface further enhances the specificity towards T cells with great potency. The three-component dCas9-SAM CRISPRa system can be co-encapsulated into one LNP and can induce strong latency reversal in a cell line model for HIV latency. These results provide compelling justification for the further assessment of CRISPRa-LNP as a 'shock and kill' strategy.

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Trafficking towards antimicrobials

Joen Luirink[1]

1. Vrije Universiteit, Amstelveen, Netherlands

In diderm bacteria, multiple secretion systems have evolved to transfer proteins across the entire cell envelope. One of these systems, type V secretion, uses a relatively simple two-step mechanism for secretion and surface display of virulence factors. It is also known as Autotransport as it functions relatively autonomously and has therefore attracted attention for recombinant protein secretion and display.

We have investigated the mechanics of type V secretion but in this talk I will focus on applications of this basic research that relate to the development of antimicrobials. First, I will discuss how we use the system for surface display of heterologous antigens to create live recombinant vaccines and derived Outer Membrane Vesicles (OMVs). Second, I will describe how we use the system to develop a stress-based high throughput assay that can identify inhibitors of type V secretion and outer membrane protein biogenesis.

23

Can a meningococcal vaccine prevent gonorrhoea?

Kate L Seib[1]

1. Institute for Glycomics, Griffith University, Gold Coast, QLD, Australia

Gonorrhoea, the sexually transmissible infection caused by *Neisseria gonorrhoeae*, is a global public health concern with an estimated 82 million new infections each year worldwide. Gonorrhoea antimicrobial resistance continues to increase and there is no proven effective vaccine

available. However, there is increasing evidence from observational cohort studies that the serogroup B meningococcal vaccine 4CMenB (trade name Bexsero®), licenced to prevent invasive disease caused by *Neisseria meningitidis*, may provide cross-protection against the closely related bacteria *N. gonorrhoeae.* We are conducting a randomised control trial with 4CMenB in gay and bisexual men (MenGo; ACTRN12619001478101) to evaluate the efficacy of two doses of 4CMenB given 3 months against *N. gonorrhoeae* infection. We are also characterising vaccine-induced immune responses and have shown that vaccination with 4CMenB induces antibodies that can recognise and kill *N. gonorrhoeae* in vitro. Due for completion in 2024, the MenGo study will inform future gonorrhoea prevention strategies. If 4CMenB is proven to be effective against *N. gonorrhoeae*, this could help achieve the World Health Organization's target for reducing gonorrhoea incidence by 90% by 2030 and improve sexual and reproductive health worldwide.

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Developing a functional cure for HTLV1 in a humanized mouse model

James Cooney[1], Cody Allison[2], Simon Preston[2], Damian Purcell[3], Lloyd Einsiedel[3], Marcel Doerflinger[1], Marc Pellergini[1]

1. Walter and Eliza Hall Institute for Medical Research, Parkville, VIC, Australia

2. Walter and Eliza Hall Institute, Parkville, VIC, Australia

3. Department of Microbiology and Immunology, Doherty Institute for Infection and Immunity, The University of Melbourne, Parkville

Satisfactory preventative or therapeutic drugs are lacking for HTLV-1, a retrovirus closely related to HIV. As a result infected individuals frequently present with serious complications including rapidly progressive Adult T Cell Leukaemia (ATL), inflammation of the spinal cord (HTLV-1-assocaited myelitis, HAM) and chronic bronchiectasis. HTLV-1 subtype c (HTLV-1c) is highly prevalent in indigenous communities in Central Australia, with infection rates unparalleled globally. Novel approaches that both inhibit viral replication and reduce the number of HTLV-1 infected cells are urgently required.

We investigated the efficacy of antiretroviral and pro-apoptotic BH3 mimetic compounds as preventative and therapeutic agents in a humanised mouse model of HTLV-1 subtype c (HTLV-1c) infection, the first of its kind. We characterised infection in this model and compared disease to the globally prevalent HTLV-1 subtype a (HTLV-1a). Tenofovir, a reverse transcriptase inhibitor, significantly reduced HTLV-1 transmission in vivo at clinically relevant doses and attenuated de novo viral spread and disease progression during early infection in combination with dolutegravir, an integrase inhibitor. HTLV-1 infection was associated with dysregulation of the intrinsic apoptotic pathway at the transcriptional level, and we found that pharmacological inhibition of MCL-1, but not BCL-2, BCL-xL or BCL-w, killed HTLV-1-infected cells in vitro and in vivo, significantly delaying disease progression in combination with tenofovir and dolutegravir. Our data demonstrate that combination antiretroviral and MCL-1 antagonism may represent an effective, clinically relevant, curative strategy against HTLV-1. We are now expanding our efforts towards next-generation therapeutics with decreased treatment off-target effects and increased cell specificity by utilizing precsion targeting of T cells by LNP-based delivery of RNA therapeutics against MCL-1.

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Resistance to last line antibiotics impairs STING-dependent sensing of MRSA in dendritic cells.

Nazneen Jahan[1], Timothy Patton[2], Jhih-Hang Jiang[1], Anton Peleg[1], Dominic De Nardo[1], Meredith O'Keeffe[1]

1. Monash University, Clayton, VIC, Australia

2. Microbiology and Immunology, University of Melbourne, Melbourne

Methicillin resistant Staphylococcus aureus (MRSA) is one of the leading causes of hospital acquired infections and is considered as the most frequent cause of bacteraemia worldwide. In recent years, MRSA has acquired resistance to the last line antibiotic daptomycin (Dap) and this is commensurate with reduced innate immune responses to MRSA. Being at the interface between innate and adaptive immune responses, dendritic cells are thus central to the immune protection against MRSA. We have previously shown that clinical isolates resistant to Daptomycin (DapR) induced a compromised dendritic cell response to MRSA compared to Daptomycin sensitive (DapS) paired isolates. In this work we have explored the molecular mechanism by which MRSA strains impede dendritic cell activation. We have found that acquisition of daptomycin resistance in MRSA, inhibits the ability of dendritic cells to recognise the DapR MRSA via the cGAS/STING signalling pathway. The DapR MRSA does not actively inhibit the intracellular signalling pathways, but rather, the DapR MRSA fails to produce detectable cyclic dinucleotides, 3'3'-cGAMP, secreted at high levels by DapS MRSA, and which are potent stimulators of STING.

Our work, thus highlights important insights for the mechanism of differential recognition of clinical isolates of DapS and DapR MRSA by dendritic cells. These data provide clear molecular evidence for antimicrobial resistance directly regulating innate immune responses.

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Specific and off-target immune responses following COVID-19 vaccination with ChAdOx1-S and BNT162b2 COVID-19 vaccines

Nicole L Messina[1, 2], Susie Germano[1], Rebecca McElroy[1], Rhian Bonnici[1], Branka Grubor-Bauk[3], David J Lynn[4, 5], Ellie McDonald[1], Suellen Nicholson[6], Kirsten P Perrett[2, 7, 8], Laure F Pittet[1, 2, 9], Rajeev Rudraraju[10], Natalie E Stevens[4, 5], Kanta Subbarao[10, 11], Nigel Curtis[1, 2, 12, 13]

1. Infection, Immunity and Global Health theme, Murdoch Children's Research Institute, Parkville, Victoria, Australia

2. Department of Paediatrics, The University of Melbourne, Parkville, Victoria, Australia

3. Viral Immunology Group, University of Adelaide and Basil Hetzel Institute for Translational Health Research, Adelaide, Australia

4. Precision Medicine Theme Australia, South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia

5. Flinders Health and Medical Research Institute, Flinders University, Adelaide, South Australia, Australia

6. Victorian Infectious Diseases Reference Laboratory, The Royal Melbourne Hospital, The Peter Doherty Institute for Infection and Immunity, Melbourne, Australia

7. Population Allergy, Murdoch Children's Research Institute, Parkville, Victoria, Australia

8. Department of Allergy and Immunology, The Royal Children's Hospital Melbourne, Parkville, Victoria, Australia

9. Paediatric Infectious Diseases Unit, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland

10. Department of Microbiology and Immunology, University of Melbourne at The Peter Doherty Institute for Infection and Immunity, Melbourne, Australia

11. WHO Collaborating Centre for Reference and Research on Influenza, Peter Doherty Institute for Infection and Immunity, Melbourne, Australia

12. Infectious Diseases Unit, The Royal Children's Hospital Melbourne, Parkville, Victoria, Australia

13. on behalf of the BRACE trial,

The primary goal of vaccination is to induce antigen-specific or cross-protective immune responses against a target pathogen. However, several vaccines, particularly live-attenuated vaccines such as Bacillus Calmette-Guerin (BCG), have beneficial off-target effects. With widespread administrations of COVID-19 vaccines, potential off-target immunological effects have important implications for global health. In this sub-study of participants enrolled in an international randomised controlled trial (BRACE trial, NCT04327206)[1], we sought to determine if the adenovirus vector ChAdOx1-S (Oxford-Astra Zeneca) vaccine and modified mRNA BNT162b2 (Pfizer-BioNTech) vaccine have off-target effects on immune responses to unrelated (heterologous) pathogens.

Blood samples were collected from 264 healthcare workers before and 28 days after vaccination with ChAdOx1-S or BNT162b2 vaccine. SARS-CoV-2-specific antibodies were measured by ELISA[2,3] and whole blood cytokine responses following *in vitro* stimulation with specific (irradiated SARS-CoV-2)[4] or heterologous stimuli (e.g. heat-killed (HK) pathogens or TLR agonists) were measured by multiplex bead array.

ChAdOx1-S vaccination was associated with increased cytokine responses to HK *C. albicans* and HK *S. aureus* and decreased cytokine responses to HK *E. coli* and BCG. Cytokines with altered responses included those involved in T cell responses, lymphocyte homeostasis, pro-inflammatory responses, growth factors and chemokines. BNT162b2 vaccination was associated decreased cytokine responses to HK *E. coli* and had variable effects on cytokine responses to BCG and R848. Despite decreased responses to BCG and HK *E. coli* following both vaccines, clusters of cytokines positively correlated with the strongly increased SARS-CoV-2-specific cytokine responses. Direct comparison of the two vaccines also revealed differences in specific and off-target effects of ChAdOx1-S and BNT162b2, manifest as differential cytokine responses, particularly to BCG, HK *E. coli*, and R848 stimulation.

Overall, ChAdOx1-S and BNT162b2 vaccines alter in vitro cytokine responses to unrelated pathogens, indicative of off-target immunological effects. In contrast to the generalized boosting of immune responses to unrelated pathogens proposed for BCG vaccination, off-target effects of ChAdOx1-S and BNT162b2 vaccination are pathogen-dependent and are likely mediated by different mechanisms. These findings have implications for vaccine selection and future vaccine development strategies.

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Influenza vaccine responses to A(H1N1)pdm09 antigens in 2020 and 2021 among repeatedly vaccinated healthcare workers

Stephany Sanchez-Ovando[1], Annette Fox[1], Sheena Sullivan[1], Louise Carolan[1], Kanta Subbarao[1], Jessica Hadiprodjo[1], Arseniy Khvorov[1]

1. Doherty Institute, Pascoe Vale South, VICTORIA, Australia

Background

Repeated administration of influenza vaccines appears to incrementally attenuate immunogenicity and effectiveness, especially when successive vaccines are antigenically similar. Although these effects appear to be worse for A(H3N2), they are also observed for A(H1N1)pdm09, which has shown increasing antigenic diversity in recent years.

Methods

A cohort of Australian health care workers (HCWs) was followed for post-vaccination antibody responses across two years during which influenza did not circulate (2020-2021). Vaccine administered in 2020 contained an A/Brisbane/02/2018-like H1N1 antigen, while in 2021 an antigenically distinct A/Victoria/2570/2019-like antigen was included. Pre-vaccination, 14 days and 7 months post-vaccination sera were assessed in haemagglutination inhibition (HI) assay against influenza A(H1N1)pdm09 vaccine viruses from the corresponding years to assess pre/post vaccination antibody titres. Differences in titre were compared by prior vaccination history.

Results

A total of 1384 HCWs contributed sera in the two years. Among them, 96 were previously unvaccinated (vaccinated in 0/5 prior years) and 778 were frequently vaccinated (≥5/5 prior years). While frequent vaccination attenuated titres and titre rises in both years, the effect was substantially diminished in 2021. Notably, only 16% of frequently vaccinated versus 80% of previously unvaccinated HCWs seroconverted in 2020 versus 80% and 86%, respectively in 2021.

The 2021 vaccine strain differed from all prior H1N1pdm09 vaccines at HA positions N129D, K130N and N156K, which are within prominent antigenic sites. Additionally, only the 2021 vaccine strain had 185I, which was present in seasonal H1N1s. We are currently investigating whether these substitutions facilitated a stronger or more specific immune response through mechanisms such as escape from memory dominance or recall of

memory against prior seasonal strains. Sera are being titrated against viruses from the alternate year, and against reverse genetics viruses bearing single substitutions. PBMC's are being assessed to compare the frequency and phenotype of H1 HA reactive B cells induced.

Conclusions

The H1N1 vaccine antigen used in 2021 induced substantially greater antibody responses than the 2020 antigen, particularly among frequently vaccinated HCW. Investigations are underway to understand how antigenic changes in the 2021 antigen may have enhanced immunogenicity.

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Glycolysis of airway macrophages controls lung homeostasis and responses to aeroallergen

Gesa J Albers[1], Christina Michalaki[1], Amy F Lloyd[2], Patricia P Ogger[1], Simone A Walker[1], Robert Gray[3], John M Halket[3], Linda V Sinclair[2], Cormac McCarthy[4], Timothy S C Hinks[5, 6], Clare M Lloyd[1], Adam J Byrne[1, 4]

1. National Heart and Lung Institute, Imperial College London, London, United Kingdom

2. Cell Signalling and Immunology, Universtity of Dundee, Dundee, United Kingdom

3. School of Health, Sport and Bioscience, University of East London, London, United Kingdom

4. Conway Institute and School of Medicine, University College Dublin, Dublin, Ireland

5. Respiratory Medicine Unit, Nuffield Department of Medicine and National Institute for Health Research Oxford Biomedical Research Centre, University of Oxford, Oxford, United Kingdom

6. Clinical and Experimental Sciences, University of Southampton Faculty of Medicine, Sir Henry Wellcome Laboratories, and the NIHR Southampton Respiratory Biomedical Research Unit, Southampton University Hopsital, Southampton, United Kingdom

The lungs form a highly dynamic microenvironment where airway macrophages (AMs) are key players in regulating the balance between tissue homeostasis and immune activation. Macrophage function is tightly controlled by cellular metabolism, yet it remains unclear how these processes in highly specialised tissue-resident cell populations such as AMs contribute to health and disease. Here, we show that glycolysis is a fundamental pathway that regulates lung homeostasis and responses to inhaled allergen. Employing multi-omics approaches and macrophage-specific targeting *in vivo*, we found that glycolytic activity in AMs was required to restrain T2 responses at homeostasis. Moreover, exposure to a variety of allergens, including the common aeroallergen house dust mite (HDM), drove glycolysis in AMs, and inhibition of glycolytic activity in AMs alleviated HDM-driven pulmonary inflammation. In line with this, we observed profound alterations in the airway metabolites specifically of HDM-sensitised patients with asthma compared to non-HDM sensitised patients or healthy controls. Finally, we found that HDM-driven glycolysis in AMs was dependent on TLR2. Thus, these data highlight a strong link between glycolysis in AMs, AM-mediated homeostatic processes and T2 inflammation in the lungs and propose a dual role for AM glycolysis in mediating lung homeostasis and inflammation. Therefore, our findings suggest that precise modulation of the optimal level of glycolysis is crucial for maintaining lung homeostasis and regulating airway inflammation and opens up new therapeutic avenues for the treatment of respiratory diseases.

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Defining pro-inflammatory antibody effector functions during viral infection

Tyron Esposito[1], Kathrin Eschke[1], Benjamin Liang[2], Dominique Mcconnachie[1], Daniel Watterson[2], Naphak Modhiran[2], Larisa Labzin[1]

1. Institute for Molecular Bioscience, The University of Queensland, St Lucia, QLD, Australia

2. School of Chemistry and Molecular Biology, The University of Queensland, Brisbane, QLD, Australia

Publish consent withheld

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A1 is induced by pathogen ligands to limit myeloid cell death and NLRP3 inflammasome activation

Mary Speir[1, 2], Hazel Tye[1, 2], Timothy A Gottschalk[1, 2], Daniel S Simpson[4, 3], Tirta M Djajawi[1, 2], Pankaj Deo[5], Rebecca L Ambrose[1, 2], Stephanie A Conos[1, 2], Jack Emery[1, 2], Isabella Kong[4, 3], Marco J Herold[4, 3], Jaclyn S Pearson[1, 2, 6], Najoua Lalaoui[4, 3], Thomas Naderer[5], James E Vince[4, 3], Kate E Lawlor[1, 2, 4, 3]

1. Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, VIC, Australia

2. Department of Molecular and Translational Science, Monash University, Clayton, VIC, Australia

3. The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia

4. Department of Medical Biology, The University of Melbourne, Parkville, VIC, Australia

5. Department of Biochemistry and Molecular Biology, Monash University, Clatyon, VIC, Australia

6. Department of Microbiology, Monash University, Clayton, VIC, Australia

Programmed cell death plays an integral role in alerting the innate immune system to a microbial threat and promoting pathogen clearance. Activation of intrinsic apoptosis is widely thought to promote the non-immunogenic phagocytic clearance of infected cells. However, with the recent discovery that apoptotic signalling can crosstalk with the NLRP3 inflammasome[1], it remains to be seen how intrinsic apoptosis is regulated during infection and whether it can induce inflammation. Here, we identify that the pro-survival BCL-2 family member, A1, combines with distinct family members to regulate intrinsic BAX/BAK-mediated cell death in macrophages and inflammatory monocytes upon Gram-negative bacterial lipopolysaccharide (LPS) sensing. We show that due to its tight transcriptional and post-translational regulation, A1 acts as a molecular rheostat to regulate intrinsic apoptosis and the subsequent NLRP3 inflammasome-dependent and -independent activation and release of IL-1β. Importantly, *Neisseria gonorrhoeae*-derived outer membrane vesicles (NOMVs), which are known to damage the mitochondria and trigger intrinsic apoptosis[2,3], also induced A1 expression in inflammatory monocytes to limit cell death and IL-1β activation. In experimental models, the absence of A1 enhanced IL-1β activation to

intraperitoneal NOMV injection and impaired the clearance of the lower respiratory tract intracellular pathogen, *Legionella pneumophila* (Δfla mutant). These results suggest that myeloid cells upregulate A1 expression in response to Gram-negative bacteria to limit early and potentially detrimental cell death and inflammatory responses. With the increasing prevalence of antibiotic resistant bacteria, these findings have implications for the development and deployment of drugs targeting cell death regulators as host-directed antimicrobial therapeutics.

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2. 2. Deo P et al. 2018 Plos Pathogens 14: e1006945
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Interferon epsilon as a novel anti-viral agent in the testis

Rukmali Wijayarathna[1, 2], Eveline de Geus[3], Rosemary Genovese[1], Linden J Gearing[2, 3], Michelle Tate[2, 3], Paul J Hertzog[2, 3], Mark P Hedger[1, 2]

1. Centre For Reproductive Health, Hudson Institute of Medical Research, Melbourne, Victoria, Australia

2. Dept. of Molecular and Translational Sciences, School of Clinical Sciences, Monash University, Melbourne, Victoria, Australia

3. Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Melbourne, Victoria, Australia

Many viruses including Zika, HIV, and SARS viruses can cause persistent infections of the testis. Spermatogenic cells were thought to lack anti-viral protective mechanisms including interferons (IFN) or IFN-stimulated genes (ISGs). Challenging this dogma, we discovered that interferon-epsilon (IFNε), a type-I IFN first identified in female reproductive epithelia, is constitutively expressed by spermatogenic cells and macrophages in mouse and human testes. We also discovered that mice lacking IFNε are more susceptible to Zika virus induced testicular inflammation. In this study, we examined the mechanisms of IFNε-mediated anti-viral defence in the testis.

Sertoli cells constitute the supporting epithelium for spermatogenic cells in the testis. A human Sertoli cell line (HSerc, ScienCell) was infected with Zika virus (MOI 5 or 10). Cultures were treated with 100IU recombinant human IFNε 12h before (prophylactic-IFNε) or 1h after infection (therapeutic-IFNε), or diluent alone (controls). Cells and media were harvested 24h or 48h post-infection for RNAseq, qPCR, and plaque assays.

IFNε treatment increased Sertoli cell anti-viral responses and reduced viral infection, with prophylactic-IFNε being more effective than therapeutic-IFNε treatment. Plaque assays and viral RNA qPCR showed that prophylactic-IFNε reduced viral load by approximately 98%. Therapeutic-IFNε reduced viral RNA by 70% and infectious virus by 97%. Sertoli cells expressed IFNAR1 and 2 receptors required for IFNε signalling. At 24h, both IFNε-treatments significantly increased anti-viral effector genes (ISG15, OAS1, IFI35, RSAD2), reduced induction of pro-inflammatory cytokines (CXCL10, CXCL11), and supported expression of Sertoli cell functional genes (INHA). Notably, anti-viral and inflammatory responses were relatively lower at 48h after IFNε treatment, compared with 24h, attributable to the reduced viral load. Genes for IFNβ and IFNλ, but not IFNα, were induced by the virus at the time points examined.

These data indicate that IFNε induces anti-viral effector responses and reduces inflammatory responses in Sertoli cells, demonstrating the importance of constitutive expression of IFNε in the testis to limit viral infection and inflammatory damage.

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Antibody-mediated enhancement of *Pseudomonas aeruginosa* acute and chronic infections

Emma Ledger[1], Sarah Hickson[1], Johannes Hoehensteiger[1], Wenkang Feng[1], Timothy J Wells[1]

1. Frazer Institute, The University of Queensland , Brisbane, QLD , Australia

Antibodies are well known for their role in protecting individuals against infection, however in certain cases the production of specific antibody can actually promote disease. Antibody-dependent enhancement (ADE) of infection is well described for viral disease; however, antibodies that enhance bacterial disease are relatively unknown. *Pseudomonas aeruginosa* is a bacterial pathogen that is increasingly resistant to last resort antibiotics and new treatments are desperately needed. We previously identified patients that produced a specific antibody that protected their infecting *P. aeruginosa* from killing by the immune system. These 'cloaking antibodies' target lipopolysaccharide on the bacterial surface. Importantly, patients with these antibodies have worse lung function and outcomes that patients with normal complement-killing. Here I'll discuss my recent research into the clincal impact of cloaking antibodies in *P. aeruginosa* bloodstream infections and in people with cystic fibrosis. Recent evidence of a novel mechanism linking these antibodies to inflammation will also be shown.

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Stromal-Immune interactions regulating pulmonary immunity

Clare Lloyd[1]

1. Imperial College, South Kensington, United Kingdom

Many lung diseases are characterized by airway inflammation in conjunction with pathological tissue remodeling. There is growing recognition of the importance of type2 inflammation in tissue healing, with archetypal type2 cells thought to be integral to the tissue repair process. Moreover, the local extracellular matrix influences cell function in vivo, impacting the nature of the ensuing inflammatory response We have determined that this local environment provides directional cues that dictate movement of cells within the lung, and this impacts the course and magnitude of local tissue inflammation. This presentation will focus on the cellular and molecular interactions that govern development of inflammation and repair in the lung.

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Prospects for a vaccine for the prevention of Hepatitis C.

Heidi Drummer[3, 2, 1]

1. Department of Microbiology and Immunology, University of Melbourne, Melbourne, VIC, Australia

2. Microbiology, Monash University, Clayton, VIC, Australia

3. Burnet Institute, Melbourne, VIC, Australia

Hepatitis C causes a chronic infection causing progressive liver damage and cancer, and is transmitted via contaminated blood. Despite the availability of direct acting antivirals that cure hepatitis C infection in >98% of treatments, 1.5 million new infections occur each year, and 58 million people globally live with hepatitis C, including almost 4 million adolescents. To achieve elimination of hepatitis C, a vaccine to prevent infection is required. A vaccine for hepatitis C must provide broad cellular and humoral immunity effective against the 8 genotypes (30% difference at nucleotide level) and at least 86 subtypes (20% difference at nucleotide level) and represents a major challenge. To address this challenge, we have explored the use of recombinant protein, virus like particles, viral vectored vaccines and mRNA to deliver HCV sequences to generate immune responses in small animal studies. Our results suggest that E1E2 uses multiple immune evasion mechanisms to minimise the production of neutralizing antibodies, instead enhancing the production of type-specific and non-neutralizing antibodies. Protein engineering is required to focus and enhance the immune response on broadly neutralizing antibody epitopes. For cellular immunity, delivery of multiple conserved epitopes across the HCV sequence results in the generation of polyspecific and polyfunctional CD4+ and CD8+ T cell responses. Combining B and T cell immunogens into a single vaccine generates broadly reactive neutralizing antibodies and T cell immunity, however, alters antibody specificity. While the development of a vaccine to prevent HCV is theoretically possible, experimentally testing efficacy in humans may be the biggest hurdle to overcome, and likely only be possible using a human challenge model.

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Exploring the female genital tract mycobiome in young South African women using metaproteomics

Tamlyn K Gangiah[1], Arghavan Alisoltani[1], Matthys Potgieter[1], Liam Bell[2], Elizabeth Ross[2], Arash Iranzadeh[1], Zac McDonald[2], Imane Allali[1, 3], Smritee Dabee[1, 4], Shaun Barnabas[1], Jonathan M Blackburn[1], David L Tabb[1, 6, 5], Linda-Gail Bekker[1, 7], Heather B Jaspan[1, 4], Jo-Ann S Passmore[1, 8, 9], Nicola Mulder[1, 10], Lindi Masson[12, 1, 8, 11]

1. Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa

2. Centre for Proteomic and Genomic Research, Cape Town, South Africa

3. Laboratory of Human Pathologies Biology, Department of Biology and Genomic Center of Human Pathologies, Mohammed V University, Rabat, Morocco

4. Seattle Children's Research Institute, Seattle, USA

5. Bioinformatics Unit, South African Tuberculosis Bioinformatics Initiative, Stellenbosch University, Stellenbosch, South Africa

6. DST–NRF Centre of Excellence for Biomedical Tuberculosis Research, Stellenbosch University, Stellenbosch, South Africa

7. Desmond Tutu HIV Centre, Cape Town, South Africa

8. Centre for the AIDS Programme of Research in South Africa, Durban, South Africa

9. National Health Laboratory Service, Cape Town, South Africa

10. Centre for Infectious Diseases Research (CIDRI) in Africa Wellcome Trust Centre, University of Cape Town, Cape Town, South Africa

11. Central Clinical School, Monash University, Melbourne, Australia

12. Burnet Institute, Melbourne, VIC, Australia

Female genital tract (FGT) diseases such as bacterial vaginosis (BV) and sexually transmitted infections are prevalent in South Africa, with young women being at an increased risk. Since imbalances in the FGT microbiome are associated with FGT diseases, it is vital to investigate the factors that influence FGT health. The mycobiome plays an important role in regulating mucosal health, especially when the bacterial component is disturbed. However, we have a limited understanding of the FGT mycobiome since many studies have focused on bacterial communities and have neglected low abundance taxonomic groups, such as fungi. To reduce this knowledge deficit, we present the first large-scale metaproteomic study to define the taxonomic composition and potential functional processes of the FGT mycobiome in South African reproductive-age women. We examined FGT fungal communities present in 123 women by collecting lateral vaginal wall swabs for liquid chromatography-tandem mass spectrometry. From this, 39 different fungal genera were identified, with *Candida* dominating the mycobiome (53.2% relative abundance). We observed changes in differential abundance at the protein, genus, and functional (gene ontology biological processes) level between BV states. In women with BV, *Malassezia* and *Conidiobolus* proteins were more abundant, while *Candida* proteins were less abundant compared to BV negative women. Correspondingly, Nugent score was negatively associated with total fungal protein abundance. The clinical variables, Nugent score, pro-inflammatory cytokines, chemokines, vaginal pH, *Chlamydia trachomatis*, and the presence of clue cells were associated with fungal community composition. The results of this study reveal the diversity of FGT fungal communities, setting the groundwork for understanding the FGT mycobiome.

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A population genomic model for measuring antigenic escape and predicting serotypes for malaria vaccine candidates

Myo T Naung[1, 3, 2, 4], Balu Balan[1, 3], Swapnil Tichkule[1, 3], Andrew J Guy[5], Somya Mehra[2], Somesh Mehra[2], Alison Paolo Bareng[2, 4], Matthew Adams[6], Benson Kiniboro[7], Inoni Betuela[7], Moses Laman[7], Leanne J Robinson[1, 3, 2, 7, 8], Rory Bowden[1, 3], Ivo Mueller[1, 3], Shannon Takala-Harrison[6], Alyssa E Barry[1, 3, 2, 4]

1. Walter and Eliza Hall Institute, Parkville, Victoria, Australia

2. Malaria Elimination, Burnet Institute, Melbourne, Victoria, Australia

3. Department of Medical Biology, University of Melbourne, Melbourne, Victoria, Australia

4. IMPACT/School of Medicine, Deakin University, Geelong, VIC, Australia

5. School of Science, RMIT University, Melbourne

6. Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, Maryland, USA

7. Vector Borne Diseases Unit, Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea

8. Monash University, Melbourne, Victoria, Australia

The design of efficacious malaria vaccines is hindered by the antigen diversity contributing to immune escape. To identify specific antigens and their polymorphisms driving immune escape, we developed a novel population genomic model that quantifies allelic turnover within a host. The approach included massively parallel Nanopore long read targeted sequencing of *Plasmodium falciparum* antigen genes from clinical and asymptomatic infections of two longitudinal paediatric cohorts from Papua New Guinea. Genetic diversity was characterised in a total of 34 genes in 2-4 consecutive infections for each of 240 children resulting in sequence data from 464 *P. falciparum* isolates. To identify immune escape polymorphisms, we applied a stringent variant calling pipeline, and a novel method to compare significant differences in the turnover rate of variant alleles within-hosts compared to that in the general parasite population. Positive hits at known immune escape polymorphisms in *apical membrane antigen 1* (*ama1*) and concordance with population genetic, biochemical, and structural predictions were used to validate our model. Overall, blood-stage antigens had a higher proportion of immune escape polymorphisms (an average of 30%) than antigens expressed in other lifecycle stages (an average of 1%). Filtering the PNG sequence dataset for these polymorphisms allowed a 30-fold reduction of the diversity for each antigen gene converting an average of 124 (range: 6 - 485) genotypes to 4 (range: 1-8) predicted serotypes. Layering this information onto global parasite genomic data to identify the most common serotypes in natural parasite populations will allow recommendations for the formulation of multivalent *P. falciparum* vaccines that may overcome the limited vaccine efficacy associated with high diversity. This provides a framework for improving malaria vaccine design and providing a deeper understanding of infection dynamics and immune escape in malaria.

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Understanding persistence of *Streptococcus pneumoniae* serotype 6B in Mongolia following vaccine introduction

Paige Skoko[1], Laura Boelsen[1], Sam Manna[1, 2, 3], Stephanie Lo[4], Casey Pell[1], Belinda Ortika[1], Jason Hinds[5], Kim Mulholland[6, 2, 7], Tuya Mungun[8], Claire von Mollendorf[6, 2], Stephen Bentley[4], Catherine Satzke[1, 2, 3]

1. Translational Microbiology, Murdoch Children's Research Institute, Parkville, Australia

2. Department of Paediatrics, The University of Melbourne, Parkville, Australia

3. Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Parkville, Australia

4. Parasites and Microbes, Wellcome Trust Sanger Institute, Hinxton, United Kingdom

5. Institute for Infection and Immunity, St George's, London, United Kindgom

6. New Vaccines, Murdoch Children's Research Institute, Parkville, Australia

7. Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, United Kingdom

8. National Centre for Communicable Diseases, Ministry of Health, Ulaanbaatar, Mongolia

Streptococcus pneumoniae (the pneumococcus) is a leading cause of death for children under five years of age. Colonisation of the nasopharynx by the pneumococcus is usually asymptomatic, however, the pneumococcus can disseminate to other anatomic sites to cause diseases such as otitis media, pneumonia, sepsis and meningitis. Pneumococcal conjugate vaccines (PCVs), targeting a subset of pneumococcal capsular types (serotypes), are effective at reducing the disease burden. However, some vaccine-serotypes persist, a phenomenon that is poorly understood, particularly in Asia. Here, we investigate vaccine-serotype persistence post-PCV introduction in Mongolia.

Hospital-based pneumonia surveillance (2015-2021) was conducted across four Mongolian districts to measure the effect of vaccine introduction in 2016. Nasopharyngeal swabs (n=15,183) were collected from children with pneumonia and a subset (n=6,545) were screened for pneumococci and serotyped using DNA microarray. To understand the genetic background of circulating serotypes, we used DNA microarray to infer genetic lineage (using Global Pneumococcal Sequence Cluster, GPSC) and examined lineage changes post-PCV. Using an infant mouse model of disease, we explored differences in colonisation and disease dynamics between lineages, by infecting C57BL/6 mice (five days old) intranasally with pneumococci, testing three isolates per lineage to date.

Overall, vaccine-serotype prevalence reduced post-PCV introduction. However, some individual vaccine-serotypes (6A, 6B, 19F and 23F) persisted and remain a likely cause of pneumonia. Within serotype 6B, GPSC23 was the dominant lineage pre-PCV (16/21, 76% in 2015), which was replaced by GPSC6 (15/18, 83% in 2020) post-PCV, $p<0.0001$. In our mouse model, GPSC23 colonised to a higher pneumococcal density in the nasopharynx than GPSC6 ($p=0.037$) at seven days post-infection. GPSC23 had a higher tendency to transition to disease, with 92% (22/24) of mice becoming moribund compared with 7% (2/28) in the GPSC6 lineage ($p<0.0001$). Tissues were collected from 18 moribund mice, finding GPSC23 had disseminated throughout the body including to the lungs (n=14), blood (n=13), brain (n=13) and middle ear (n=13).

Despite persistence of vaccine-serotype 6B, our data indicates that a lineage replacement occurred, and the emerging lineage may be less virulent. Future experiments will examine the process of lineage replacement and the genetic differences between lineage.

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Towards broad-spectrum henipavirus vaccines & therapeutics: rational design of prefusion F glycoproteins

Ariel Isaacs[1], Yu Shang Low[1], Kyle L Macauslane[1], Joy Seitanidou[1], Cassandra L Pegg[1], Jun J Hen[1], Stacey TM Cheung[1], Benjamin Liang[1], Connor AP Scott[1], Michael J Landsberg[1, 2], Ben L Schulz[1], Keith J Chappell[1, 3, 2], Naphak Modhiran[1], Daniel Watterson[1, 2]

1. School of Chemistry & Molecular Biosciences, University of Queensland, St Lucia, Queensland, Australia

2. Australian Infectious Disease Research Centre, Brisbane, QLD, Australia

3. The Australian Institute for Biotechnology and Nanotechnology, University of Queensland, St Lucia, Queensland, Australia

In August 2022, a novel henipavirus (HNV) named Langya virus (LayV) was isolated from patients with severe pneumonic disease in China. This virus is closely related to Mòjiāng virus (MojV), and both are divergent from the deadly bat-borne HNV members, Nipah (NiV) and Hendra (HeV) viruses. The spillover of LayV is the first instance of a HNV zoonosis to humans outside of NiV and HeV, highlighting the continuing threat this genus poses to global human health. In this work, we make use of the molecular clamp2 technology to stabilize the prefusion form of the LayV and MojV F glycoproteins. We couple this with cryogenic electron microcopy to solve the first high resolution structure of these antigens and perform a glycoproteomic analysis to characterize the glycan profiles. We determined the prefusion structures of MojV and LayV F proteins to 2.66 and 3.37 Å, respectively. We show that despite sequence divergence from NiV, the F proteins adopt an overall similar structure but are antigenically distinct as they do not react to known antibodies or sera. Glycoproteomic analysis revealed that while LayV F is less glycosylated than NiV F, it contains a glycan that shields a site of vulnerability previously identified for NiV. We leveraged this information to design a trivalent prefusion F HNV vaccine, and demonstrated broad-reactivity against several emerging HNVs. Concurrently, we isolated novel human-derived antibodies that target LayV F and G proteins and offer cross-reactivity to MojV. Our findings shed light on the structures and antigenic profiles of major vaccine targets of LayV and MojV. Our results carry implications for broad-spectrum HNV vaccines and therapeutic development, and we demonstrate that a multivalent HNV approach is likely required to combat emerging viruses.

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Specific gut microbiota modulates intestinal epithelial cell signalling and inflammation

Michelle Chonwerawong[1, 2], Gemma DAdamo[1, 2], Linden J Gearing[1, 2], Vanessa Rossetto Marcelino[3, 4], Jodee A Gould[1, 5], Emily L Rutten[1, 2], Trevor J Wilson[5], Tamblyn Thomason[1, 2], Emily L Gulliver[1, 2], Kitty McCaffrey[1, 2], Eva Chan[1, 2], Garrett Ng[1, 2], Paul J Hertzog[1, 2], Edward M Giles[1, 6], Samuel C Forster[1, 2]

1. Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, VIC, Australia

2. Department of Molecular and Translational Sciences, Monash University, Clayton, VIC, Australia

3. Melbourne Integrative Genomics, The Univerity of Melbourne, Melbourne, VIC, Australia

4. Department of Microbiology and Immunology, Peter Doherty Institute, Melbourne, VIC, Australia

5. MHTP Medical Genomics Facility, Hudson Institute of Medical Research, Clayton, VIC, Australia

6. Department of Paediatrics, Monash Health, Clayton, VIC, Australia

The gastrointestinal microbiome plays key roles in the development and modulation of innate immune responses. Despite these known associations, mechanistic understanding of the host-microbiota interactions in the gut remains poorly understood. This greatly limits our ability to functionally define the role of the microbiome in heterogenous diseases associated with the gut such as inflammatory bowel disease (IBD). We performed site-specific bacterial culturing on patient intestinal biopsies (IBD and control) and integrated this culture-dependent approach with bacterial metagenomic and host transcriptional analysis. We found that specific bacterial clades that were closely related phylogenetically, were associated with distinct host inflammatory signatures. Co-culturing these bacteria with Caco2 epithelial cells and colonic organoid monolayers demonstrated a clade-specific cell death phenotype associated with the unfolded protein response (UPR) and endoplasmic reticulum (ER) stress pathways which are signalling processes known to be associated with regulation of epithelial cells and intestinal immunity implicated in IBD. This phenotype was dependent on direct bacterial-cell contact with Caco2 cells; however, the response was abrogated upon treatment of cells with heat-killed bacteria. In addition, we demonstrated that the cytokine IL-6, was induced following stimulation of Caco2 cells with clade-specific bacteria. Cell-free conditioned media from bacterial-stimulated epithelial cells also resulted in transcriptional modulation of ER stress pathways in Caco2 cells suggesting that there may be microbial and host mediators previously unknown that act to regulate epithelial cell death and stress signalling associated with intestinal inflammatory responses. Given that there are significant knowledge gaps in the pathophysiology of IBD and the microbiome, a comprehensive understanding of how specific bacteria modulate host cell responses will provide insight into the development of targeted microbial-based treatments for IBD and for microbiome-related diseases beyond the gut.

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Building a Pipeline of High-Impact Products to Prevent, Diagnose and Treat Bacterial Infections

Erin Duffy[1]

1. CARB-X, Boston University, USA

The emergence of antibiotic-resistant bacteria is not a new topic; however, the urgency to discover and develop new high-impact products to prevent, diagnose and treat infections caused by them is high. In 2022, the world learned of the global burden of antibiotic-resistant bacteria from a paper published in the Lancet: in a single year (2019), 1.27 million deaths were directly attributable to infections caused by antibiotic-resistant bacteria, while an estimated 4.95 million deaths were associated with it. However, the visible clinical and preclinical pipeline of products to keep pace with the emergence of antibiotic-resistant bacteria is thin. CARB-X, a sponsored program at Boston University, is a global biopharmaceutical accelerator charged with building and supporting a pipeline of discovery and early-development projects and products of potential high-impact therapeutics, preventatives and diagnostics in this arena. Funded by four international governments (US, UK, DE and CA) and three foundations (the Wellcome Trust, the Bill and Melinda Gates Foundation and the Novo Nordisk Foundation), CARB-X is expected to deliver compelling products, primed for advanced development, for the infections (and bacteria that cause them) where the burden of mortality and morbidity are highest. The CARB-X model features non-dilutive funding and a novel support model that extends the project team to include the expertise necessary to advance a program from early-stage discovery and into clinical trials. From 1,400 initial applications, CARB-X has selected and accelerated 97 individual programs, covering traditional antibiotic programs, non-traditional treatment and prevention programs, vaccines and rapid diagnostics. After 6 full years of operation, CARB-X has initiated or completed 14 first-in-human studies for treatment and prevention, with several studies ongoing. Two of these programs are in advanced clinical-development studies. Two diagnostics have obtained CE marking, indicating that they are deemed to meet EU health, safety and environmental protection standards; 3 additional diagnostics are conducting clinical trials.

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Antimicrobial resistance (AMR)

Trevor Lithgow[1]

1. Monash University, Clayton, VIC, Australia

Antimicrobial resistance (AMR) is a global threat and, while the situation in Australia is dire with 1031 deaths directly attributable to AMR in 2020, globally things are far worse. Within the 4.95 million deaths associated with AMR globally, a terrible irony is that the Indo-Pacific region includes many of the countries most affected by AMR (India, Bangladesh and China) and the country so far least affected (Australia). Low investment stands in the way to sustainable solutions to the AMR crisis e.g. US venture capital invested 16X more into oncology versus antimicrobial drugs; MRFF invested less than 1% of its budget into AMR solutions. This, despite numerous innovations and solutions ready for deployment.

We have been working from the proposition that AMR is building in our environments: homes, workplaces, schools, and only thereafter is found in our healthcare settings. We will present data from surveys of the run-off into waterways around Melbourne that shows AMR pathogens such as *Klebsiella* are easily cultivated. In partnership with Traditional Owners, we have used the same water sources to isolate new bacteriophages (phages) and extensive analysis shows that these phages can be readily evolved to broaden their host range (i.e. to kill more types of Klebsiella) and can be selected to have extended shelf-life. These versatile phages and the antibacterial products that can be derived from them offer prospects for sustainable solutions in decolonizing patients, treating drug-resistant infections and decontaminating clinical equipment such as ventilators.

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Cracking the Gram-negative cell envelope to revive otherwise ineffective antibiotics

Yaoqin Hong[1, 2], **Wee Boon Tan**[3], **Zhe Hu**[4], **Jilong Qin**[1], **Shu Sin Chng**[3], **Makrina Totsika**[1, 2]

1. Centre for Immunology and Infection Control, QUEENSLAND UNIVERSITY OF TECHNOLOGY, Herston, Queensland, Australia

2. Max Planck Queensland Centre, QUEENSLAND UNIVERSITY OF TECHNOLOGY, Herston, Queensland, Australia

3. Department of Chemistry, National University of Singapore, Singapore

4. South China Agricultural University, Guangzhou, Guangdong, China

The bacterial cell envelope is a crucial defense mechanism, shielding bacteria from external stress, aiding nutrient acquisition, energy generation, toxin expulsion, and cell division coordination. It is not only a validated drug target but also a central player in acquired and intrinsic antibiotic resistance. In Gram-negative bacteria, this envelope consists of three successive layers: an inner phospholipid membrane, a rigid peptidoglycan layer, and an outer membrane (OM), which is an asymmetric bilayer of phospholipid and lipopolysaccharide (LPS). The OM acts as a formidable barrier, excluding antibiotics and rendering infections challenging to treat. Understanding the synthesis and maintenance of this complex envelope is pivotal for disrupting its function, rendering the organism susceptible to otherwise ineffective treatments, or ultimately killing the bacterium (1).

Fatty acids serve as the fundamental building blocks for structural membrane lipids, and their synthesis presents an attractive antimicrobial target, given its distinct pathways in prokaryotes and eukaryotes. Previously, we identified FabH, a component of fatty acid synthesis, as the gatekeeper of OM barrier function (2). In the absence of FabH, Gram-negative bacteria become vulnerable to antibiotics that were previously ineffective and can be resensitized to last-resort antibiotics. Our research focuses on refining antimicrobials that target the fatty acid pathway. We delve into the nature of antibiotic hypersensitivity in *fabH* null mutants, both in laboratory K-12 and clinical multi-drug resistant *Escherichia coli* strains (2). In this work, we reveal that the compromised cell envelope is not solely due to a lack of fatty acids, but instead, triggered by the specific types of fatty acids deemed unsuitble to construct LPS to sufficiently fill into the outer leaflet of the OM. Furthermore, the poor OM quality is substantially reversed through dismantling cationic lateral intermolecular LPS interactions, OM asymmetry maintenance or enhanced acetyl-CoA pool. Our work supports a balance model for the criticality of structural lipids across both faces of the OM, highlighting its essential role in bacterial survival and the enigmatic evolutionary trajectory in prototypical OM development. Our research offers valuable insights into how to effective disrupting defence of Gram-negative bacteria, potentially leading to more effective drug discovery strategies.

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Exogenous arginine enhances the diagnosis of 'undetectable' polymyxin-dependent *Acinetobacter baumannii*

Meiling Han[2, 1], **Yasser Alssadi**[2, 1], **Jinxin Zhao**[2, 1], **Yan Zhu**[2, 1], **JING LU**[2, 1], **Xukai Jiang**[2, 1], **Rhys Dunstan**[2, 1], **Anton Le Brun**[3], **Christopher Barlow**[2, 1], **Phillip Bergen**[2, 1], **Hsin-Hui Shen**[2, 1], **Trevor Lithgow**[2, 1], **Darren Creek**[4, 5], **Tony Velkov**[1], **Jian Li**[1]

1. Faculty of Medicine, Nursing & Health Sciences, Monash University, Melbourne, VIC, Australia

2. Monash University, Clayton, Melbourne, VIC, Australia

3. Australian Nuclear Science and Technology Organisation, Sydney, NSW, Australia

4. Monash University, Parkville, VIC, Australia

5. Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, VIC, Australia

Polymyxins are last-line antibiotics against the top-priority pathogen *Acinetobacter baumannii*, yet polymyxin-dependent resistant isolates have emerged. These unique strains are unculturable on agar plates without polymyxins, escaping the current clinical diagnosis procedure and causing treatment problem. In the present study, we integrated multi-omics (*incl.* genomics, transcriptomics and metabolomics) to examine the mechanism of polymyxin-dependent growth of *A. baumannii* and discovered that arginine metabolism is a critical factor. Specifically, the arginine degradation pathway was significantly altered in polymyxin-dependent strains compared to wild-type strains, with critical metabolites (*e.g.*, L-arginine and L-glutamate) severely depleted and the expression of *astABCDE* operon significantly increased. Our stable isotope labelling study with $^{13}C_6$-L-arginine

as the sole carbon source demonstrated significantly higher uptake of L-arginine and faster generation of L-glutamate in polymyxin-dependent strain AB5075D compared to the wild-type strain AB5075S, indicating increased metabolic activity due to arginine supplementation which led to suppressed polymyxin dependence. Deletion of the first gene *astA* in the arginine degradation pathway of AB5075D produced similar effects to arginine supplementation, namely substantial growth enhancement and decreased polymyxin dependence, with the downstream genes *astB/D/E* and related metabolites (*e.g.*, N^2-succinyl-L-arginine, N^2-succinyl-L-ornithine and L-ornithine) significantly depleted. Our membrane lipidomics results showed decreased phosphatidylglycerol (PG) proportion and increased phosphatidylethanolamine (PE) proportion in the outer membrane (OM) of the *astA* deletion mutant AB5075D^{DastA} compared to its parent AB5075D. The downregulation of *pldA* (encoding an OM phospholipase) and decreased abundance of lysoPE and *sn*-glycerol-PE due to *astA* deletion supported the increased PE level in the OM of AB5075D^{DastA}. Our molecular dynamics simulations and neutron reflectometry studies showed that the above membrane lipid change decreased the interaction with polymyxins. Overall, this study elucidates the molecular mechanism of how arginine metabolism impacts polymyxin-dependence in *A. baumannii* and provides critical knowledge to improve the diagnosis and treatment of 'undetectable' polymyxin-dependent *A. baumannii* in patients.

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Distribution of phenotypic antimicrobial resistance in the gastrointestinal microbiome.

Emily L Gulliver[1, 2], Jodee A Gould[1, 2], Emily L Rutten[1, 3], Linden J Gearing[1, 2], Wei Khoo[1, 3], Sean M Solari[1, 2], Remy B Young[4, 1], Jamia Hemphill[4, 1], Tamblyn Thomason[1, 2], Margarette Williamson[4, 1], Samuel C Forster[1, 2]

1. Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Victoria, Australia

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

3. Department of Molecular and Translational Science, Monash University, Clayton, Victoria, Australia

4. Department of Microbiology, Monash University, Clayton, VIC, Australia

In 2019, 4.95 million deaths were associated with antimicrobial resistance (AMR) worldwide. The human gastrointestinal microbiome has been identified as a source of acquired resistance in pathogens. Previously, culture-independent metagenomics was the primary method for identifying antimicrobial resistance in gastrointestinal microbiota. However, this approach cannot identify novel AMR genes or determine their expression or functionality within the cell. To overcome these limitations, our study utilised novel culturing on YCFA media in anaerobic conditions coupled with microbial genomics to identify phenotypic antibiotic resistance in the microbiome. This method was used to culture 10 faecal samples from healthy individuals, with and without six common orally administered antimicrobials (Amoxicillin, Amoxicillin-Clavulanic acid, Cefalexin, Ciprofloxacin, Clindamycin and Doxycycline). The resultant 1058 colonies were picked, identified through 16S rRNA sequencing and resistance to each of the antimicrobials was confirmed in broth growth. Genes associated with resistance or sensitivity to the antimicrobials were identified through combining whole genome sequencing data and phenotypic (growth in broth) data of 98 isolates. There were 2050 genes associated with resistance to Amoxicillin-Clavulanic acid, 310 genes associated with resistance to Ciprofloxacin, 220 genes associated with resistance to Clindamycin, one gene associated with resistance to Doxycycline and no genes found to be associated with resistance to Amoxicillin or Cefalexin resistance. Of the genes associated with resistance, four are known antimicrobial resistance genes, including the Clindamycin resistance gene *ermF*. Using this as a positive control, the putative Clindamycin resistance genes NADPH-flavin oxidoreductase and an operon containing SAM-methyltransferase and *tetR* were cloned into expression vectors in *E. coli* to determine difference in MIC. In this study advanced culturing coupled with microbial genomics has been used to identify putative, uncharacterised, antimicrobial resistance genes.

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