CONTENTS

WELCOME FROM THE CONFERENCE CO-CONVENORS

Dear Colleagues and Friends,

On behalf of the organising committee, we welcome you to our 13th Lorne Infection & Immunity conference. We are delighted by 13 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 innate or adaptive immunity, who study the mechanisms that regulate immune responses determining the fate of infection, and who apply this knowledge to preventing and treating 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 of the past decade. 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 for this field of science enables us to hold this meeting each year. In this difficult economic climate, we are especially appreciative of your investment in this conference. This 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 13 years and we are delighted to welcome Moderna to Australia and this meeting.

We thank each of the conference's subcommittees, detailed on the following pages of this program. They have each worked tirelessly to bring this meeting about. The Program Committee, co-chaired by Dr Michelle Boyle and Dr Cameron Stewart, have led the development of the conference's program, selection of invited speakers, presentations from abstracts, and the meeting's overall structure. The Development Committee takes responsibility for its strategic direction, budget and sponsorship and registration targets. The Student and ECR Committee, chaired by Dr Andrew Currie and Deputy Chair, Prof Justine Mintern, have developed a new program of activities for the conference's students and ECRs. Thanks go to Dr Ebony Monson and Mahtab Eivazitork, the co-chairs of the student and ECR sub-committee, and all members of this team, who have organised the student / ECR pizza night, networking lunch and other activities. Each of these committees continues to be supported by our Program Manager, Dr Rebecca Smith and ASN's Gemma-Ann Taylor, Lek Krongsing, Gabrielle Dyson and team. Thanks to you all.

With best wishes,

Heidi Drummer and Richard Ferrero, Lorne Infection and Immunity Conference Co-Convenors

WELCOME FROM THE PROGRAM CHAIRS

Dear Colleagues and Friends,

We are pleased to welcome you to our 13th 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 today.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.

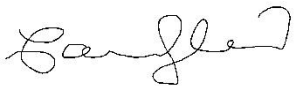
In developing the program for 2023 we built on the tradition of the Lorne Infection and Immunity conference in bringing together researchers working at the interface of microbiology and immunology. We have also developed sessions around emerging themes such as pandemic preparedness, clinical translation and the impact of climate change on infectious disease research. We hope you enjoy these emerging topics and invite 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 international visitors who are here with us in person. 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 is with mixed feelings that we make the following special announcement. Prof Heidi Drummer, co-convenor of six Lorne Infection and Immunity Conferences (2018 – 2023) and the Victorian Infection and Immunity Network since 2017, will be stepping down from these roles at the conclusion of this meeting. A debt of thanks goes to Heidi for her diligence, unflappable demeanor and wise guidance of the VIIN and Lorne conferences for these many years. We are particularly grateful to her for her championship of diversity, equity and accessibility in our activities, as well as her steady hand in leading the development of new processes to help streamline and make more transparent our operations. All of these things have helped shape VIIN and the Lorne I&I meeting into places where all scientists can feel welcome and valued, which is a wonderful legacy to leave in a professional contribution, as well as in life more generally.

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 now pass on the baton to Begona Heras and Makrina Totsika to chair the Program committee for the 2024 conference and stand ready to help them in any way we can.

Kind regards,

Michelle Boyle 

Michelle Boyle and Cameron Stewart

ORGANISING COMMITTEE 2023

CO-CONVENORS

Heidi Drummer Burnet Institute	**Richard Ferrero** Hudson Institute of Medical Research

PROGRAM COMMITTEE

PROGRAM CHAIRS	COMMITTEE MEMBERS
Michelle Boyle, QIMR Berghofer Medical Research Institute **Cameron Stewart,** CSIRO Australian Animal Health Laboratory	**Begoña Heras,** La Trobe University **Matt Johnansen,** Centenary Institute **Si Ming Man,** Australian National University **Eugene Maraskovsky,** CSL Ltd **Nicole Moreland,** The University of Auckland **Wai-Hong Tam,** Walter and Eliza Hall Institute **Makrina Totsika,** Queensland University of Technology **Ana Traven,** Monash University **Daniel Utzschneider,** Peter Doherty Institute for Infection and Immunity, University of Melbourne

STUDENT & ECR COMMITTEE

CHAIR	COMMITTEE MEMBERS
Andrew Currie, Murdoch University **DEPUTY CHAIR** **Justine Mintern,** University of Melbourne	**Greg Moseley,** Monash University **Danny Wilson,** University of Adelaide **ECR COMMITTEE MEMBER** **Ebony Monson,** La Trobe University **STUDENT COMMITTEE MEMBER** **Mahtab Eivazitork,** University of Melbourne **SUB-COMMITTEE OF STUDENTS AND ECRS** **Ebony Monson,** La Trobe University (Co-Chair) **Mahtab Eivazitork,** University of Melbourne (Co-Chair) **Shoaib Anwaar,** University of Queensland **Muhammad Ikhtear Uddin,** Monash University **Wen Shi Lee,** The University of Melbourne **Akachukwu Onwuka**, The University of Melbourne **Kevin John Selva,** University of Melbourne, Doherty Institute **Modhusudon Shaha,** Monash University **Catherine Tsai,** University of Auckland

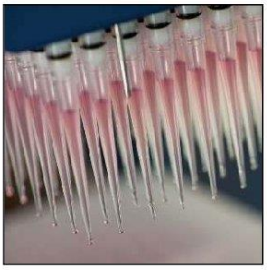
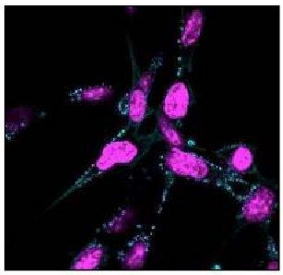
BUSINESS DEVELOPMENT COMMITTEE

CHAIRS	COMMITTEE MEMBERS
Heidi Drummer, Burnet Institute **Richard Ferrero,** Hudson Institute of Medical Research	**Antje Blumenthal,** The University of Queensland Diamantina Institute **Darren Creek,** Monash Institute of Pharmaceutical Sciences **Philip Hansbro**, Centenary Institute, University of Technology Sydney, Hunter Medical Research Institute and The University of Newcastle

Victorian Infection & Immunity Network

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

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


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

VIIN Contributors include:

WEHI brighter together

MONASH University

THE UNIVERSITY OF MELBOURNE

murdoch children's research institute

Burnet Institute Medical Research. Practical Action.

HUDSON INSTITUTE OF MEDICAL RESEARCH

Doherty Institute A joint venture between the University of Melbourne and Melbourne Health

LA TROBE INSTITUTE FOR MOLECULAR SCIENCE

LA TROBE UNIVERSITY

VIDRL Victorian Infectious Diseases Reference Laboratory

DEAKIN UNIVERSITY

CSIRO

RMIT UNIVERSITY

For enquiries contact: info@viin.org.au

INVITED SPEAKERS

INTERNATIONAL INVITED SPEAKERS

PROF MARYLYN ADDO, *University Medical Center Hamburg-Eppendorf*

Prof. Addo studied Medicine at the University of Bonn with study years abroad in Strasbourg, France and Lausanne, Switzerland (Erasmus exchange program). After completing a Master's Degree in "Applied Molecular Biology of Infectious Diseases" (MSc AMBID, on a DAAD scholarship) and the Diploma in Tropical Medicine and Hygiene (DTM & H) at the London School for Hygiene and Tropical Medicine, she pursued postdoctoral research years with focus on HIV Immunology at the Massachusetts General Hospital (MGH)/Harvard Medical School (HMS) in Boston, USA, funded through the DFG Emmy Noether program. After completing her specialist training at MGH and Brighams and Womens Hospital in internal medicine (2007) and infectious diseases (2010), she worked as staff attending physician in infectious diseases at the MGH until 2013. From 2010-2013 she also worked as a PI and research group leader at the Ragon Institute of MGH, MIT and Harvard and as an Assistant Professor in Medicine at Harvard Medical School. Since her appointment as first Professor of the German Center for Infection Research (DZIF) (W2 Emerging Infections) in 2013, Professor Addo has headed a translational research program on virus immunology and vaccine development for "Emerging Infections" at the University Medical Center Hamburg-Eppendorf (UKE). In 2017 she was appointed to the W3 professorship of Infectious Diseases at the UKE and is currently Head of the Infectious Diseases Division at the UKE Department of Internal Medicine. Since January 2022 she serves as the Founding Director of the new Institute for Infection Research and Vaccine Development (IIRVD) at the UKE. Her research group focuses on immunity to infectious diseases and early vaccine development of vaccines against emerging or re-emerging pathogens such as Ebola, MERS and now SARS-CoV-2. She lives in Hamburg, Germany and has two children.

PROF JOANNE FLYNN, *University of Pittsburgh School of Medicine*

Prof JoAnne Flynn has a Bachelor of Science in Biochemistry, from the University of California at Davis and a PhD from University of California at Berkeley in Microbiology and Immunology. Dr. Flynn's first post-doc was with Dr. Magdalene So at the Scripps Clinic Research Institute and then a Howard Hughes Research Associate with Dr. Barry Bloom at Albert Einstein College of Medicine where she began her studies in tuberculosis. Dr. Flynn joined the Department of Microbiology and Molecular Genetics at the University of Pittsburgh School of Medicine in 1994 and in 2019 was awarded the title of Distinguished Professor. Dr. Flynn directs a NIH T32 Training Program and has multiple grants from NIH and the Gates Foundation. She is a current Section Editor for PLoS Pathogens and former member of the NIH NIAID Board of Scientific Counselors. She served as a Councilor for the American Association of Immunologists and as President in 2018. Dr. Flynn is a Fellow of the American Academy of Microbiologists and a Distinguished Fellow of the American Association of Immunology. She has published over 200 papers. Dr. Flynn won the University of Pittsburgh School of Medicine Distinguished Mentor Award in 2018 and Distinguished Research Award in 2019. Her research in tuberculosis is focused on immunology, host-pathogen interactions, vaccines, and drugs, and she has developed and used non-human primate models for TB research for more than 20 years. Dr. Flynn's research uses cutting-edge tools and technologies to investigate the complexities of Mycobacterium tuberculosis infection, with a particular focus on lung and lymph node granulomas, vaccines and treatments.

PROF SHABAANA KHADER, *Washington University in St Louise*

Prof Shabaana has broad background in Immunology, with specific training and expertise in the area of pulmonary inflammation and mucosal host defense, specifically in the field of mycobacterial infections. She has published several key findings in the area of immunity to tuberculosis in high impact journals such as Nature, Nature Immunology, Nature Microbiology, Science Translational Medicine, Immunity, Journal of Experimental Medicine, Journal of Clinical Investigation, Nature Communications, American Journal of Respiratory Medicine and Critical Care Medicine, and Mucosal Immunology, and these studies have been heavily cited in the literature. Her lab has been a pioneer in determining the parameters that mediate protective vaccine-induced immunity against TB. For example, her study describing a novel role for the cytokine Interleukin-17 in generating vaccine-induced immunity against tuberculosis that was published in Nature Immunology, has been cited over 1400 times. In the prior funding period of this R01, work from her lab has demonstrated that Th17 vaccine responses are critical for vaccine-induced immunity against TB, considerably changing the landscape of TB vaccine development. In addition, over the past 15 years her team have identified the immune factors necessary for Th17 mediated TB vaccine responses and delineated the protective-dependent mechanisms that contribute to Mtb control, and delineated drug resistance mutations that modulate host immune responses.

As evident from her publications and funding record, her lab has a long, strong and successful history of collaborating with leaders in the field of lung and vaccine biology, mucosal immunology and host immune responses. In addition, she is deeply committed to training and mentoring the next generation of scientists and faculty working in the research area of infectious diseases in general, and HIV/TB in particular.

PROF MARION KOOPMANS, *Department of Viroscience*

She is the head of the Viroscience department of Erasmus MC, a Centre of Excellence in Virology. Her research focuses on unravelling the modes of transmission of viruses among animals and between animals and humans, and the use of pathogenic genomic information to unravel these pathways and to signal changes in transmission or disease impact.

She is PI of VEO, a large H2020 funded (16Meuro) project aiming to create a Versatile Emerging infectious disease Observatory for the generation and distribution of high-quality actionable information for evidence-based early warning, risk assessment and monitoring of emerging infectious diseases (EIDs) and antimicrobial resistance (AMR) (https://www.veoeurope.eu) and scientific director of the newly established PDPC (https://convergence.nl/nl/pandemicdisaster-preparedness-center/)

As an expert advisor for the WHO on foodborne diseases and emerging disease outbreaks, she serves as expert on the WHO emergency committee on COVID-19, the One Health High Level Expert Panel (OHHLEP). She is director of a WHO collaborating centre for emerging viral diseases and a driving force of the scientific advisory group for the WHO R&D Blueprint for action to prevent epidemics by fast-tracking the availability of effective tests, vaccines and medicines. She is head of the designated National Reference Laboratory for high threat viral pathogens for the European Commission. She has published >550 papers in international format and is active in science advocacy.

PROF ANNETTE OXENIUS, *Institute of Microbilogy*

Prof. Annette Oxenius, PhD, received her university degree in biochemistry and molecular biology at the University of Zurich in 1993 and in 1997 she completed her PhD in immunology at the Institute of Experimental Immunology at ETH Zurich. After a postdoc at the University of Oxford, UK, she was elected assistant professor for immunology at the Institute of Microbiology of the ETH in 2002, was promoted to associate professor in 2007 and to full professor in 2012. Currently she is the Head of the Department of Biology at ETH Zurich. Her research focuses on the regulation of immune responses in the context of acute and persistent viral infections in experimental mouse models.

A/PROF LISA WAGAR, *University of California Irvine*

Lisa Wagar is an Assistant Professor in the Department of Physiology & Biophysics at the University of California Irvine. Dr. Wagar earned her PhD from the University of Toronto and completed a postdoctoral fellowship at Stanford University in Dr. Mark Davis's lab. The foundation of her lab's research is investigating how the specialized microenvironment of lymphoid tissues regulates antigen-specific adaptive immune responses. Dr. Wagar's group uses an immune organoid model derived from primary human lymphoid tissues to study how a variety of host factors and antigen factors contribute to inter-individual differences in vaccine responses. The long-term goal is to use leverage this information for improved and accelerated vaccine design.

PROF HAO WU, *Harvard Medical School and Boston Children's Hospital*

Hao Wu is Asa and Patricia Springer Professor at Harvard Medical School and Boston Children's Hospital, and an elected member of the US National Academy of Sciences and the American Academy of Arts and Sciences. The Wu laboratory of structural and mechanistic immunology delineates the assembly of supramolecular complexes in immunity, primarily focusing on innate immune pathways, in particular the inflammasome pathway. Her critical research provides new opportunities for drug targeting by therapeutic intervention of supramolecular complexes. Her work has received a number of honors including Dorothy Crowfoot Hodgkin Award from the Protein Society, Seymour & Vivian Milstein Award for Excellence in Interferon and Cytokine Research from the International Cytokine and Interferon Society, and William B. Coley Award for Distinguished Research in basic and tumor immunology from the Cancer Research Institute. She serves on the Scientific Advisory Council of the Cancer Research Institute and the Editorial Board of Science and Cell.

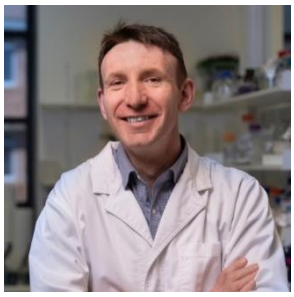
NATIONAL INVITED SPEAKERS

DR KATIE ANDERS, *World Mosquito Program*

Dr Katie Anders is an epidemiologist and the Director of Impact Assessment at the World Mosquito Program, based at Monash University. In this role she leads a program of epidemiological and implementation research to demonstrate the effectiveness, durability, cost-effectiveness and scalability of WMP's Wolbachia mosquito replacement method for control of dengue and other Aedes-borne viruses. She has previously worked at the Oxford University Clinical Research Unit in Ho Chi Minh City, Vietnam, where her research was focussed on the epidemiology of dengue and other viral infections in young children, and prior to that in infectious disease surveillance at the (former) UK Health Protection Agency in London.

PROF JULIE BINES, *Murdoch Children's Research Institute*

Professor Julie Bines is Professor of Paediatrics at the University of Melbourne, a Paediatric Gastroenterologist at the Royal Children's Hospital Melbourne and leads the Enteric Disease Group at Murdoch Children's Research Institute. Professor Bines has focused on the development of the human neonatal rotavirus vaccine, RV3-BB vaccine, aimed at preventing rotavirus disease from birth in infants worldwide. She has led clinical trials in Australia, New Zealand, Indonesia and Malawi. Professor Bines is Director of the WHO Collaborative Centre for Child Health and the WHO Rotavirus Regional Reference Laboratory for the Western Pacific Region and leads the Australian Rotavirus Surveillance Program. In response to the CVID pandemic Professor Bines has explored the role of SARS-CoV-2 infection of the gut and in the potential for wastewater and environmental surveillance of potential pathogens of global significance including SARS-CoV-2, typhoid.



PROF IAN COCKBURN, *Telethon Kids Institute*

Ian Cockburn received his PhD from the University of Edinburgh in which he discovered a new malaria resistance gene among individuals in Papua New Guinea. In 2004 he moved to Johns Hopkins University where his post-doctoral work focused on CD8+ T cells and their ability to kill malaria parasites in the liver. HIs contributions include the first intravital imaging of pathogen killing in vivo, which paved the way for the identification of tissue resident T cells in the liver as major mediators of protection against malaria. Ian established his laboratory at the Australian National University in 2013 where he established a new program of research on B cell responses to malaria. Key achievements have been the biophysical analysis of antibody binding to the circumsporozoite protein, and the identification of the factors that regulate memory responses to malaria vaccines.

DR AMY CHUNG, *The University of Melbourne*

Dr Amy Chung leads the Systems Serology laboratory at the Department of Microbiology and Immunology, University of Melbourne at the Doherty Institute. Dr Chung completed her PhD at the University of Melbourne in 2011 and was awarded an NHMRC CJ Martin, American Australian Fellowship, and MGH Discovery Fellowship) to conduct a postdoc at the Ragon Institute of MGH, MIT and Harvard. She returned to the University of Melbourne, Peter Doherty Institute in 2015 and was awarded an NHMRC CDF and amfAR Mathilde Krim II Fellowship (only Australian ever awarded) that helped her to establish her own laboratory in 2019. She is currently an NHMRC EL2 Investigator and Dame Kate Campbell Fellow. Her research focuses upon the application of cutting-edge experimental technologies, combined with advanced computational analysis to holistically examine functional antibodies against a range of infectious diseases including HIV, Mycobacterium Tuberculosis, Malaria and COVID-19.

A/PROF NADEEM KAAKOUSH, *The University of New South Wales*

A/Prof Nadeem Kaakoush leads the Host-Microbiome Interactions group in the School of Biomedical Sciences at the University of New South Wales. He completed his doctoral studies in pathology (infectious diseases) and was invited to continue his work at the Institut Pasteur in France through a trainee fellowship. He was then awarded a NHMRC ECF to investigate the role of the microbiota in inflammatory bowel diseases. In 2016, he received a Cancer Institute NSW CDF to establish his own group investigating the human gastrointestinal microbiome. In 2019, he was awarded a UNSW Scientia appointment and his current research focuses on the microbiome in an array of gastrointestinal diseases, and methods to manipulate the microbiome to improve health outcomes.

PROF ALEXANDER KHROMYKH, *The University of Queensland*

Prof Alexander Khromykh has a PhD degree in Molecular Virology. He worked as a Postdoctoral Fellow at the University of Ottawa in Canada and then joined Sir Albert Sakzewski Virus Research Centre in Brisbane and became a laboratory Head. He was awarded NH&MRC Senior Research Fellowship and moved his laboratory to University of Queensland to take up an appointment with the School of Chemistry and Molecular Biosciences (SCMB) where he holds a Professor of Virology position.

He was the founding Director of SCMB's Centre for Infectious Disease Research and is currently the Deputy Director of the Australian Infectious Diseases Research Centre and the Director of Centre of Excellence in the Global Virus Network. His research interests include molecular mechanisms of replication of positive-strand RNA viruses (primarily West Nile, Zika and SARS-CoV-2 viruses), virus-host interactions and vaccine development. He has authored >150 publications including papers in Science, Nature Biotechnology, Nature Microbiology, Science Translational Medicine and 5 book chapters.

DR GLENN MARSH, *CSIRO VIC*

Dr Glenn Marsh is a Principal Research Scientist within the Dangerous Pathogens Team at CSIRO based at the Australian Centre for Disease Preparedness in Geelong. Glenn has a strong interest in working with pathogens that require high levels of containment and is trained to work with risk group pathogens in a PC4 laboratory.

Glenn's research areas include development of animal models for a range of zoonotic human pathogens such as Ebola and Hendra viruses, vaccine and therapeutic development and understanding of the mechanisms of pathogenesis.

PROF GILDA TACHEDJIAN, *Department of The Life Sciences Discipline And Retroviral Biology*

Professor Gilda Tachedjian is Head of the Life Sciences Discipline and Retroviral Biology and Antivirals Group at the Burnet Institute and is an adjunct Professor at Monash University. She is recognized for her contributions to HIV biology, antivirals and prevention research. Prof Tachedjian obtained her PhD in Microbiology at Monash University and completed a NHMRC CJ Martin Postdoctoral Fellowship at Columbia University in New York. She was recruited to the Burnet Institute in 2002 and was awarded a NHMRC RD Wright followed by NHMRC Senior Research Fellowships. In 2012 Prof Tachedjian was the recipient of the Frank Fenner Award from the Australian Society for Microbiology, is the immediate past President of the Australasian Virology Society and co-founded the Melbourne HIV Exchange (mHIVE) and the Genital Microbiome Consortium. Prof Tachedjian's current research interests are focused on defining how the vaginal microbiome and their

metabolites modulate susceptibility to HIV and other STIs through their antimicrobial and immunobiological properties, developing a new drug class for HIV treatment and prevention, and studying bat viruses and their interplay with host antiviral factors.

A/PROF JAMES VINCE, *The Walter and Eliza Hall Institute of Medical Research*
A/Prof James runs a laboratory at the Walter and Eliza Hall Institute (WEHI) in Melbourne, Australia, and is the Science Director of Mermaid Bio, a new biotech startup with its headquarters in Munich. At WEHI his laboratory studies the molecules that govern the often-connected processes of cell death and inflammation. These processes normally protect against microbial infection and allow tissue repair. However, the loss of control of cell death and inflammation underpins diseases such as cytokine shock syndromes (e.g. sepsis and severe COVID-19), arthritis and cancer. Their goal is to use genetics, biochemistry, and disease modelling to understand how cell death and inflammation are controlled, and how they influence each other, as a basis for new treatments.

SPONSORS 2023

GOLD SPONSOR

CSL™ moderna®

SILVER SPONSOR

NEW ENGLAND BioLabs® Cell Signaling TECHNOLOGY®

EXHIBITORS

AUSTRALIAN biosearch Your Life Science Specialists

bio-strategy delivering technology

BMG LABTECH The Microplate Reader Company

CYTEK TRANSCEND THE CONVENTIONAL

GENESEARCH e*FREEZER

Lonza PEPROTECH OUR SUPPORT, YOUR DISCOVERY

MP

MIMOTOPES The Peptide Company

Promega

TECAN.

TransnetYX Serving research. Saving time.

TrendBio Partnerships in Science

CAREER DEVELOPMENT AWARD SPONSOR

microorganisms an Open Access Journal by MDPI

STUDENT & ECR PRIZE SPONSOR

vaccines an Open Access Journal by MDPI

The Australian Society for Microbiology bringing Microbiologists together VIC BRANCH

COFFEE CART SPONSOR

BD

FAMILY ROOM SPONSOR

WEHI brighter together

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

Gemma-ann Taylor
ASN Events
Email: gemma-ann.t@asnevents.net.au
Mobile: 0490 514 632

Lek Krongsing
ASN Events
Email: lek.k@asnevents.net.au
Mobile: 0431 991 151

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)

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.

COVID-19 Guidelines

The Lorne Infection and Immunity Conference has put in place a number of measures for the management of ongoing risks arising from the COVID-19 pandemic.

Mask wearing indoors and in crowded outdoor areas is strongly recommended. One N-95 mask has been provided to all registrants. Rapid antigen tests and additional N-95 masks will be available at the registration desk for those who require them.

If you feel unwell, please do not attend the conference. Your registration can be converted to a virtual registration or refunded.

If you feel unwell onsite, please do not attend further sessions, but undertake a COVID test and follow the instructions below. If you return a positive result and need medical care or advice on resources to support you in isolation, please use the following:

- Great Ocean Road Health in Lorne: 5289 4333 or 5289 4300 (after hours)
- Nurse on Call: 1300 60 60 24
- Emergency Services: 000
- Victorian Government Coronavirus Hotline at 1800 675 398

Instructions after taking a COVID test: In the case that you test positive for COVID-19 while at the conference or in the 48 hours after the conference, we are obliged to ask that you contact **gemma-ann.t@asnevents.net.au** to alert her of this fact. In the case a positive case is reported at the conference or within 48 hours after the conference, delegates will be notified by email from ASN Events that a positive COVID-19 person has been in attendance at the conference (and in which sessions/venues where that information is provided) and that delegates should follow local guidelines about testing, isolation and seeking medical care. No other identifying information about the affected person will be communicated. We ask that delegates who test positive for COVID-19 at or after the conference refrain from attempting to disseminate this information on social media, but instead alert the conference secretariat, as above.

CONFERENCE APP

Lorne I&I 2023 has a conference app that includes all information contained in this book and live updates of the program.

To view this app, go to http://infection-and-immunity-2023.m.asnevents.com.au/

DISPLAYING YOUR POSTER

Science Bite Presentations

These presenters will have their posters available for the full duration of the conference though have been advised to be available for discussion during the poster session of the day they presented their oral presentation.

Poster Presentations

Presenters for the first poster session (Wednesday, 15th February at 8:00pm) will display their poster at the start of the conference and will remove them after morning tea on Thursday.

Presenters for the second poster session (Thursday, 16th February at 8:00pm) will display their poster from lunch on Thursday until morning tea on Friday.

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 Dinner – Pizza Night

The Heritage Room or Seagrass Lawn (weather permitting), Mantra Lorne
Wednesday, 15th February, 6:30pm – 8:00pm

General Delegate Dinner

Lorne Central, 54-56 Mountjoy Pde, Lorne
Wednesday, 15th February, 6:30pm – 8:00pm

Student and ECR Networking Lunch

The Heritage Room or Seagrass Lawn (weather permitting), Mantra Lorne
Thursday 16th February, 12:10PM - 1:20PM

Conference Dinner

Lorne Common, 45 Otway St, Lorne
Thursday 16th February, 6:00pm – 8:00pm

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 **Gold 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 **Gold Sponsor**

Website: www.modernatx.com/en-AU

New England Biolabs **Silver Sponsor**

Website: www.nebiolabs.com.au/

Created "by scientists for scientists", NEB is renowned for providing exceptional product quality and unsurpassed technical support. We offer the largest selection of recombinant and native enzymes for genomic research. Our ever-expanding catalogue also includes products related to PCR, gene expression, NGS, synthetic biology, glycobiology, epigenetics and RNA analysis. NEB Australia is also a distributor for Cell Signaling Technology, a manufacturer of antibodies, and related reagents, for studying the molecular pathways that impact human health.

AUSTRALIAN BIOSEARCH **Exhibitor**

Webiste: www.aust-biosearch.com.au/

Bio-Strategy **Exhibitor**

Website: www.bio-strategy.com.au

Bio-Strategy offers a comprehensive range of products for your laboratory research, analysis and production. Our portfolio includes technical instrumentation, high quality laboratory equipment, consumables, chemicals and reagents & assays.

Founded in 2003, Bio-Strategy is an established locally-owned ISO 9001:2015 accredited distributor delivering technology throughout Australasia. Market sectors include Diagnostic Laboratories, Academic Research, Government Institutes, Applied Testing and Industry.

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.

Cytek Biosciences **Exhibitor**

Website: www.cytekbio.com

Cytek Biosciences is committed to providing powerful flow cytometry solutions that enable scientists to rapidly and efficiently obtain deep biological insights through high quality, high parametric datasets.

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

Phone: +61 3 9565 1111

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!

MP Biomedicals **Exhibitor**

Website: www.mpbio.com

MP Biomedicals is dedicated to giving scientists and researchers innovative, quality tools and superior service to aid them in their quest for ground-breaking discovery.

MP Biomedicals offers a comprehensive line of life science, fine chemical, and diagnostic products. Some of our product offerings include FastPrep-24™ 5G bead beating grinder and lysis system, MPure-32 automated magnetic bead-based nucleic acid extraction system and a wide range of high-performance purification kits. Stop by our booth, see FastPrep--24™ 5G in action and discuss your research requirements with our experts. Alternatively, get in touch with us at custserv.au@mpbio.com or 1800249998.

Promega **Exhibitor**
Website: www.promega.com.au/

TECAN **Exhibitor**
Website: https://www.tecan.com/
Tecan is a leading global provider of automated laboratory instruments and solutions. Our systems and components help people working in clinical diagnostics, basic and translational researchand drug discovery bring their science to life. In particular, we develop, produce, market andsupport automated workflow solutions that empower laboratories to achieve more.

Transnetyx **Exhibitor**
Website: www.transnetyx.com
Transnetyx serves research and the efficient care of animals through genetic services (Automated Genotyping, Genetic Monitoring, Microbiome). With Transnetyx, labs and facilities can focus on efficiently and responsibly progressing reproducible research forward while effectively saving valuable time and resources.

TrendBio **Exhibitor**
Website: www.trendbio.com.au
Company logos are also attached in the email. Company profile remains the same as previous years: TrendBio is a specialised instrument and consumable supplier servicing the Australian and New Zealand scientific and diagnostic markets. With leading products across the divisions of Genomics, Proteomics, Cell Biology, Imaging, Biologics and Chemistry. We are committed to establish trusted partnerships with the research communities to empower your innovative work. For more information, visit http://www.trendbio.com.au or contact us at info@trendbio.com.au

BD **COFFEE CART SPONSOR**
Website: www.bdbiosciences.com/en-au/home-page
BD is a global medical technology company that is advancing the world of health™ by improving medical discovery, diagnostics and the delivery of care. BD leads in patient and healthcare worker safety and the technologies that enable medical research and clinical laboratories. The company provides innovative solutions that help advance medical research and genomics, enhance the diagnosis of infectious disease and cancer, improve medication management, promote infection prevention, equip surgical and interventional procedures.

WEHI (Walter and Eliza Hall Institute) **Parents Room Sponsor**
Website: www.wehi.edu.au
WEHI is where the world's brightest minds collaborate and innovate to make life-changing scientific discoveries that help people live healthier for longer. Our medical researchers have been serving the community for more than 100 years, making transformative discoveries in cancers, infectious and immune diseases, developmental disorders and healthy ageing.
WEHI brings together diverse and creative people with different experience and expertise to solve some of the world's most complex health problems. With partners across science, health, government, industry and philanthropy, we are committed to long-term discovery, collaboration and translation. At WEHI, we are brighter together. Find out more at www.wehi.edu.au

The Australian Society for Microbiology Victoria Branch **Award Sponsor**
Website: www.theasmvic.org.au/

15th February 2023

Session 1: Welcome and Plenary I

1:00PM - 2:00PM — Heritage Ballroom

Chairs: Michelle Boyle & Cameron Stewart

Gilda Tachedjian (*Burnet Institute, Melbourne, VIC, Australia*)
Vaginal microbiome and HIV susceptibility — abs# 1

Session 2: Innate Immunity

2:00PM - 3:50PM — Heritage Ballroom

Chairs: Si Ming Man & Larisa Labzin

Shabaana Khader (*The University of Chicago, Chicago, IL, United States*)
Role of iBALT in Tuberculosis — abs# 2

James Vince (*Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia*)
Programmed cell death crosstalk in a monogenetic inflammatory disease — abs# 3

Ashley Mansell (*Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Melbourne, VIC, Australia*)
ADS032, the first dual NLRP1 and NLRP3 inflammasome inhibitor for the treatment of infectious and inflammatory diseases — abs# 4

Sarah E Garnish (*University of Melbourne, Department of Medical Biology, Parkville, VIC, Australia*)
MLKL mutation causes defective immune response during bacterial infection — abs# 5

Thomas E Schultz (*The University of Queensland Diamantina Institute, The University of Queensland, Brisbane, QLD, Australia*)
Probing the molecular basis of curbing TLR4-driven inflammatory responses — abs# 6

Nicky de Weerd (*Hudson Institute of Medical Research, Clayton, VIC, Australia*)
IFNλ1 utilises interferon alpha/beta receptor 2 to induce signalling and anti-viral defence in lung epithelial cells — abs# 7

Afternoon Tea

3:50PM - 4:20PM — Convention Centre

Session 3: Adaptive Immunity

4:20PM - 6:30PM — Heritage Ballroom

Chairs: Daniel Utzschneider & Danika Hill

Session sponsored by

Lisa Wagar (*University of California Irvine, Irvine, CA, United States*)
Predicting immunogenicity using human tonsil organoids — abs# 8

Amy Chung (*University of Melbourne, Doherty Institute, Melbourne, VIC, Australia*)
High-throughput characterization of antibody responses in SARS-CoV-2 infection and vaccination abs# 9

Alexandra Spencer (*The Jenner Institute, University of Oxford, Oxford, United Kingdom*)
Exploiting the tropism of viral vectored vaccines for optimal induction of liver resident T cells and long-term protection against malaria — abs# 10

Danika L Hill (*Monash University, Melbourne, VIC, Australia*)
Systems immunology of T follicular helper cells reveals impaired BCL6 function in ageing. — abs# 11

Tom S Fulford (*Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, University of Melbourne, Parkville, VIC, Australia*)
Butyrophilin 3A1 interacts with the Vγ9Vδ2+ T cell receptor — abs# 12

Annette Oxenius *(ETH Zürich, Zurich, ZURICH, Switzerland)*
T cell immunity in cytomegalovirus infection — abs# 13

Student Pizza Night

6:30PM - 8:00PM — Mantra Lawn Area

General Delegates Dinner

6:30PM - 8:00PM — Lorne Central
54-56 Mountjoy Parade, Lorne VIC 3232

Poster Session I

8:00PM - 9:30PM — Convention Centre

16th February 2023

Session 4: Plenary II and Hartland Oration

8:30AM - 9:30AM Heritage Ballroom
Chairs: Eugene Maraskovsky & Justine Mintern

Session sponsored by

Hao Wu (*Boston Children's Hospital and Harvard Medical School, Boston, MA, United States*)
Inner workings of the inflammasome engine abs# 14
Harshini Weerasinghe (*Infection Program and the Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Victoria, Australia*)
Eat to compete: Understanding immunometabolic interactions that drive *Candida auris* infection abs# 15

Session 5: Hertzog Oration

9:30AM - 10:00AM Heritage Ballroom
Chair: Richard Ferrero

Thomas Kufer (*University of Hohenheim, Stuttgart, BW, Germany*)
Brief update on non-inflammasome NLRs abs# 16

Morning Tea

10:00AM - 10:30AM Convention Centre

Session 6: Pandemic Preparedness I

10:30AM - 11:50AM Heritage Ballroom
Chairs: Begoña Heras & Vinod Sundaramoorthy

Glenn Marsh (*Australian Animal Health Laboratory, CSIRO Health and Biosecurity, Geelong, VIC, Australia*)
Preparing for disease X abs# 17
Katie Anders (*World Mosquito Program, Monash University, Clayton, VIC, Australia*)
Deploying Wolbachia-infected mosquitoes to control - and potentially eliminate? - dengue and other Aedes-borne diseases abs# 18
Katherine Kedzierska (*Department of Microbiology & Immunology, University of Melbourne at Doherty Institute, Parkville, VIC, Australia*)
Robust and prototypical immune responses towards COVID-19 BNT162b2 vaccine in First Nations Australian people are impacted by co-morbidities abs# 19
Diana S Hansen (*Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia*)
Integrated systems immunology approach identifies impaired effector T cell memory responses as a key feature of progression to severe dengue haemorrhagic fever abs# 20

Lunch
11:50AM - 1:00PM Convention Centre

Student and ECR Networking Lunch
11:50AM - 1:00PM Mantra Lawn Area

Break
1:00PM - 2:20PM

Afternoon Tea
2:20PM - 2:50PM Convention Centre

Session 7: Science Bites I
2:50PM - 3:15PM Heritage Ballroom
Chair: Danny Wilson & Thomas Schultz

Sharon L Clark (*School of Medicine, The University of Western Australia, Perth, WA, Australia*)
Otitis-Prone children have reduced Haemophilus influenzae protein D-specific memory B-cells compared to non-otitis-prone children but no reduction in overall T follicular helper cell proportions. abs# 51

Jessica Engel (*QMIR Berghofer Medical Research Institute, Brisbane, QLD, Australia*)
Using single-cell RNA sequencing to understand tissue- and drug- specific CD4+ T cell responses in experimental visceral leishmaniasis abs# 53

Jessica Horton (*Burnet Institute, Melbourne, VIC, Australia*)
Monocytes as effectors of antibody-mediated functions in malaria infection abs# 54

Dawson Ling (*Malaria Virulence and Drug Discovery Group, Burnet Institute, Melbourne, VIC, Australia*)
Identifying the mechanism of action of a novel invasion-blocking compound in Plasmodium falciparum abs# 55

Costanza Tacoli (*Population Health and Immunity Division, Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC, Australia*)
Assessing the feasibility, specificity and sensitivity of a serological testing and treatment approach to eradicate P. vivax in Cambodia abs# 56

Catherine Tsai (*Molecular Medicine and Pathology, University of Auckland, Auckland, New Zealand*)
A novel recombinant Lactococcus lactis mucosal vaccine platform based on group A streptococcus pili abs# 57

Madeleine Wemyss (*Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, VIC, Australia*)
Salmonella Typhimurium induces cIAP1 degradation to promote death in macrophages abs# 58

Session 8: Science Bites II
2:50PM - 3:15PM Heritage Dining Room
Chairs: Gregory Moseley & Matt Johansen

Michaela S Bulloch (*The University of Melbourne, Parkville, VIC, Australia*)
Doxycycline inhibits both apicoplast and mitochondrial translation in Plasmodium falciparum abs# 59
Zahra Elahi (*Department of Anatomy and Physiology, University of Melbourne, Melbourne, VIC, Australia*)
The Human Dendritic Cell Atlas: An integrated transcriptional tool to study human dendritic cell biology abs# 60
Cheng Xiang Foo (*Mater Research, University of Queensland, Woolloongabba, QLD, Australia*)
GPR183 antagonism reduces macrophage infiltration in SARS-CoV-2 infection abs# 61
Jessica J Harrison (*University of Queensland, St Lucia, QLD, Australia*)
A novel chimeric virus for vaccine and diagnostic application for the Japanese encephalitis outbreak in Australia abs# 62
Sonia McAlister (*Wesfarmers Centre of Vaccines and Infectious Diseases, Telethon Kids Institute, The University of Western Australia, Perth,WA, Australia*)
Safety and immunogenicity of a 2+1 DTPa infant vaccination schedule in Australian infants. abs# 63
Joseph Menassa (*Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, VIC, Australia*)
TREML4 receptor regulates inflammation and innate immune cell death during polymicrobial sepsis abs# 64
Natalia Sampaio (*Hudson Institute of Medical Research, Clayton, VIC, Australia*)
MDA5 guards against infection by surveying cellular RNA homeostasis abs# 65

Mini-break
3:15PM - 3:20PM

Session 9: Microbiomes
3:20PM - 5:15PM Heritage Dining Room
Chairs: Philip Hansbro & Lindi Masson

Nadeem O Kaakoush (*University of New South Wales, Kensington, NSW, Australia*)
Microbiome manipulation in the treatment of ulcerative colitis abs# 21
Remy B Young (*Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, VIC, Australia*)
Key commensal species breakdown phenolic compounds to support community stability within the gastrointestinal microbiome abs# 22
Jamia Hemphill (*Department of Microbiology, Monash University, Clayton, VIC, Australia*)
Investigating antimicrobial activity by bacterial members of the human gastrointestinal microbiome abs# 23
Howard C H Yim (*Microbiome Research Centre, UNSW, Sydney, NSW, Australia*)
Association of Fecal DNA Virome with the Development of Colorectal Neoplasia abs# 24
Jenna Wilson (*Disease Elimination Program, Life Sciences Discipline, Burnet Institute, Melbourne, VIC, Australia*)
Functional differences between cervicovaginal Lactobacillus species that protect against HIV infection abs# 25
Praveena Thirunavukkarasu (*Infection and Immunity program & Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Melbourne, VIC, Australia*)
Understanding the molecular recognition of Bacteroides fragilis glycosphingolipids by Natural Killer T-cell receptor abs# 26
Sara K Di Simone (*Ritchie Centre, Hudson Institute of Medical Research, Clayton, VIC, Australia*)
Culturing the neonatal microbiome has revealed similarities between bacteria colonising the respiratory tract and gut abs# 27

Sean Solari (*Centre of Innate Immunity and Infectious Disease, Hudson Institute of Medical Research, Clayton, VIC, Australia*)
Phylogeny-based metagenomic analyses increases the resolution of investigations of the human microbiome abs# 28

Session 10: Bugs, Viruses and Parasites

3:20PM - 5:15PM Heritage Ballroom
Chairs: Darren Creek & Elinor Hortle

Alexander A Khromykh (*School of Chemistry and Molecular Bisociences, University of Queensland, Brisbane, QLD, Australia*)
Reverse genetics of SARS-CoV-2 abs# 29
Danushka Marapana (*Walter and Eliza Hall Institute of Medical Research, Parkville, Melbourne, VIC, Australia*)
Identifying critical E3 ubiquitin ligases in human malaria parasites abs# 30
Stephen W Scally (*Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia*)
PCRCR complex is essential for invasion of human erythrocytes by Plasmodium falciparum abs# 31
Aurelie ATD Tsee Dawson (*Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia*)
Imaging host-cell invasion by Toxoplasma gondii: What is the role of the host membrane and cytoskeleton? abs# 32
D. Herbert Opi (*Burnet Institute of Medical Research and Public Health, Melbourne, VIC, Australia*)
Identification of targets of protective antibody responses against Plasmodium vivax malaria using a multifunctional antibody profiling approach abs# 33
Arthika Manoharan (*Department of Infection, Immunity and Inflammation, School of Medical Sciences, The University of Sydney, Sydney, NSW, Australia*)
Disarming Proteus mirabilis and blowing its cover: N acetylcysteine inhibits P. mirabilis urease activity and prevents catheter encrustation in catheter associated UTIs. abs# 34

Mini-break

5:15PM - 5:20PM

Session 11: Pandemic Preparedness II

5:20PM - 5:45PM Heritage Ballroom
Chair: Begoña Heras & Vinod Sundaramoorthy

Marion Koopmans (*Erasmus MC - afdeling Viroscience, Rotterdam, ZH, Netherlands*)
Pandemic preparedness: time for a focus on animals? abs# 35

Conference Dinner

6:00PM - 8:00PM Lorne Common (Footy Oval)

Sponsored by
moderna

Poster Session II

8:00PM - 9:30PM Convention Centre

17th February 2023

Session 12: Clinical Translation

8:30AM - 10:05AM Heritage Ballroom
Chairs: Nikki Moreland & Heidi Drummer

Marylyn Addo (*University Medical Center Hamburg-Eppendorf, Hamburg, Germany*)
Dissecting immunity to MVA-MERS-S, a novel vaccine candidate against the Middle East respiratory syndrome (MERS) abs# 36
Julie Bines (*Paediatrics, University of Melbourne, Parkville, VIC, Australia*)
From discovery to prevention: The development journey of the RV3-BB Rotavirus Vaccine abs# 37
William Petri (*University of Virginia, Charlottesville, VA, USA*)
Role of Type 2 Immunity in COVID-19 abs# 38
Katharina Ronacher (*Stellenbosch University, Cape Town, South Africa*)
Oxidised Cholesterols Drive Macrophage Infiltration into the Lung during Bacterial and Viral Respiratory Infections abs# 39

Morning Tea

10:05AM - 10:40AM Convention Centre

Session 13: Host Pathogen Interactions

10:40AM - 12:40PM Heritage Ballroom
Chairs: Makrina Totsika & Tim Barnett

JoAnne Flynn (*University of Pittsburgh, Pittsburgh, PA, United States*)
Unraveling protection against tuberculosis abs# 40
Ian Cockburn (*Australian National University, Canberra, ACT, Australia*)
Antibody and B cell responses to a complex pathogen abs# 41
Rebecca Edgar (*School of Medicine, Deakin University, Waurn Ponds, VIC, Australia*)
Genetic and chemical validation of Plasmodium falciparum aminopeptidase PfA-M17 as an anti-malarial drug target in the haemoglobin digestion pathway abs# 52
Michael Gantier (*Hudson Institute of Medical Research, Clayton, VIC, Australia*)
Pharmacological inhibition of TBK1/IKKε blunts COVID-19 immunopathology abs# 42
Paul R Gilson (*Burnet Institute, Melbourne, VIC, Australia*)
Inhibitors of the START-domain lipid transfer protein potently block intraerythrocytic development in newly invaded Plasmodium falciparum merozoites. abs# 43
Nirmal Robinson (*CECAD, University of Cologne, Cologne, Germany*)
Mitochondrial Sirtuin 4 regulates inflammation during Salmonella Typhimurium infection abs# 44

Closing Remarks, Prizes, Awards and Photos

12:40PM - 1:00PM Heritage Ballroom
Chairs: Richard Ferrero & Heidi Drummer

POSTER LISTING

Science Bite presenters from Session 7 and odd numbered poster presenters will be available at the Mantra for discussions during the poster session on Wednesday.

Science Bite presenters from Session 8 and even numbered poster presenters will be available at the Mantra for discussions during the poster session on Thursday.

Science Bites

Sharon L Clark *(School of Medicine, The University of Western Australia, Perth, WA, Australia)*
Otitis-Prone children have reduced Haemophilus influenzae protein D-specific memory B-cells compared to non-otitis-prone children but no reduction in overall T follicular helper cell proportions. abs# 51

Jessica Engel *(QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia)*
Using single-cell RNA sequencing to understand tissue- and drug- specific CD4+ T cell responses in experimental visceral leishmaniasis abs# 53

Jessica Horton (*Burnet Institute, Melbourne, VIC, Australia*)
Monocytes as effectors of antibody-mediated functions in malaria infection abs# 54

Dawson Ling (*Malaria Virulence and Drug Discovery Group, Burnet Institute, Melbourne, VIC, Australia*)
Identifying the mechanism of action of a novel invasion-blocking compound in Plasmodium falciparum abs# 55

Costanza Tacoli (*Population Health and Immunity Division, Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC, Australia*)
Assessing the feasibility, specificity and sensitivity of a serological testing and treatment approach to eradicate P. vivax in Cambodia abs# 56

Catherine Tsai (*Molecular Medicine and Pathology, University of Auckland, Auckland, New Zealand*)
A novel recombinant Lactococcus lactis mucosal vaccine platform based on group A streptococcus pili abs# 57

Madeleine Wemyss (*Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, VIC, Australia*)
Salmonella Typhimurium induces cIAP1 degradation to promote death in macrophages abs# 58

Michaela S Bulloch (*The University of Melbourne, Parkville, VIC, Australia)*
Doxycycline inhibits both apicoplast and mitochondrial translation in Plasmodium falciparum abs# 59

Zahra Elahi (*Department of Anatomy and Physiology, University of Melbourne, Melbourne, VIC, Australia*)
The Human Dendritic Cell Atlas: An integrated transcriptional tool to study human dendritic cell biology abs# 60

Cheng Xiang Foo (*Mater Research, University of Queensland, Woolloongabba, QLD, Australia*)
GPR183 antagonism reduces macrophage infiltration in SARS-CoV-2 infection abs# 61

Jessica J Harrison (*University of Queensland, St Lucia, QLD, Australi*a)
A novel chimeric virus for vaccine and diagnostic application for the Japanese encephalitis outbreak in Australia abs# 62

Sonia McAlister (*Wesfarmers Centre of Vaccines and Infectious Diseases, Telethon Kids Institute, The University of Western Australia, Perth, WA, Australia*)
Safety and immunogenicity of a 2+1 DTPa infant vaccination schedule in Australian infants. abs# 63

Joseph Menassa (*Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, VIC, Australia*)
TREML4 receptor regulates inflammation and innate immune cell death during polymicrobial sepsis abs# 64

Natalia Sampaio (*Hudson Institute of Medical Research, Clayton, VIC, Australia*)
MDA5 guards against infection by surveying cellular RNA homeostasis abs# 65

Poster Session 1

Yara N Abo (*Murdoch Children's Research Institute, Melbourne, VIC, Australia*)
Strategic and scientific contributions of human challenge trials for vaccine development: facts versus fantasy abs# 101

Shoaib Anwaar (*The University of Queensland Diamantina Institute, Brisbane, Australia*)
Elucidating the contribution of regulatory T cells to the prevention of Cutaneous Squamous Cell Carcinoma abs# 103

Kristian T Barry (*Hudson Institute of Medical Research, Clayton, VIC, Australia*)
Activation of the NLRP3 inflammasome by Hendra Virus C-protein abs# 105

Rob Bischof (*Allergenix Pty Ltd, Clayton, VIC, Australia*)
Features of a novel sheep model of ALI/ARDS induced by pulmonary instillation of live Streptococcus pneumoniae abs# 107

Hayley Bullen (*Burnet Institute, Melbourne, VIC, Australia*)
Antimalarial compounds the 2-Anilino 4-Amino substituted quinazolines, are irresistible and promiscuous. abs# 109

Suzanne Butcher (*Stem Cell Systems, Department of Anatomy and Physiology, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville, VIC, Australia*)
An integrated atlas of innate immune cell responses to activating stimuli reveals time-, dose- and ligand-dependent axes of inflammation. abs# 111

Eva Chan (*Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, 27-31 Wright Street, Clayton, VIC, Australia*)
Establishing human small intestinal monolayers to study EPEC infection abs# 113

Rachel MY Cheong (*School of Clinical Sciences, Monash University, Melbourne, VIC, Australia*)
Smith-specific TCR-Tregs successfully treat lupus nephritis in a humanised model of disease abs# 115

Julia Chitty (*Centre of Innate Immunity and Infectious Disease, Hudson Institute of Medical Research, Clayton, VIC, Australia*)

Caroline Soliman (*Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Parkville, Victoria, Australia*)
Characterisation of CD1c-restricted T cells recognising mycobacterial lipids abs# 197

Risa Takahashi (*Molecular Medicine and Pathology and Maurice Wilkins Centre, The University of Auckland, Auckland, New Zealand*)
Understanding the innate immune response to Group A Streptococcus pili abs# 199

Sushama Telwatte (*The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia*)
Single-cell RNAseq analysis of HIV reservoir cells from viremic and virally-suppressed individuals living with HIV abs# 201

Emma C. Tovey Crutchfield (*Inflammation Department, The Walter and Eliza Hall Institute, Melbourne, VIC, Australia*)
MLKL deficiency protects against sterile inflammation and immunosenescence in aged mice abs# 203

Anna Truong (*Chemistry, Duke University, Durham, NC, United States*)
Covalent probe reveals Ubc13 interactome in the malaria parasite abs# 205

Holly K Ung (*Ritchie Centre, Hudson Institute of Medical Research, Clayton, VIC, Australia*)
Functional IL-37 receptor cell surface expression on immune cell subsets in health and disease by flow cytometry abs# 207

Joshua von Ameln (*Hudson Institute for Medical Research, Clayton, VIC, Australia*)
Structural and functional characterisation of a novel gasdermin protein abs# 209

Alisha M Wilson (*Telethon Kids Institute, Subiaco, WA, Australia*)
Pilus tip protein AP1 essential for Group A Streptococcus adhesion to the tonsil epithelium abs# 211

Ka Yuen (*School of Veterinary Science, The University of Queensland, Gatton, QLD, Australia*)
Kinetics of the innate antiviral response of equine peripheral mononuclear cells in response to Ross River virus abs# 213

Poster Session 2

Grace Androga (*Burnet Institute, Melbourne, VIC, Australia*)
Development of a Lactobacillus therapeutic approach for HIV prevention in women abs# 102

George Ashdown (*Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia*)
Characterising macrophage proteomic signatures of tuberculosis infection abs# 104

Miguel MAB Berrocal (*University of Melbourne, Carlton, VIC, Australia*)
Characterization of a novel Transcriptional Start Site in human myeloid cells that generates unique NRG1 isoforms abs# 106

Luzia Bukali (*Immunology and Infection, QIMR Berghofer Medical Research Institute, Bowen Hills, QLD, Australia*)
Understanding CD4+ T cell mediated immune regulation in malaria: Assessing the immunomodulatory potential of Ruxolitinib during controlled human malaria infection. abs# 108

ORAL ABSTRACTS

1

Vaginal microbiome and HIV susceptibility

Gilda Tachedjian[1, 2]

1. Burnet Institute, Melbourne, VIC, Australia

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

The risk of acquiring sexual transmitted infections (STI) including HIV is modulated by the vaginal microbiota. Women colonised with optimal vaginal bacterial communities, typically dominated by a beneficial Lactobacillus spp., have a decreased risk of acquiring and transmitting HIV compared to women colonised with 'non-optimal" microbiota (i.e. bacterial vaginosis, BV), which promote subclinical genital inflammation driving increased HIV risk. BV is characterised by a depletion of Lactobacillus spp. and a high relative abundance or polymicrobial anaerobes. While studies have focused on the association between vaginal microbiota and HIV risk, relatively little is known about the role of microbiota metabolites in modifying the vaginal microbiome and environment to help protect against STIs including HIV. Major distinguishing features between women colonised with optimal vaginal microbiota compared to women with BV includes dramatically increased vaginal levels of lactic acid (~120 mM vs £20 mM), while short chain fatty acids are elevated in women with BV. Our working hypothesis is that lactic acid, produced by optimal lactobacilli, is a bioactive that plays a key role in preventing HIV/STI acquisition. Here, data will be presented demonstrating that lactic acid has potent bactericidal activity against BV-associated bacteria but not lactobacilli, in vivo and ex vivo HIV virucidal activity, and immune modulatory properties on cervicovaginal epithelium that reduces production of soluble pro-inflammatory mediators linked with increased HIV risk. RNAseq and meta-proteomics studies show that lactic acid directly promotes epithelial barrier function by upregulating intracellular junctional molecules that could prevent paracellular penetration of pathogens. These findings highlight the multifaceted properties of a vaginal microbiota metabolite and suggest potential use of lactic acid and/or lactic acid producing bacteria to optimise the vaginal environment and microbiome to promote sexual and reproductive health including their use as adjuncts to reduce acquisition of STIs including HIV.

2

Role of iBALT in Tuberculosis

Shabaana Khader[1]

1. The University of Chicago, Chicago, ILLINOIS, United States

Mycobacterium tuberculosis (Mtb), the causative agent of the disease tuberculosis (TB), is estimated to infect one-fourth of the world's population, resulting in approximately 1.5 million deaths each year. The emergence of multidrug- and extensively drug-resistant Mtb strains and the variable efficacy of the currently used vaccine, M. bovis Bacille Calmette Guerin (BCG), are barriers to the global control of TB. Thus, there is a critical need to better understand the mechanisms of TB immunopathogenesis, as such mechanisms can be targeted to improve host control of Mtb infection. Although granulomas have long been considered a hallmark of both latent TB infection (LTBI) and clinical pulmonary TB (PTB), the immunological differences between protective granulomas and non-protective granulomas have only recently begun to emerge. Our recent data suggest that the presence of B-cell follicles in inducible bronchus-associated lymphoid tissue (iBALT)-containing granulomas is indicative of protective granulomas that mediate Mtb control during LTBI. In contrast, infiltrating neutrophils producing proinflammatory molecules are characteristic of non-protective granulomas during PTB. In this talk, Dr. Khader will challenge the current paradigm that TB granulomas are generally protective and advocate a new model for TB immunopathogenesis in which protective granulomas contain iBALT whereas non-protective granulomas are neutrophilic or exhibit immune activation.

3

Programmed cell death crosstalk in a monogenetic inflammatory disease

Sebastian Hughes[1], Meng Lin[2], Ashley Weir[1], Bing Huang[2], Liya Xiong[2], Ngee-Kiat Chua[1], Jiyi Pang[1], Jascinta Santavanond[3], Rochelle Tixeira[3], Marcel Doerflinger[1], Yexuan Deng[1], Natasha Silke[1], Stephanie Conos[4], Daniel Frank[1], Daniel Simpson[1], James Murphy[1], Kate Lawlor[4], John Silke[1], Marc Pellegrini[1], Marco Herold[1], Ivan Poon[3], Seth Masters[1], Mingsong Li[2], Qing Tang[5], Yuxia Zhang[2], Maryam Rashidi[1], Lanlan Geng[2], James Vince[1]

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

2. Department of Gastroenterology, Guangzhou Women and Children's Medical Center, Guangzhou, Guangdong, China

3. Department of Biochemistry and Genetics, La Trobe University, Melbourne, Victoria, Australia

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

5. The First Affiliated hospital of Guangxi Medical University, Nanning, Guangxi, China

Genetic lesions in X-linked Inhibitor of Apoptosis (XIAP) pre-dispose humans to cell death-associated diseases, including severe inflammatory bowel disease and the cytokine storm syndrome hemophagocytic lymphohistiocytosis. Here, we report that patients lacking XIAP can present with heightened levels of both apoptotic and pyroptotic cell death markers in diseased tissue. Using models of XIAP deficiency, we genetically show that only the combined deletion of several cell death modalities abrogates excess cell death and associated inflammasome-driven inflammatory IL-1β activation. Interestingly, our results also reveal that mitochondrial and death receptor apoptosis signalling trigger inflammasome responses via distinct mechanisms, despite both pathways converging of apoptotic caspase-3 and -7. These findings uncouple the mechanisms of cell death and inflammasome activation resulting from extrinsic and intrinsic apoptosis,

reveal how XIAP loss can co-opt dual cell death programs, and uncover strategies for targeting the cell death and inflammatory pathways that result from XIAP deficiency.

4

ADS032, the first dual NLRP1 and NLRP3 inflammasome inhibitor for the treatment of infectious and inflammatory diseases

Ashley Mansell[1, 2, 3], Callum Docherty[1], Anu Fernando[4], Sarah Rosli[1], Maggie Lam[1], Michelle Tate[1], Christopher Murphy[3], Adriano Rossi[4]

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

2. Adiso Therapeutics, Concord, MA, USA

3. Adiso Therapeutics, Concord, MA, USA

4. University of Edinburgh Centre for Inflammation, , Queen's Medical Research Institute, Edinburgh, United Kingdom

Inflammasomes induce maturation of the inflammatory cytokines IL-1β and IL-18, whose activity is associated in the pathophysiology of a wide range of infectious and inflammatory diseases including influenza A virus (IAV), SARS CoV2, pulmonary, neurodegenerative, metabolic and cardiovascular diseases, monogenic autoinflammatory syndromes and cancer. As validated therapeutic targets for the treatment of acute and chronic inflammatory diseases, there has been intense interest in developing small molecule inhibitors to target inflammasome activity.

We here describe ADS032 as the world's first dual NLRP1 and NLRP3 inhibitor. ADS032 is a rapid, reversible and stable small molecule inflammasome inhibitor that directly binds both NLRP1 and NLRP3, reducing secretion and maturation of IL-1β in mouse macrophages, human blood-derived macrophages, stimulated with both NLRP1 and NLRP3 agonists. ADS032 suppressed IL-1β secretion from human primary bronchial epithelial cells in response to the NLPR1 agonists anisomycin and poly I:C, and NLRP3 agonists nigericin and silica.

Demonstrating its therapeutic potential, *in vivo* administered ADS032 reduced IL-1β and TNF levels in the serum of mice both systemically and intranasally challenged with LPS and reduced pulmonary inflammation in an acute model of lung silicosis. Importantly, while we previously found that ablating NLRP3 functionality with potent inhibitors such as MCC950 had both positive and detrimental disease outcomes during IAV infection; we found that mice treated with ADS032 protected mice from lethal IAV challenge when administered at any time post-infection, displaying increased survival and reduced pulmonary inflammation.

Ex vivo treatment of human alveolar macrophages and intra-pulmonary imaging demonstrate rapid and effective target engagement with a fluorescently tagged ADS032, and efficacy in inhibiting NLRP3, highlighting impending clinical translation.

ADS032 is therefore a potential drug to treat NLRP1- and NLRP3-associated inflammatory diseases in first in human trials and a novel tool to allow examination of the role of NLRP1 in human disease.

5

MLKL mutation causes defective immune response during bacterial infection

Sarah E Garnish[1, 2], Katherine R Martin[1, 2], Maria Kauppi[1, 2], Rebecca Ambrose[3, 4], Vik Ven Eng[3, 4], Andre L Samson[1, 2], Jaclyn S Pearson[3, 4, 5], John Silke[1, 2], James M Murphy[1, 2], Joanne M Hildebrand[1, 2]

1. University of Melbourne, Department of Medical Biology, Parkville, Australia

2. The Walter and Eliza Hall Institute of Medical Research, Officer, VIC, Australia

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

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

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

Necroptosis is an inflammatory form of programmed cell death that originated as a pathogen defence mechanism. During bacterial and viral infections, the necroptotic executioner protein, MLKL, has been identified as both a protector against- and progressor of- infection. Across the globe, 2-3% of humans carry a single nucleotide polymorphism, *Ser132Pro,* in *MLKL* that confers a gain in necroptotic function. In human cells this is detected under pharmacological and regulatory inhibition. In mouse cells is detected as an enhanced sensitivity to IFN- β induced death. In a CRISPR-Cas9 generated mouse model, $Mlkl^{S131P}$, we observed that this gain-of-function mutation manifests *in vivo* changes to the immune response, defective emergency haematopoiesis, and impaired bacterial clearance. At steady state, $Mlkl^{S131P}$ homozygotes present with significant reductions in the inflammatory monocyte population in the bone marrow. Following myelosuppressive irradiation or during competitive bone marrow transplants, a broader hematopoietic defect is observed across all immune cells. This $Mlkl^{S131P}$ driven hematopoietic dysfunction results in defective mobilisation of innate and adaptive immune cells under inflammatory conditions. Specifically, we show that $Mlkl^{S131P}$ homozygosity reduces the capacity to clear *Salmonella* during infection, with the increased bacterial burdens present in the spleen and liver accompanied by reduced quantities of peripheral lymphocytes and monocytes. Collectively, these findings indicate mutations that alter *MLKL* function can disrupt the integral inflammatory and immune responses thus, raising important questions as to health implications for the proportion of the population that are carriers.

6

Probing the molecular basis of curbing TLR4-driven inflammatory responses

Thomas E Schultz[1], Chien-Hsiung Yu[1], Meg L Donovan[1], Makrina Totsika[2], Matthew J Sweet[3], Antje Blumenthal[1]

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

2. School of Biomedical Sciences, Faculty of Health, Queensland University of Technology, Brisbane, QLD, Australia

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

Publish consent withheld

7

IFNλ1 utilises interferon alpha/beta receptor 2 to induce signalling and anti-viral defence in lung epithelial cells

Nicky de Weerd[1], Julian Vivian[2], Tony Matthews[1], Amina Ismail[1], Tatiana Soares da Costa[3], Jodee Gould[1], Jamie Gearing[1], Natalia Sampaio[1], Daniel Garama[1], Daniel Gough[1], Eveline de Geus[1], Qian Zhang[4], Jean-Laurent Casanova[4], Rune Hartmann[5], Meredith O'Keeffe[6], Charles Rice[4], Paul Hertzog[1]

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

2. St Vincent's Institute, Fitzroy, VIC, Australia

3. Waite Research Institute, The University of Adelaide, Urrbrae, South Australia, Australia

4. St Giles Laboratory of Human Genetics of Infectious Disease, The Rockefeller Institute, New York, USA

5. Aarhus University, Aarhus, Denmark

6. Monash Biomedical Discovery Institute, Clayton, VIC, Australia

Type I and type III interferons (IFNs) together regulate the early response to viral infection including SARS-CoV-2. Despite displaying structural and functional similarities, type I and type III IFNs operate via different heterodimeric receptor complexes. Type I IFNs (IFNα, IFNβ, etc) bind ubiquitously expressed IFN alpha/beta receptor (IFNAR)1 and IFNAR2. In contrast, type III IFNs (IFNλ1-4) bind a unique high affinity receptor, IFN lambda receptor (IFNLR)1 which displays restricted cellular expression and a widely expressed low affinity receptor shared with the Interleukin(IL)10 cytokine superfamily (IL10R2).

Here we report that the type I IFN receptor IFNAR2 is required for mediating the anti-viral properties of the type III IFN, IFNλ1 on lung epithelial cells. Using microscale thermophoresis, we observed high affinity binding of IFNλ1 to the extracellular domain of IFNAR2; a characteristic not displayed by IFNλ3 or IFNλ4. We used direct protein-protein interactions and mass spectrometry to show that IFNAR2 co-operates with IFNLR1 to bind IFNλ1, forming a novel ternary protein complex. Furthermore, IFNλ1, but not IFNλ3, utilise IFNAR2 on the surface of lung epithelial cells for efficient expression of interferon regulated genes (IRGs) including key anti-viral genes such as *MX2, OAS2, BST2, STAT1, STAT2, IFITM1* and *IFITM3*. Finally, we demonstrate that IFNλ1, but not IFNλ3, requires IFNAR2 on lung epithelial cells to upregulate cell-surface levels of the viral restriction factor IFITM3 and to efficiently protect cells from viral infection.

Our data shows that IFNλ1 binds IFNAR2 to form a novel protein complex that is required for efficient IFNλ1-mediated gene induction and anti-viral activity in lung epithelial cells.

8

Predicting immunogenicity using human tonsil organoids

Lisa Wagar[1]

1. University of California Irvine, Irvine, CA, United States

Highly effective vaccines elicit specific, robust, and durable adaptive immune responses and ideally stimulate local immune responses at the site of infection to mediate optimal protection. However, it has been challenging to understand and design vaccine candidates with these properties beyond empirical testing in animal models and validation in clinical trials. To make advances in informed vaccine design, it is critical that we understand the cellular dynamics underlying human adaptive immune responses to different antigen formats. Tonsils are considered both lymphoid and mucosal tissues; they are also accessible from otherwise-healthy patients. In this study, we sought to understand how antigen-specific B and T cells are recruited to adaptive immune responses within the mucosal site. Using a human tonsil organoid model, we tracked the dynamics of the adaptive immune response to influenza vaccine and virus modalities. Each antigen format elicited distinct B and T cell responses, including differences in the magnitude, diversity, phenotype, function, and breadth. These differences culminated in striking changes in the corresponding antibody response. We show that a major source of response variability related to antigen format is the ability to recruit naive vs. memory B cells. These findings have important implications for vaccine design and the generation of protective immune responses in the upper respiratory tract.

9

High-throughput characterization of antibody responses in SARS-CoV-2 infection and vaccination

Amy Chung[1]

1. University of Melbourne, Doherty Institute, Melbourne, VIC, Australia

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19 disease, remains a challenge to global public health. Emerging viral variants with mutations in the spike protein can facilitate immune escape from vaccine-induced immunity. Antibodies can mediate protection through neutralisation and Fc-mediated functions. Unlike neutralizing Abs that must target specific anti-viral regions that prevent infection, antibodies that mediate Fc effector functions can target any viral surface antigen and are therefore less affected by viral mutational escape.

To facilitate surveillance and improved understanding of the importance of both neutralizing and Fc effector antibody functions against SARS-CoV2 and emerging variants, we developed a suite of high throughput antibody assays capable of assessing neutralization and the Fc profiles (isotype, subclass, Fc Receptor binding) to an extensive panel of SARS-CoV-2 proteins including variants of concern. Antibody responses from both plasma and mucosal samples were characterized across a range of clinical cohorts including SARS-CoV2 infected and vaccinated individuals.

Here we demonstrate that SARS-CoV-2 antibody responses are influenced by numerous factors including age, disease severity, immunogenetics, prior antigen exposure (including number of vaccine doses and/or prior SARS-CoV2 infection), along with vaccine platform. Our data highlights the numerous factors that modulate humoral immunity and provides insights into ways to improve SARS-CoV-2 vaccination strategies across different populations.

10

Exploiting the tropism of viral vectored vaccines for optimal induction of liver resident T cells and long-term protection against malaria

Alexandra Spencer[1], Marta Ulaszewska[1], Andrés Noé[1], Sarah Sebastian[1], Adrian Hill[1]

1. The Jenner Institute, University of Oxford, Oxford, United Kingdom

Vaccines are amongst the most cost-effective tools in reducing the burden of infectious diseases. With the ability to induce both cellular and humoral immunes responses, viral-vectored vaccines have become a promising approach against a variety of diseases. While the standard route of vaccination, intra-muscular injection, is highly effective at inducing circulating responses and protecting from severe disease, there is increasing evidence that improved vaccine efficacy will come by targeting the immune response to the site of pathogen exposure. We have previously shown that intravenous Adenovirus administration leads to induction of liver resident T cells that alone are able to protection against liver-stage malaria.

In this study we aimed to identify the key factors driving the induction and maintenance of Trm cells to optimise viral vector vaccination regimens for translation to the clinic. We compared three different viral vectors, Adenovirus (Ad), modified vaccinia Ankara (MVA) poxvirus and Adeno-associated virus (AAV), for their capacity to target the immune response to the liver. Quantity and quality of the immune response was measured by flow cytometry while vaccine efficacy was assessed in a mouse malaria challenge model.

Although the kinetics of antigen expression differed between vectors, an intravenous targeting dose of all vectors induced high numbers of antigen specific T cells in the liver and protection from malaria. However, optimal induction of liver Trm cells was dependant on the level of antigen presentation in the liver during the induction phase, with liver tropic vectors (Ad and AAV) better able to target T cells to the liver. While the local inflammatory microenvironment induced by different vectors did not appear to impact Trm numbers, differences in the cytokine profiles between vectors was observed. Sporozoite exposure enhanced the maintenance of long-lived protective T cells but only following Ad and AAV vector administration, suggesting an underlying relationship between antigen expression and Trm maintenance. As Adenoviral vectors are being developed for a broad variety of disease, we are now exploring their capacity to target the immune response to other organs.

11

Systems immunology of T follicular helper cells reveals impaired BCL6 function in ageing.

Danika L Hill[1, 2]

1. Monash University, Melbourne, VICTORIA, Australia

2. Babraham Institute, Cambridge, United Kingdom

Antibody production following vaccination can provide protective immunity to subsequent infection from pathogens such as influenza. However, circumstances where antibody formation is impaired after vaccination, such as in older people, require us to better understand the cellular and molecular mechanisms that underpin successful vaccination in order to improve vaccine design for at risk groups. By studying responses to seasonal influenza vaccination, we show that T follicular helper (Tfh) cell differentiation is impaired in older people. This age-dependent defect was not due to contraction of the TCR repertoire with age, and instead involved the aberrant expression of genes normally repressed by BCL6 in Tfh cells, such as Lamin A. Together, our data suggests that impaired BCL6 function limits Tfh differentiation and restrains humoral responses to immunisation in older people.

12

Butyrophilin 3A1 interacts with the Vγ9Vδ2$^+$ T cell receptor

Tom S Fulford[1], Caroline Soliman[1], Rebecca G Castle[1], Marc Rigau[1, 2], Zheng Ruan[1], Olan Dolezal[3], Rebecca Seneviratna[1], Hamish G Brown[4], Eric Hanssen[4, 5], Shihan Li[1], Samuel J Redmond[1], Amy Chung[1], Michael A Gorman[5], Andrew Hammet[6], Michael W Parker[5, 7], Onisha Patel[8, 9], Tom S Peat[3], Janet Newman[3], Andreas Behren[10], Nicholas A Gherardin[1], Dale I Godfrey[1], Adam P Uldrich[1]

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

2. University of Bonn, Bonn, Germany

3. Biomedical Manufacturing, Commonwealth Scientific and Industrial Research Organisation, Parkville, VIC, Australia

4. Ian Holmes Imaging Centre, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne, VIC, Australia

5. Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne, VIC, Australia

6. CSL Limited, Bio21 Institute, Parkville, VIC, Australia

7. St. Vincent's Institute of Medical Research, Fitzroy, VIC, Australia

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

9. Department of Medical Biology, University of Melbourne, Parkville, VIC, Australia

10. Olivia Newton-John Cancer Research Institute, Heidelberg, VIC, Australia

Whilst αβ T cell recognise MHC-Ag complexes for immunity, very little is known about the Ag-presentation elements of γδ T cells. Recently, butyrophilin (BTN) molecules have emerged as key regulators of γδ T cell immunity, however, the mechanism by which they activate the γδTCR is unknown. In humans, most circulating γδ T cells are phosphoantigen (pAg)-reactive Vγ9Vδ2$^+$ T cells, which play a critical role in immunity to most bacterial and apicomplexan parasites infections as well as cancer. To explore how BTNs co-ordinate γδ T cell immunity, we solved the crystal structure of Vγ9Vδ2$^+$ TCR in complex with BTN member 2A1 (BTN2A1), revealing that BTN2A1 engages the side of the γδTCR. Intriguingly, we also found that a second ligand, namely BTN3A1, can bind the exposed apical surface of Vγ9Vδ2$^+$ TCR alongside BTN2A1. However, BTN3A1 binding only occurred following cross-linking with an agonist anti-BTN3A1 mAb, or alternatively, following mutation of a negative regulatory residue, Lys53 of the TCR δ-chain. Unexpectedly, BTN2A1 and BTN3A1

ectodomains were also shown to interact directly with each other in *cis*, forming heteromers on the surface of Ag-presenting cells, and that this BTN2A1–BTN3A1 interaction depends upon the same epitopes that BTN2A1 and BTN3A1 each use to engage γδTCR. We propose that this heteromer represents a 'closed state', which impairs the ability of γδTCR to bind the BTN complex. Indeed, either forced separation or locking together of BTN2A1 and BTN3A1 resulted in enhanced or abrogated γδTCR reactivity, respectively. Finally, we demonstrate that pAg can modulate γδTCR affinity for the BTN complex, suggesting that pAg-sequestration can lead to a conformational change in the BTN ectodomains enabling γδTCR to bind. Our findings reveal a new paradigm in immune activation, whereby γδ T cells recognise BTN complexes following conversion of BTN epitopes from an closed-state into a permissive-state.

13

T cell immunity in cytomegalovirus infection

Annette Oxenius[1], Senta Walton[1], Nicole Torti[1], Josua Oderbolz[1], Nathan Zangger[1], Rahel Gertsch[1]

1. ETH Zürich, Zurich, ZURICH, Switzerland

CMV infection results first in lytic replication which is controlled by CMV-specific T cells, followed by establishment of viral latency. CMV can sporadically reactivate in latently infected cells which is quickly controlled by CMV-specific T cells. CMV infection induces an atypical CD8 T cell response, characterized by the accumulation of a subset of highly protective CD8 T cells exhibiting an effector-like phenotype, a process termed "memory inflation". Their activated phenotype is caused by repetitive antigen encounter invoked by sporadic viral reactivation events. Although the half-life of inflationary T cells in mice is estimated to be around 6-12 weeks, the pool of inflationary T cell stabilizes at constant frequencies, implying continuous replenishment. A small subset of $Tcf1^+$ inflationary MCMV-specific T cells, enriched in lymph nodes, feeds into the pool of inflationary CD8 T cells after antigen exposure.

Secretory glands are an attractive target tissue for viruses to persist and exploit mucosal secretions for dissemination, such as the salivary glands (SGs) in case of CMV. Murine CMV (MCMV) infection has revealed that virus-mediated MHC class I downregulation on infected epithelial cells renders the SG uniquely resistant to CD8 T cell mediated virus control. Instead, CD4 T cells are required to cease virus replication through the secretion of IFNγ and TNF. However, the question why and how long-lasting productive virus infection is maintained in the SG remains open. One important aspect that has so far not received enough attention is information about in situ tissue immunity in the SGs during MCMV infection, including spatial information about infection foci, distribution of infiltrating virus-specific T cells, sites of antigen recognition and IFNγ production. Using advanced microscopy methods in combination with mathematical modelling, we propose a scenario in which MCMV antigens in the SGs are sensed by virus-specific CD4 T cells only in an indirect manner, after remnants of previously infected cells have been engulfed by local antigen-presenting cells (APCs). This leads to locally confined IFNγ secretion, affording protection only in restricted areas. Eventual control occurs if local IFNg-concentrations allow sufficient accumulation of protected sites, and thus restriction of viral spread on an organ-wide range.

14

Inner workings of the inflammasome engine

Hao Wu[1]

1. Boston Children's Hospital and Harvard Medical School, Boston, MA, United States

Inflammasomes are supramolecular complexes that activate caspase-1 and other inflammatory caspases. A family of proteins known as NLRs (NACHT and LRR-containing) comprise main sensor proteins for inflammasomes, including the PYD-containing NLRs – NLRP3, NLRP6 and NLRP1. How these proteins induce inflammasome activation remain unclear. In this talk, I will present both published and unpublished data, and elaborate on what we have learned in the mechanisms of activation of these NLRs, compare and contrast these mechanisms and raise questions that await to be addressed.

References:

1. Andreeva L, David L, Rawson S, Shen C, Pasricha T, Pelegrin P, Wu H (2021). NLRP3 cages revealed by full-length mouse NLRP3 structure control pathway activation. ***Cell*** 184: 6299-6312
2. Hollingsworth LR, Sharif H, Griswold AR, Fontana P, Mintseris J, Dagbay KB, Paulo JA, Gygi SP, Bachovchin DA, Wu H (2021). DPP9 sequesters the C terminus of NLRP1 to repress inflammasome activation. ***Nature*** 592: 778-783
3. Xia S, Zhang Z, Magupalli VG, Pablo JL, Dong Y, Vora SM, Wang L, Fu T-M, Jacobson MP, Greka A, Lieberman J, Ruan J, Wu H (2021). Gasdermin D pore structure reveals preferential release of mature interleukin-1. ***Nature*** 593: 607-611
4. Shen C, Li R, Negro R, Cheng J, Vora SM, Fu TM, Wang A, He K, Andreeva L, Gao P, Tian Z, Flavell RA, Zhu S,Wu H (2021). Phase Separation Drives RNA Virus-Induced Activation of the NLRP6 Inflammasome. ***Cell*** 184: 5759-5774

15

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

Harshini Weerasinghe[2, 1], Claudia Simm[1, 3], Tirta Djajawi[4], Irma Tedja[2, 1], Tricia L Lo[1, 3], David Shasha[5], Naama Mizrahi[5], Françios AB Olivier[2, 1], Mary Speir[4], Kathryn Lawlor[4], Ronen Ben-Ami[5, 6], Ana Traven[1, 3]

1. Infection Program and the Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Victoria, Australia

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

3. Centre to Impact AMR, Monash University, Melbourne, Victoria, Australia

4. Hudson Institute of Medical Research, Melbourne, Victoria, Australia

5. Infectious Diseases Unit, Tel Aviv Sourasky Medical Centre, Tel Aviv, Israel

6. Sachler School of Medicine, Tel Aviv University, Tel Aviv, Israel

Opportunistic fungal pathogens that cause human disease pose a serious threat to public health. The rapid emergence of drug resistant pathogenic fungi such as *Candida auris,* along with the overuse of commercially available antifungal drugs and inadequacies in drug

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

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

16

Brief update on non-infammasome NLRs

Thomas Kufer[1]

1. University of Hohenheim, Stuttgart, BW, Germany

Coming soon

Nod-like receptors (NLR) represent an important family of cytosolic pattern-recognition receptors (PRRs) that contribute to innate and adaptive immune responses in mammals. In my presentation I will focus on NLR signalling of NOD1 and NLRC5.

The receptor interacting serine/threonine kinase 2 (RIPK2) is essential for linking activation of the pattern recognition receptors NOD1 and NOD2 to cellular signalling events. We recently demonstrated that RIPK2 forms detergent insoluble complexes in the cytosol of host cells upon infection with invasive enteropathogenic bacteria. I will summarize our current understanding of NOD1 activation and of RIPosome formation.

Others and we identified NLRC5 as the key transcriptional regulator of major histocompatibility (MHC) class I genes. Our recent observations suggest novel roles for NLRC5 also in metabolic traits. I will discuss our recent findings, revealing that Nlrc5 contributes to weight gain and adipose tissue development in mice which involves transcriptional enhancement of PPARγ targets by NLRC5 that is co-regulated by Sin3A.

17

Preparing for disease X

Glenn Marsh[1, 2]

1. Australian Animal Health Laboratory, CSIRO Health and Biosecurity, Geelong, VIC, Australia

2. CSIRO, Geelong, VIC, Australia

Disease X is a “placeholder name” that was first used by the World Health Organization (WHO) in a 2018 on their shortlist of blueprint priority diseases of pandemic concern requiring R&D. The term was used to represent a hypothetical, unknown pathogen that could cause a future epidemic or pandemic. The term was used to encourage the infectious disease R&D community to develop broad approaches to planning for future pandemic, with technology being sufficiently flexible to adapt to an unknown pathogen (e.g., broader vaccines and manufacturing facilities). The emergence of SARS-coronavirus 2 and resulting the COVID-19 global pandemic was the first pathogen to meet the definition of a ‘Disease X’ emergence.

CSIRO has a current focus on preparedness for future emergence of pandemic pathogens, focussing in the areas of pre-clinical testing and manufacturing. The development of appropriate animal models for vaccine and therapeutic testing is a bottle-neck that is often faced prior to the assessment of vaccine and therapeutic candidates, with this being a more challenging issue when it comes to emergence. The talk will focus on the aspects that must be considered when developing animal models to generate data required by regulators for vaccine approvals, and what can be done now to be better prepared for the next Disease X emergence.

18

Deploying *Wolbachia*-infected mosquitoes to control - and potentially eliminate? - dengue and other Aedes-borne diseases

Katie Anders[1, 2]

1. World Mosquito Program, Monash University, Clayton, Victoria, Australia

2. School of Public Health and Preventive Medicine, Monash University, Melbourne, Victoria, Australia

Dengue is an arboviral disease transmitted by *Aedes aegypti* mosquitoes, which causes a substantial health and economic burden for half of the world's population. *Ae. aegypti* also transmit chikungunya, Zika and yellow fever viruses, and its geographic range is expanding due to climate change, urbanisation and increased global connectivity. The World Mosquito Program (WMP) is a global not-for-profit initiative that aims to provide an innovative new tool for the prevention of Aedes-borne diseases using *Wolbachia*, a common insect bacteria. Stable infection of *Ae. aegypti* with *w*Mel-strain *Wolbachia* significantly reduces their ability to transmit medically-important arboviruses including dengue, Zika and chikungunya, and manipulates the mosquitoes’ reproductive outcomes such that *Wolbachia* introgresses into wild *Ae. aegypti* populations following short-term open releases. I will present results from a cluster randomised trial in Indonesia and non-randomised field releases in 11 countries showing that releases of *Wolbachia*-infected mosquitoes have resulted in successful and sustained *Wolbachia* introgression into local *Ae aegypti* populations, and significant reductions in the

incidence of dengue and other Aedes-borne diseases. The potential for large-scale *Wolbachia* implementation to lead to local elimination of dengue will be discussed.

19

Robust and prototypical immune responses towards COVID-19 BNT162b2 vaccine in First Nations Australian people are impacted by co-morbidities

Katherine Kedzierska[1], Wuji Zhang[1], Lukasz Kedzierski[1], Brendon Chua[1], Mark Mayo[2], Claire Lonzi[2], Vanessa Rigas[2], Bianca Middleton[2], Hayley McQuilten[1], Louise Rowntree[1], Lilith Allen[1], Ruth Purcell[1], Hyon Xhi Tan[1], Jan Petersen[3], Priyanka Chaurasia[3], Francesca Mordant[1], Kanta Subbarao[1], Florian Krammer[4], Allen Cheng[5], Adam Wheatley[1], Stephen Kent[1], Jamie Rossjohn[5], Amy Chung[1], John Boffa[6], Adrian Miller[7], Sarah Lynar[2], Jane Nelson[2], Oanh Nguyen[1], Jane Davies[2]

1. Department of Microbiology & Immunology, University of Melbourne at Doherty Institute, Parkville, VIC, Australia

2. Menzies School of Health Research, Darwin, TN, Australia

3. Infection and Immunity Program and Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, Vic, Australia

4. Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, USA

5. Department of Infectious Diseases, The Alfred Hospital and Central Clinical School, Monash University, Melbourne, Australia

6. Central Australian Aboriginal Congress, Alice Springs, ACT, Australia

7. Indigenous Engagement, CQUniversity, Townville, QLD, Australia

Higher morbidity and mortality rates from pandemic COVID-19 are disproportionately observed in high-risk groups, including Indigenous people globally. We evaluated immune responses in Australian First Nations people and non-Indigenous individuals after COVID-19 BNT162b2 vaccination, and in First Nations people hospitalized with COVID-19. Our study provides evidence that First Nations people elicit effective immune responses following BNT162b2 vaccination: neutralizing antibodies, anti-RBD-antibodies, SARS-CoV-2 Spike-specific B cells, $CD4^+$ and $CD8^+$ T cells measured by activation-induced marker (AIM) assay, IFNγ/TNF production and peptide-HLA tetramer staining directly *ex vivo*. In First Nations participants, RBD IgG antibody titres positively correlated with the body mass index, while negatively correlated with age. Importantly, however, reduced SARS-CoV-2 antibody axis (RBD-antibodies, spike-specific B cells, T follicular helper (Tfh) cells) was found in vaccinated First Nations participants with chronic conditions (diabetes, renal disease). This was strongly associated with altered IgG glycosylation and increased IL-18 levels. First Nations people hospitalized with COVID-19 elicited broad immunity, including RBD/nucleoprotein-antibodies, antibody-secreting cells, Tfh cells, tetramer-specific $CD4^+$/$CD8^+$ T cells with prominent TCR motifs and peptide-stimulated AIM^+ T cell frequencies. Our study provides key insights into immune responses following SARS-CoV-2 infection and vaccination in Indigenous people, links for the first-time antibody glycosylation levels to reduced antibody titres post COVID vaccination and emphasizes the importance of vaccine-induced T cells in individuals with co-morbidities.

20

Integrated systems immunology approach identifies impaired effector T cell memory responses as a key feature of progression to severe dengue haemorrhagic fever

Stephanie I Studniberg[1], Lisa J Ioannidis[1], Emily Eriksson[1], Alexandra Garnham[1], Tedjo Samono[2], Diana S Hansen[1]

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

2. Eijkman Institute for Molecular Biology, Jakarta, Indonesia

Dengue fever (DF) is one of the most common mosquito-borne viral diseases in the world. Typical symptoms of (DF) include fever accompanied by headache, muscle pains, rash, cough, and vomiting. A proportion of cases can progress to severe dengue, known as dengue haemorrhagic fever (DHF), associated with increased vascular permeability, thrombocytopenia, and haemorrhages. To date, there are no accurate means to predict which patients will progress to severe dengue at first presentation, meaning that in endemic areas, health facilities are overwhelmed with patients admitted for observation, posing a huge socio-economic burden on health systems. Strategies aimed at predicting disease progression are urgently needed to improve patient management. To address this issue, we pursued a systems immunology approach integrating plasma cytokine profiling, high-dimensional mass cytometry and peripheral blood mononuclear cell (PBMC) transcriptomic analysis in a prospective study of individuals from a dengue-endemic area of Indonesia, that progressed to develop either uncomplicated DF or DHF. Elevated levels of inflammatory chemokines as well as high frequencies of non-classical monocytes were associated with progression to DHF. Furthermore, circulating levels of $CD4^+$ non-classical monocytes predicted risk of severe disease at the onset of fever, after a primary and secondary dengue virus infection. Progression to severe dengue was also associated with an important transcriptional signature featuring impaired T cell activation and cell division as well as reduced glycolysis and oxidative stress response. In contrast, protection from DHF was associated with high frequencies of $CD4^+$ and $CD8^+$ effector memory T cells expressing elevated levels of the co-stimulatory molecules ICOS and CD27, as well as high numbers of $CXCR3^+$ T_{H1}-polarised T follicular helper cells. Our research demonstrated that efficient effector T cell memory activation plays a critical role in protection from DHF during secondary dengue infections. The results also provide proof of concept for the potential of system immunology approaches to identify discrete populations in the blood predicting increased risk of DHF to develop diagnostic tools for early detection of complicated cases at point of care.

21

Microbiome manipulation in the treatment of ulcerative colitis

Nadeem O Kaakoush[1]

1. University of New South Wales, Kensington, NSW, Australia

Faecal microbiota transplantation (FMT) is effective at treating recurrent *Clostridioides difficile* infection (rCDI) and is now recommended within clinical guidelines. Although success rates are consistently high for rCDI (>90%), efficacy in treatment of ulcerative colitis (UC), one of the main subtypes of inflammatory bowel diseases, have been less impressive. In those with mild to moderate UC, FMT induces

remission in 30-40% of patients, emphasising the need to identify microbial and host markers that are associated with treatment success. I will present our work assessing the utility of FMT in the treatment of UC and understanding what properties make FMT successful in these patients. This includes discussion of our donor selection strategies, randomised clinical trials of FMT in UC, studies on microbial markers of therapeutic response in patients as well as donor efficacy, and more recently, studies on host markers of disease remission. Our findings may be of importance in optimising donor selection and designing future live microbial therapeutics for UC, and in the discovery of potential druggable targets in UC.

22

Key commensal species breakdown phenolic compounds to support community stability within the gastrointestinal microbiome

Remy B Young[1, 2], Tamblyn Thomason[1, 3], Jodee Gould[1, 3], Emily Rutten[1, 3], Marina Iacovou[1, 3], Samuel Costello[4, 5], Emily Gulliver[1, 3, 2], Vanessa Rossetto Marcelino[1, 3], Samuel Forster[1, 3]

1. Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Victoria, 3168, Australia

2. Infection and Immunity Program, Monash Biomedicine Discovery Institute and Department of Microbiology, Monash University, Clayton, Victoria, 3800, Australia

3. Department of Molecular and Translational Sciences, Monash University, Clayton, Victoria, 3800, Australia

4. Department of Gastroenterology, The Queen Elizabeth Hospital, Woodville, South Australia, 5011, Australia

5. School of Medicine, University of Adelaide , Adelaide, South Australia, 5005, Australia

Compositional changes in the commensal bacteria of the human gastrointestinal tract have long been associated with a plethora of conditions, including inflammatory bowel disease, diabetes and obesity. Manipulating these bacterial communities, termed the microbiome, represents a potential avenue for disease treatment. Diet and dietary interventions have the ability to change the microbiome, however, there is limited knowledge on how dietary compounds impact the growth of individual bacterial species and underpin community structures within the gastrointestinal tract. This has restricted our ability for targeted modulation of the microbiome through dietary interventions, and limited the scope for microbiome-based therapies broadly.

Applying a novel high-throughput screening technique, we aimed to undertake functional analysis of dominant microbiome species to understand how dietary compounds impact individual isolates and the microbiome community structure. Initial phenotypic analysis investigating the response of 23 common and phylogenetically diverse gastrointestinal bacteria to 43 dietary compounds highlights strain-level growth responses and nutrient dependencies in these isolates for the first time. We identified a group of four phenolic compounds, which are associated with health in the human diet, that were inhibitory to the growth of over 80% of the bacteria tested. Widespread inhibition by these compounds was confirmed through screening of a further 119 commensals, with 112 (94%) isolates showing inhibition in at least one of the four phenolic compounds. In order to identify potential bacterial candidates that may prevent community disruption by this phenolic compound induced inhibition, genomic analyses were applied to resistant species whole genomes to identify isolates with the potential to breakdown phenolic compounds. Co-culture experiments validated the role of these key species in preventing the inhibition of susceptible isolates by phenolic compounds. This work has contributed to a foundational understanding of the complex interactions that may greatly impact the use of dietary interventions for microbiome therapies.

23

Investigating antimicrobial activity by bacterial members of the human gastrointestinal microbiome

Jamia Hemphill[1, 2], Marina Yakou[3], Jodee Gould[4, 2], Emily Gulliver[4, 2], Samuel Forster[4, 2]

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

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

3. Olivia Newton-John Cancer Research Institute , Heidelberg, Victoria, Australia

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

To combat the increasing prevalence of antimicrobial resistance in bacterial pathogens, discovery of novel antimicrobial molecules is vital. In microbial communities, bacteria have developed various methods to increase their competitive advantage, including the production of antimicrobial molecules. We have exploited some of these antimicrobials to develop our current clinical antibiotics, however there are still many bacterial communities that have not yet been investigated for novel antimicrobials, including the human gastrointestinal microbiome. Due to the abundance and diversity of bacterial species in the gastrointestinal tract, competition in this environment is high, and microbiota species may benefit from the advantages provided by antimicrobial production. Genomic analysis shows that many of these species carry biosynthetic gene clusters that may encode for novel antimicrobial molecules, which can now be investigated in the laboratory due to recent advancements in culturing techniques that allow us to cultivate the previously “unculturable” members of the human gastrointestinal microbiota. This technique offers a promising approach in exploring a new source of therapeutics for the treatment and prevention of drug-resistant infections. In this study, we used a high throughput culturing method, to screen a panel of 287 commensal bacterial isolates for antimicrobial activity against eight multi-drug resistant strains of four species of gastrointestinal pathogens: *Clostridiodes difficile, Escherichia coli, Enterococcus faecium,* and *Klebsiella pneumoniae*. Of the isolates screened, 148 (52%) exhibited inhibition of at least one pathogen strain. From these isolates, 20 candidates that displayed inhibition of all four pathogen species were selected for further validation of inhibitory activity, which confirmed inhibition in six candidates. To determine mechanism of inhibition, the cell free supernatant of these candidates was used in a well-diffusion assay, however these exhibited variable results. Further investigation into the requirements for antimicrobial production in these commensal strains is required and will aid in the characterisation of the molecules responsible for inhibition, which could lead to development of novel antibiotics.

24

Association of Fecal DNA Virome with the Development of Colorectal Neoplasia

Yingshi Li[1], Fan Zhang[1], Huimin Zheng[1], Sanjna Kalasabail[1], Chloe Hicks[1], Ka Yee Fung[2], Adele Preaudet[2], Tracy Putoczki[2], Julia Beretov[3], Ewan K A Millar [3], Emad El-Omar[1], Xiao-Tao Jiang[1], Howard C H Yim[1]

1. Microbiome Research Centre, UNSW, Sydney, New South Wales, Australia

2. Personalised Oncology Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia

3. Department of Anatomical Pathology, NSW Health Pathology, St George Hospital, Kogarah, NSW, Australia

Background Alterations of the gut virome has been associated with human colorectal cancer (CRC)[1,2] , but the timing and mechanism of such alterations remain unknown. To answer this question, we performed a longitudinal study on an azoxymethane (AOM)-induced CRC murine model.

Method Four adult A/J mice per sex were intraperitoneally injected with 8mg/kg AOM or PBS weekly for 6 weeks, and the colons of the mice were monitored biweekly by colonoscopy until 24 weeks after the initial injection. At least a pair of endoscopically normal and tumour tissues were biopsied during colonoscopies of each mouse at different time points. Tissues were formalin-fixed, paraffin-embedded, stained with hematoxylin and eosin (H&E), and examined by a pathologist blindly. Stool samples from weeks 0, 10, 12, 14, 20, and 24 were subjected to DNA extraction and whole metagenomic analysis. The number of tumours was calculated by analysis of colonoscopic videos, and the tumour size was scored based on the diameter of the colon lumen occupied by a tumour.

Results The number and size of the tumours increased as the mice aged in the AOM-treated group, as compared to the control group. Tumours were first observed in the AOM group in week 12. Significantly lower alpha diversity and shift in viral profile were observed when tumours first appeared. Novel viruses from the genera *Brunovirus*, and *Hpunavirus* were identified to be positively associated with tumour growth and enriched at a late time point in the AOM group. On the other hand, members from *Lubbockvirus* were negatively correlated with the tumour growth. Network analysis revealed two clusters of bacteriophages in the AOM virome. While one cluster positively correlated with tumour growth, the other cluster negatively correlated with tumor growth.

Conclusion Our findings suggest that the gut virome alters along with colorectal tumour formation and provide strong evidence of a potential role for bacteriophage in the development of colorectal neoplasia.

1. Nakatsu G, Zhou H, Wu WKK, Wong SH, Coker OO, Dai Z, Li X, Szeto CH, Sugimura N, Lam TY, Yu AC, Wang X, Chen Z, Wong MC, Ng SC, Chan MTV, Chan PKS, Chan FKL, Sung JJ, Yu J. Alterations in Enteric Virome Are Associated With Colorectal Cancer and Survival Outcomes. Gastroenterology. 2018 Aug;155(2):529-541.e5.
2. Hannigan GD, Duhaime MB, Ruffin MT 4th, Koumpouras CC, Schloss PD. Diagnostic Potential and Interactive Dynamics of the Colorectal Cancer Virome. mBio. 2018 Nov 20;9(6):e02248-18.

25

Functional differences between cervicovaginal *Lactobacillus* species that protect against HIV infection

Jenna Wilson[1], Arghavan Alisoltani[2, 3], Monalisa T Manhanzva[2, 3], Grace Androga[1], Matthys Potgieter[2, 5, 4], Liam Bell[6], Elizabeth Ross[6], Arash Iranzadeh[2, 4], Imane Allali[7, 8, 4], Nicola Mulder[2, 9, 4], Smritee Dabee[2, 3, 10], Shaun L Barnabas[3, 11], Hoyam Gamieldien[2, 3], Jonathan M Blackburn[2, 5], Linda-Gail Bekker[2, 12], Heather B Jaspan[2, 10, 13], Jo-Ann S Passmore[2, 3, 14, 15], Lindi Masson[1, 2, 3, 14, 16]

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

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

3. Division of Medical Virology, Department of Pathology, University of Cape Town, Cape Town, South Africa

4. Computational Biology Division, Department of Integrative Biomedical Science, University of Cape Town, Cape Town, South Africa

5. Division of Chemical and Systems Biology, Department of Integrative Biomedical Sciences, University of Cape Town, Cape Town, South Africa

6. Centre for Proteomic and Genomic Research, Cape Town, South Africa

7. Faculty of Sciences, , University Mohammed V, Rabat, Morocco

8. Laboratory of Human Pathologies Biology, Department of Biology and Genomic Center of Human Pathologies, Mohammed V University, Rabat, Morocco

9. Centre for Infectious Diseases Research (CIDRI) in Africa Wellcome Trust Centre, University of Cape Town, Cape Town, South Africa

10. Seattle Children's Research Institute, University of Washington, Washington, USA

11. Famcru, University of Stellenbosch, Cape Town, South Africa

12. Desmond Tutu HIV Centre, University of Cape Town, Cape Town, South Africa

13. Division of Immunology, Department of Pathology, University of Cape Town, Cape Town, South Africa

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

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

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

Background: Young South African women are at disproportionate risk of bacterial vaginosis (BV) and HIV infection, and strategies to reduce these outcomes are urgently needed. Cervicovaginal *Lactobacillus* species such as *L. crispatus* protect against HIV, while *L. iners* is considered less protective. To understand this difference, this study compared the protein and functional profiles of cervicovaginal *L. iners* and *L. crispatus* communities in young South African women.

Methods: Vaginal swab samples collected from women (aged 16-22 years) with cervicovaginal microbiota dominated by either *L. crispatus* (n=19) or *L. iners* (n=50), were analysed using liquid chromatography-tandem mass spectrometry, a custom cervicovaginal database and MaxQuant. Data analysis comprised moderated t-tests, principal component analysis and hierarchical clustering.

Results: A total of 218 *L. iners* and 276 *L. crispatus* proteins were identified, and of the 105 proteins shared by both species, 39 were significantly differentially abundant. Proteins including glucose-6-phosphate isomerase and LPXTG-motif anchor-domain protein were overabundant in *L. crispatus* communities, and enolase and L-lactate dehydrogenase overabundant in *L. iners*. Comparison of *L. iners* protein relative abundance between BV positive versus negative women revealed that several *L. iners* proteins involved in pathogenesis and carbohydrate metabolism were significantly upregulated in women with non-optimal microbiota. Additionally, 40 host proteins were overabundant in women with *L. iners* and 13 in those with *L. crispatus*. After excluding participants with BV, *L. iners* dominance was correlated with immune markers (IgG H chain, clusterin and calpain small-subunit 1), while *L. crispatus* cytokeratin-8 overabundance suggests greater epithelial barrier integrity.

Discussion: Metaproteomic analyses provided valuable insight into the function of *Lactobacillus* spp. *in vivo*, demonstrating significant differences in the metabolic activities of *L. iners* versus *L. crispatus*. The functional activities of *L. iners* were linked to host BV status, suggesting that bacterial gene expression is influenced by environmental factors or strain level differences.

26

Understanding the molecular recognition of Bacteroides fragilis glycosphingolipids by Natural Killer T-cell receptor

Praveena Thirunavukkarasu[1], Sungwhan F. Oh [2, 3], Hee Bum 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[6, 7, 1]

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

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

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. Our structural studies on two such CD1d presented BfaGCs in complex with the type I NKT T cell receptor (TCR) revealed that the TCR adopted a parallel docking topology atop the F'-pocket of CD1d in recognising them. 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 sheds light on a better understanding of the existing symbiotic relationship between the microbes producing these endogenous lipids and the host.

References:

Sungwhan F. Oh*†, Praveena T*, Hee Bum Song, Ji-Sun Yoo, Da-Jung Jung, Deniz ErturkHasdemir, Yoon Soo Hwang, Changwon C. Lee, Jérôme Le Nours, Hyunsoo Kim, Jesang Lee, Richard S. Blumberg, Jamie Rossjohn†, Seung Bum Park†, and Dennis L. Kasper†. Host immunomodulatory lipids created by symbionts from dietary amino acids. Nature. 2021 Dec;600(7888):302-307.

* Equal first author

27

Culturing the neonatal microbiome has revealed similarities between bacteria colonising the respiratory tract and gut

Sara K Di Simone[1, 2, 3], Josephine C Owen [1, 2], Elys A Green [1, 2, 4], Rebecka P Atkinson[1, 4], Steven X Cho [1], Jodee A Gould[3], Claudia A Nold[1, 2], Samuel C Forster[5, 3], Marcel F 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 human body is home to a dynamic and evolving community of microorganisms that collectively form the microbiome. The first months of life are critical for the acquisition of the microbiome and mark the earliest interactions between our microbiota and maturing immune system. The lung, previously believed to be sterile, harbours a variety of microorganisms recently thought to be critical in these processes. Although metagenomic technologies continue to uncover the diverse microbial community in the human respiratory tract, isolate-level resolution, dynamic developmental changes, and functional analysis of the microbiota across body sites is yet to be fully achieved. Through the 'GLAM&I' (Gut and Lung and their Microbiome and Immunology) clinical study, longitudinal and time-matched bronchoalveolar lavage, nasopharyngeal swab, stool, blood, and milk samples have been sampled from 79 patients admitted to the neonatal and paediatric intensive care units at Monash Children's Hospital. To evaluate community composition, we have metagenomically sampled respiratory and stool microbial communities and cultured and purified individual bacterial isolates to allow functional validation of the interactions between the microbiota and host immune system. Capillary sequencing of the 16S rRNA gene in 5,480 individual isolates from 208

respiratory samples and 1,657 isolates from 62 stool samples has revealed 30 bacterial species shared across the respiratory tract and gut microbiomes in early life. Future work will validate the site-specific functional roles and immune interaction of these isolates. The application of culturing to the lung microbiome will improve our understanding of host-microbe interactions and support experimental validation of patient-specific bacterial species. This knowledge has the potential to provide novel therapeutics and microbiome-based medicines.

28

Phylogeny-based metagenomic analyses increases the resolution of investigations of the human microbiome

Sean Solari[1, 2], Remy Young[1, 2], Vanessa Rossetto Marcelino[1, 2], 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

The human microbiome is a complex biological ecosystem, comprising diverse communities of archaea, bacteria and viruses that play essential roles in human health, and whose disturbance has been implicated in various pathologies. Advances in bacterial culturing techniques has enabled *in vitro* characterisation of these microbiota, and while an overwhelming majority of the functional repertoire within the human microbiome remains to be characterised, it has become apparent that slight genomic differences can have disproportionately significant impacts on large-scale phenotypes. The current taxonomic standards used to describe and compare microbiota do not always capture precise genomic relationships, and may not be sufficient to accurately recapitulate these different phenotypes being observed between similar microbes. Phylogenetics provides an alternative framework for investigating microbiome composition and functional potential. In particular, clade-based analyses resolve the hierarchical differences between microbial genomes. We developed and tested a phylogenetic approach "*expam*" to culture-free shotgun metagenomic sequencing pipelines, enabling high-throughput characterisation of microbial communities at unprecedented resolution. Our benchmarking on 140 simulated metagenomes demonstrates that *expam* achieves state-of-the-art precision and recalls of 84.0% and 55.8% respectively when translated into the taxonomic setting, while also performing classification within a phylogenetic tree that provides a resolution beyond current taxonomic standards. The increasing depth of *-omics* analyses is rapidly revealing not only the immense genotypic and phenotypic diversity of the microbiome, but also the vast milieu of cells and environments within the human host with which the microbiome interacts with and relies upon. This apparent diversity of interactions between the microbiome and host environment underscores the importance of clade-specific associations based on precise genomic relationships, as such associations lay the foundation for generating mechanistic hypotheses and informing our investigation into the complex host-microbiome relationship.

29

Reverse genetics of SARS-CoV-2

Alexander A Khromykh[2, 1]

1. School of Chemistry and Molecular Bisociences, University of Queensland, Brisbane, QLD, Australia

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

The current COVID-19 pandemic is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with new variants continuously emerging. Since the beginning of pandemic, we set up PC3 facilities dedicated to SARS-CoV-2 work at the UQ's School of Chemistry and Molecular Biosciences and established a range of productive collaborations on various projects including viral detection assays, virus inactivation, and development of vaccines and antivirals (e.g.1-4).

SARS-CoV-2 is a positive strand RNA virus with a large ~30kb genome. The ability to manipulate SARS-CoV-2 genome allows to assess the role of changes present in different viral variants on various virus properties including their susceptibility to immune responses elicited by vaccinations. We have developed a circular polymerase extension reaction (CPER) methodology for in vitro assembly of full-length SARS-CoV-2 cDNA that does not require intermediate steps of cloning in bacteria and in vitro RNA transcription (5). Overlapping cDNA fragments are generated from viral RNA and assembled together with a linker fragment containing mammalian expression promoter into a circular full-length cDNA. Transfection of this cDNA into mammalian cells results in the recovery of SARS-CoV-2 virus that exhibits properties comparable to the parental virus in vitro and in vivo. We have applied CPER to generate SARS-CoV-2 variants, recombinants, and reporter viruses and are employing them for studies on virus replication and virus-host interactions.

1. 1.Amarilla et al. Front Microbiol 2021, 12:625136
2. 2.Albert et al. Environ Res 2021, 196:110944
3. 3.McMillan et al. Sci Adv 2021, 7: eabj8065
4. 4.Guimond et al. ACS Cent Sci 2022, 8:527-545.
5. 5. Amarilla et al. Nature Communications 2021, 12: 3431

30

Identifying critical E3 ubiquitin ligases in human malaria parasites

Danushka Marapana[1, 2], Simon Cobbold[1, 2], Michal Pasternak[1, 2], Stuart Ralph[3, 4], David Komander[1, 2], Alan Cowman[1, 2]

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

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

3. Department of Biochemistry and Pharmacology, University of Melbourne, Melbourne, Victoria, Australia

4. Bio21 Molecular Science and Biotechnology Institute, Melbourne, Victoria, Australia

241 million cases of human malaria disease and 627,000 deaths were reported in 2021. Malaria eradication is hindered by the complex dual-host life cycle of the causative Plasmodium spp parasite and the rapid evolution of parasite-derived antimalarial resistance. The best-in-class antimalarials target parasite proteins expressed in the symptomatic asexual blood stage of disease. These protein virulence factors and their homeostasis, encompassing protein synthesis, activity, and degradation, are critical for parasite survival within the human host.

Ubiquitination is the key post-translational modification in eukaryotic cells that governs protein degradation, localisation, and activity. This process results in the tagging of the 7.6 kDa ubiquitin molecule to lysine or methionine residues on protein substrates by a concerted enzyme cascade of E1, E2 and E3 enzymes. The E3 enzymes mediate the final key ubiquitin ligation step in this reaction and govern substrate selection. To accommodate the discriminate selection of specific substrates, most eukaryotic cells encode a diverse repertoire of E3 ligases with about 700 members expressed in human cells. Of these enzymes, the largest superfamily includes the Cullin-Ring-Ligase (CRL) complexes of E3 ligases. A single modular CRL complex contains one of 7 Cullin scaffold proteins which simultaneously engages a ubiquitin-binding RING-type protein, a substrate adaptor protein, and one of multiple substrate receptors.

Plasmodium falciparum, the most deadly human malaria parasite, encodes for the critical proteins required for ubiquitination, but we currently lack functional characterization of the key players in this pathway.

In this study, we characterised the P. falciparum CRL E3 ligases using CRISPR/Cas9 conditional gene knockouts, high-resolution imaging, and whole-cell mass spectrometry. We discovered an essential but minimal CRL repertoire in parasites, controlled by only two Cullin scaffolds. We identified a PfCullin1-linked CRL complex involved in parasite inner-membrane biogenesis and DNA replication, which remarkably recruits only one substrate receptor, compared to the ~70 receptors used interchangeably by human cells. A second CRL complex functioning through a PfCullin4 scaffold utilises a previously unidentified adaptor protein and receptors to support correct DNA replication. Collectively these results identify for the first time, the essential role of human malaria parasite E3 ubiquitin ligases.

31

PCRCR complex is essential for invasion of human erythrocytes by Plasmodium falciparum

Stephen W Scally[1], Tony Triglia[1], Cindy Evelyn[1], Benjamin A Seager[1], Michal Pasternak[1], Pailene S Lim[1], Julie Healer[1], Niall D Geogheghan[1], Amy Adair[1], Wai-Hong Tham[1], Laura F Dagley[1], Kelly L Rogers[1], Alan F Cowman[1]

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

The most severe form of malaria is caused by *Plasmodium falciparum*. These parasites invade human erythrocytes in a complex multistep process that ultimately ends with the internalization of the parasite. An essential step in this process involves the ligand PfRh5, which forms a complex with the cysteine-rich protective antigen (CyRPA) and the PfRh5-interacting protein (PfRipr; RCR complex) and binds basigin on the host cell. However, it was unclear what tethers the RCR complex to the parasite surface, as all three proteins lack a transmembrane region or GPI anchor. We identified a heteromeric disulphide-linked complex consisting of the Plasmodium Thrombospondin-Related Apical Merozoite Protein (PfPTRAMP) and the Cysteine rich Small-Secreted protein (PfCSS) and have shown it binds RCR to form a pentameric complex PCRCR. Importantly, PfPTRAMP has a single transmembrane domain and likely tethers the complex to the parasite. Using *P. falciparum* lines with conditional knockouts and invasion inhibitory nanobodies to both PfPTRAMP and PfCSS we utilised lattice light-sheet microscopy and show they are essential for parasite invasion. The PCRCR complex functions to anchor the contact between parasite and erythrocyte membranes brought together by strong parasite deformations. Furthermore, we determined the X-ray crystal structure of PfCSS in complex with one neutralizing and one non-neutralizing nanobody. Our results define the function of the PCRCR complex and identify invasion neutralising epitopes providing a roadmap for structure-guided development of these proteins for a blood stage malaria vaccine.

32

Imaging host-cell invasion by *Toxoplasma gondii*: What is the role of the host membrane and cytoskeleton?

Aurelie ATD Tsee Dawson[1], Alex AU Uboldi[1], Niall NG Geoghegan[1], Cindy CE Evelyn[1], Kelly KR Rogers[1], Chris CT Tonkin[1]

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

Toxoplasma gondii is an obligate intracellular parasite that is responsible for the human and animal disease toxoplasmosis. Toxoplasma belongs to the phylum Apicomplexa, which includes *Plasmodium* species (the agents of malaria) and *Cryptosporidium spp.* (responsible for severe diarrhea). All apicomplexan parasites undergo a unique form of motility to actively invade their target cell and create a vacuole to aid their intracellular survival. A better understanding of the host cell – parastite interaction during invasion will help to create new disease treatments. In this study, we used lattice light-sheet microscopy to capture whole cell volumes of *T. gondii* invasion using parasites expressing mCherry and the Ca^{2+} sensor GCaMP6. In addition, we applied a combination of live cell dyes and genetically encoded fluorescent reporters to label both membrane and cytoskeletal proteins in the host cell. Using this novel workflow, we are quantitatively investigating biophysical membrane changes and the reorganization of the cytoskeleton during host cell invasion by *Toxoplasma*. This study will reveal the role of the host cell during invasion of apicomplexan parasites and inform new therapies to treat diseases caused by this group of pathogens.

33

Identification of targets of protective antibody responses against *Plasmodium vivax* malaria using a multifunctional antibody profiling approach

D. Herbert Opi[1, 2, 3], Rhea Longley[4], Linda Reiling[1], Kael Schoffer[4], Yanie Tayipto[4], Ali Haghiri[5], Kaitlin Pekin[1], Jessica Brewster[4], Damien Drew[1], Gaqian Feng[1], Bruce Wines[1], Danielle Stanisic[6], Matthias Harbers[7], Takafumi Tsuboi[8], Mark Hogarth[1], Benson Kiniboro[9], Leanne Robinson[1], Julie Simpson[5], Ivo Mueller[4], James Beeson[1, 2, 11, 10]

1. Burnet Institute of Medical Research and Public Health, Melbourne, VIC, Australia

2. Department of Medicine, University of Melbourne, Melbourne, Victoria, Australia

3. Department of Immunology and Pathology, Monash University, Melbourne, Victoria, Australia

4. Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

5. Centre for Epidemiology and Biostatistics, University of Melbourne, Melbourne, Victoria, Australia

6. Institute for Glycomics, Griffith University, Southport, Queensland, Australia

7. RIKEN Center for Life Science Technologies, Division of Genomic Technologies, Yokohama, Japan

8. Division of Malaria Research, Proteo-Science Center, Ehime University, Matsuyama, Japan

9. Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea

10. Department of Infectious Diseases, Monash University, Melbourne, Victoria, Australia

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

A need for highly effective vaccines against malaria has been made more urgent following stalled progress in reducing the global burden of malaria since 2015 and increases in malaria burden from COVID-19 pandemic-related disruptions. While advances in *Plasmodium falciparum* malaria vaccine development have seen the recent approval of the RTS,S vaccine, very limited progress has been made towards development of a vaccine against *P. vivax*. No vaccines for *P. vivax* have completed testing for efficacy in malaria-endemic settings and limited candidates are in the discovery pipeline. *P. vivax* is the most widespread *Plasmodium* species and a major cause of malaria outside Africa, with over 3 billion people at risk of infection with *P. vivax* malaria. A major challenge to developing a *P. vivax* vaccine is a limited knowledge of the targets of protective immune responses. Antibodies play the major role in acquired immunity to malaria and are likely to act through three major mechanisms: direct inhibition of host cell invasion, recruitment and activation of complement, and interactions with Fcγ-receptors to promote phagocytosis or killing by immune cells. However, knowledge of functional antibody mechanisms in *P. vivax* immunity is very limited. To address this, we developed novel high throughput multiplex assays to identify the targets of functional antibodies against *P. vivax* that interact with complement and Fcγ-receptors. In a longitudinal cohort study of 1–3-year-old children from PNG, we measured antibody magnitude (IgG, IgG subclasses and IgM) and antibody functions (complement fixation, FcγR binding, opsonic phagocytosis and avidity) to 30 *P. vivax* antigens. Using these approaches, we identified known and novel antibody targets and specific functions associated with protection against clinical *P. vivax* malaria. Using statistical modelling approaches we identify important combinations of antigen-specific antibodies, both magnitude and function, that may provide maximal protection against *P. vivax* malaria. Our findings identify promising antigens for prioritisation in *P. vivax* vaccine development, and a knowledge of target-specific functional immune responses that are most important for protective *P. vivax* immunity.

34

Disarming Proteus mirabilis and blowing its cover: N acetylcysteine inhibits P. mirabilis urease activity and prevents catheter encrustation in catheter associated UTIs.

Arthika Manoharan[1], Greg Whiteley[2], Theerthankar Das[1], Jim Manos[1]

1. 1. Department of Infection, Immunity and Inflammation, School of Medical Sciences,, The University of Sydney, Sydney, NSW, Australia

2. Whiteley Corporation, Tomago, NSW, Australia

Introduction: Catheter-associated UTIs (CAUTIs) account for 80% of all hospital-acquired UTIs, with Proteus mirabilis accounting for ~44% of these. P. mirabilis secretes copious amounts of urease that causes urine alkylation. Alkylation precipitates calcium and magnesium, forming robust crystalline biofilms that block indwelling catheters, causing urine backflow, resulting in severe inflammation, pyelonephritis, and septicaemia.

Acetohydroxamic acid, the only FDA approved urease inhibitor, displays severe side effects such as mutagenicity and teratogenicity. Furthermore, antibiotic therapy does not prevent biofilm formation or catheter encrustation, thus necessitating better alternatives. Here we investigated the effect of N acetylcysteine (NAC) on P. mirabilis urease activity and catheter encrustation.

Methods: Urease activity from five clinical P. mirabilis isolates treated with NAC were quantified by the Berthelot's method. Enzyme kinetics were studied using purified urease and quantified by spectrometry. Catheter encrustation was investigated in the presence of NAC using an in vitro glass bladder model, where a 2-way Foley catheter was "infected" with P. mirabilis. Artificial urine with NAC was pumped through and "infection" of catheter was allowed to run for 120hrs or complete blockage. ICP-mass spectrometry analysis was used on catheter eye sections where elemental profiles of deposits in catheters compared. Inflammatory responses to urease and treatment by bladder cells were measured using ELISAs.

Results: NAC suppressed P. mirabilis urease activity by >3-fold at concentrations >5mM. Kinetics studies showed strong binding capacity of NAC to urease in a competitive manner. NAC significantly delayed complete blockage of catheters to >96hrs and decreased bacterial loads (>3 $\log_{10}$ CFU/mL) in catheter cross sections. Interestingly, NAC also muted IL-6 and IL-8 responses to infection by >4-fold.

Conclusion: NAC is a non-toxic antibiofilm agent with potent urease inhibitory effects. Urease inhibition effectively disarms P. mirabilis by preventing catheter blockage and biofilm formation. Moreover, NAC treatment subdues inflammation in bladder cells *in vitro,* thus possibly limiting infection severity. With its multifaceted activity, NAC allows CA-UTI treatment in a highly targeted and efficient manner without promoting antibiotic resistance.

35

Pandemic preparedness: time for a focus on animals?

Marion Koopmans[1]

1. Erasmus MC - afdeling Viroscience, Rotterdam, ZH, Netherlands

A majority of emerging diseases in humans originates in the animal world. Wild life and farm animals may harbor viruses that can directly or with some adaptations infect humans. Such zoonotic spillovers have been documented for a range of viruses, and constitute a first step in a chain of emergence. Risk of spillover is determined by a complex set of factors, collectively called drivers. Rapid changes in the balance between these factors often precede spillover events. Zoonotic spillover may lead to epidemics or pandemics when the pathogens involved have or acquire the ability to become transmissible between humans. Once that occurs, the efficiency of that process again is determined by a multifactorial process of drivers. Using recent examples, this presentation will focus on the concept of One Health, and the importance of considering the role of animals and environmental changes in emerging disease preparedness and response.

36

Dissecting immunity to MVA-MERS-S, a novel vaccine candidate against the Middle East respiratory syndrome (MERS)

Marylyn Addo[4, 1, 3, 2], **Leonie M Weskamm**[1, 5, 3], **Anahita Fathi**[1, 5, 3], **Matthijs Raadsen**[2], **Leonie Mayer**[1, 5, 3], **Asisa Volz**[6], **Gerd Sutter**[7], **Stephan Becker**[8], **Christine Dahlk**[1, 5, 3]

1. Institute for Infection Research and Vaccine Development (IIRVD), University Medical Centre Hamburg-Eppendorf, Hamburg, Germany

2. Department of Viroscience, Erasmus Medical Center, Rotterdam, The Netherlands

3. German Centre for Infection Research, Hamburg, Germany

4. University Medical Center Hamburg-Eppendorf, Germany, Hamburg, Germany

5. Department for Clinical Immunology of Infectious Diseases, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

6. Institute of Virology , University of Veterinary Medicine Hannover, Hanover, Germany

7. Division of Virology, Department of Veterinary Sciences, Institute for Infectious Diseases and Zoonoses, LMU Munich, Munich, Germany

8. Institute for Virology, Philipps University Marburg, Marburg, Germany

The Middle East respiratory syndrome coronavirus (MERS-CoV) belongs to the group of highly pathogenic beta-coronaviruses that have been identified as priority pathogens by the WHO. Infection may lead to pneumonia and multi-organ failure,and is associated with a case fatality rate of up to 35%. Since its emergence in 2012, MERS-CoV has caused multiple outbreaks and has been exported to 27 countries. However, to date there is no licensed vaccine or specific therapy available. MVA-MERS-S is a novel modified vaccinia Ankara (MVA)-based vaccine candidate against the MERS-CoV encoding for the full spike glycoprotein (S). We report data from two investigator-initiated phase-1 clinical trials (IIT) investigating the safety and immunogenicity of MVA-MERS-S. The candidate vaccine was found to be safe and well-tolerated. Neutralizing and non-neutralizing antibody responses (ADNK, ADCP) and T cell immunity were detectable following MVA-MERS immunization. Vaccine dose and immunization intervals impacted the magnitude of antibody responses. Four immunodominant MERS-CoV-S-specific linear B-cell epitopes were identified using microarrays mapping the proteome of MERS-CoV-S.

37

From discovery to prevention: The development journey of the RV3-BB Rotavirus Vaccine

Julie Bines[3, 1, 2], **RV3 Rotavirus Vaccine Program Enteric Diseases Group**[3]

1. Paediatrics, University of Melbourne, Parkville, Victoria, Australia

2. Gastroenterology and Clinical Nutrition, Royal Children's Hospital, Parkville, Victoria, Australia

3. Murdoch Childrens Research Institute, Parkville, VIC, Australia

Diarrhea is a leading cause of death and morbidity in children worldwide. Prior to the 1970's the main infectious cause was unknown and Gastroenteritis Wards were common in Children's Hospitals in Australia. As a result of a collaboration between Clinicians and Scientists at the Melbourne Royal Children's Hospital and the University of Melbourne, using a novel approach, rotavirus was identified in the small bowel biopsies of children with acute severe gastroenteritis. Following on from this landmark discovery rotavirus was subsequently identified in the stool of children with acute severe dehydrating gastroenteritis globally, providing the target for the development of vaccines for prevention of severe rotavirus disease. Today over 114 countries have introduced a rotavirus vaccine into their national or regional immunisation program.

The RV3-BB vaccine is a novel human neonatal vaccine developed at MCRI to target prevention from severe rotavirus disease from birth. Clinical trials of RV3-BB vaccine have been conducted in Australia, New Zealand, Indonesia and Malawi. RV3-BB vaccine has been shown to be safe, immunogenic and efficacious when administered in a neonatal or infant administration schedule. In Indonesian babies, RV3-BB was associated with a protective efficacy of 94% at 12 months of age and 75% at 18 months of age when administered in the neonatal schedule. RV3-BB vaccine has now been licensed to emerging country vaccine manufacturers with the aim to provide an affordable vaccine. Indonesian manufactured (PT BioFarma) RV3 vaccine is planned for introduction into the national immunisation program in Indonesia in 2023.

38

Role of Type 2 Immunity in COVID-19

William Petri[1], Jen Sasson[1], Allie Donlan[1]

1. University of Virginia, Charlottesville, VA, USA

We have discovered that COVID-19 patients with high plasma interleukin (IL)-13 levels have a significantly greater risk of needing mechanical ventilation. IL-13, which signals through the receptor IL-4Rα along with the closely related cytokine IL-4, is involved in eosinophilic inflammation, mucous secretion, goblet cell metaplasia and fibrosis, and has been regularly implicated in airway hyperresponsiveness and atopic disease. It was therefore plausible that IL-13 could exacerbate respiratory disease in COVID-19. Neutralization of IL-13 in K18-hACE2 C57Bl/6J mice protected the animals from severe infection with SARS-CoV-2, as evidenced by reduced clinical score, weight loss and mortality. RNA-seq analysis of whole lung tissue taken from infected mice who underwent IL-13 neutralization revealed the most downregulated gene to be Has1, which encodes a synthase responsible for hyaluronan (HA) production, a polysaccharide apart of the extracellular matrix that has previously been implicated in other inflammatory pulmonary diseases. This was mechanistically further supported by an increase in HA deposition in human and mouse lung upon SARS-CoV-2 infection. Additionally, neutralization of the HA receptor, CD44, led to improved survival in K18-hACE2 mice. As HA has been proposed previously in asthma as a potential culprit in airway remodeling, we hypothesize that HA, as a downstream effector of IL-13, may be involved in pulmonary dysfunction post recovery from COVID-19.

39

Oxidised Cholesterols Drive Macrophage Infiltration into the Lung during Bacterial and Viral Respiratory Infections

Katharina Ronacher[2, 1]

1. Stellenbosch University, Cape Town, South Africa

2. Mater Research Institute - The University of Queensland, Brisbane, QUEENSLAND, Australia

Immune cell recruitment to the site of infection is an integral part of an effective immune response to both bacterial and viral pathogens. However excessive immune cell infiltration and production of pro-inflammatory cytokines in the lung can result in increased lung pathology and disease severity. We recently discovered a novel and previously unrecognised mechanism of immune cell recruitment to the infected lung, which can be targeted pharmacologically to improve respiratory infection outcomes.

We showed that infection with *M. tuberculosis* (Mtb), influenza A virus (IAV) and SARS-CoV-2 leads to production of the oxidised cholesterols 25-hydroxycholesterol and 7α,25-dihydroxycholesterol in the lung through upregulation of the oxysterol-producing enzymes CH25H and CYP7B1. 7α,25-dihydroxycholesterol is the endogenous high affinity ligand for the oxysterol-sensing receptor GPR183, which is expressed on cells of the innate and adaptive immune system. We demonstrated in a preclinical model of Mtb infection that both CYP7B1 and GPR183 are required for rapid macrophage infiltration into the lung. Mice genetically deficient in GPR183 (*Gpr183*$^{-/-}$) and mice that were unable to upregulate CYP7B1 upon infection had delayed macrophage infiltration and higher mycobacterial burden during early infection.

Similarly, in *Gpr183*$^{-/-}$ mice infected with either IAV or a mouse-adapted SARS-CoV-2 the infiltration of macrophages was delayed compared to control animals. However, this was associated with a significant reduction in inflammatory cytokines and beneficial infection outcomes. Therefore, we next administered a GPR183 antagonist to C57BL/6J mice 24 hours after SARS-CoV-2 infection and found that the antagonist significantly reduced macrophage infiltration without affecting other immune cell subsets. Further, the antagonist attenuated the severity of SARS-CoV-2 infection and reduced viral loads.

Finally, analysis of single cell RNASeq data from bronchoalveolar lavage samples from healthy controls and COVID-19 patients with moderate and severe disease revealed that CH25H, CYP7B1 and GPR183 are significantly upregulated in human macrophages during COVID-19.

Together this study demonstrates that oxysterols drive macrophage infiltration and inflammation in the lung via GPR183. We provide the first preclinical evidence for therapeutic benefit of targeting GPR183 during severe COVID-19.

40

Unraveling protection against tuberculosis

JoAnne Flynn[1]

1. University of Pittsburgh, Pittsburgh, PENNSYLVANIA, United States

Tuberculosis is responsible for 1.5 million deaths per year, despite available drugs and a vaccine given to infants in most countries. The vaccine lacks effectiveness against pulmonary tuberculosis, and we still do not understand the immune responses necessary for full protection against Mycobacterium tuberculosis infection or disease. In our lab, we have developed several non-human primate (macaque) models of M. tuberculosis infection, as well as different models of protection against infection or disease. These include protection at the granuloma level, vaccine induced protection using intravenous administration of the live vaccine BCG, and concomitant immunity, which is protection against reinfection. Using various tools and technologies, we are investigating the cell types and functions underlying protection in these model systems. Each of these protection scenarios may have unique and common factors that promote protection, supporting our hypothesis that there are various paths to control and failure to control infection and disease.

41

Antibody and B cell responses to a complex pathogen

Ian Cockburn[1]

1. Australian National University, Canberra, ACT, Australia

Malaria caused by *Plasmodium* infection kills around 600,000 people each year, demonstrating a clear need for a safe and effective vaccine. In clinical trials the RTS,S and R21 subunit vaccines confer modest protection for less than a year. Another, whole parasite, vaccine performs well in malaria naïve volunteers but is less efficacious in endemic areas. These vaccines work in large measure by the generation of antibody and T cell responses to the circumsporozoite protein (CSP). To better understand the factors shaping these immune responses to both subunit and whole parasites we have developed CSP-specific BCR knockin and TCR transgenic mice. Using these tools we find that the requirements for immune responses to whole parasites are different to those for subunit vaccines. In particular, B cells interacting with whole parasites have an opportunity to obtain T cell help from diverse sources that may facilitate sustained germinal center responses. These sustained germinal center responses can generate long lived plasma cells. However, this response is also limited by antibody feedback. Our results highlight the need to study B cell responses to whole pathogens as well as simple immunogens to determine how sustained responses can be generated by vaccination.

42

Pharmacological inhibition of TBK1/IKKε blunts COVID-19 immunopathology

Tomalika Ullah[2, 1], Matt Johansen[3], Daniel Wenholz[4], Olivier Laczka[4], Philip Hansbro[3], Dominic De Nardo[5], Michael Gantier[2, 1]

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

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

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

4. Noxopharm, Chatswood, NSW, Australia

5. Department of Anatomy and Developmental Biology, Monash Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

TANK-binding kinase 1 (TBK1) is a key signalling component that drives the production of type-I interferons (IFNs), which have essential antiviral activities including against SARSCoV-2. TBK1 and its homolog IκB kinase-ε (IKKε) can also induce the production of proinflammatory factors that contribute to pathogen clearance. While initially protective, delayed engagement of type-I IFN is associated with lethal hyper-inflammation seen in severe COVID-19 patients. The contribution of TBK1/IKKε signalling to this response is unknown. We have discovered that the anticancer small molecule idronoxil inhibits both IRF3 and NF-κB-driven inflammation by disrupting the formation of TBK1/IKKε signalling complexes following STING activation. Leveraging this unique activity, we show that therapeutic administration of idronoxil suppresses cellular and molecular lung inflammation in K18-hACE2 mice challenged with SARS-CoV-2, resulting in reduced pro-inflammatory cytokine production and decreased airway fibrosis in experimental COVID-19. Our results indicate a critical role for TBK1/IKKε signalling in SARS-CoV-2 hyper-inflammation and identify a novel therapeutic intervention to limit disease severity in COVID-19 patients.

43

Inhibitors of the START-domain lipid transfer protein potently block intraerythrocytic development in newly invaded *Plasmodium falciparum* merozoites.

Paul R Gilson[1], Madeline G Dans[2], Coralie Boulet[1], William Nguyen[2], Gabby Watson[2], Zahra Razook[3], Somya Mehra[1], Greta E Weiss[1], Cindy Evelyn[2], Michael Mlodzianoski[2], Kelly Rogers[2], Alyssa E Barry[1], Wai-Hong Tham[2], Brendan S Crabb AC[1], Brad E Sleebs[2]

1. Burnet Institute, Melbourne, VIC, Australia

2. Walter and Eliza Hall Institute, Melbourne, Australia

3. Deakin University, Geelong, VIC, Australia

Malaria is a devastating infectious disease, causing about 241 million cases in 2020, tragically resulting in 627,000 deaths. The disease burden of malaria has increased in recent years, partly due to emerging parasite resistance to current front-line artemisinin-based drug treatments. As a result, new anti-malarial drugs with novel modes of action urgently need to be discovered and developed. A potential source of novel targets for new antimalarials is the stage of the asexual blood cycle when parasites briefly emerge from old red blood cells (RBCs) to rapidly invade new RBCs. Invasive extracellular merozoite stage parasites have a short half-life and drug-like molecules that can delay or prevent invasion would leave the merozoites exposed to rapid destruction by the immune system. To identify new egress and invasion inhibitors we screened the 400-compound Medicines for Malaria Venture Pathogen Box library and discovered dozens of inhibitors. We found using video microscopy of parasites treated with the aryl acetamide inhibitor MMV006833, that the drug did not specifically block invasion but rather a downstream step in which the newly invaded merozoite normally differentiates into an amoeboid, intracellular, ring-stage parasite. Selection of drug resistant parasites followed by whole genome sequencing revealed resistance mutations in the START-domain lipid transfer protein. Both introduction of the START mutations into wildtype parasites that reproduced drug resistance and evidence for the binding of recombinant START to the drug, confirmed this protein is the target. Analogues of MMV006833 that are as potent as artemisinin, have been synthesised indicating START has great potential as a future therapeutic drug target. Ongoing investigation of the drug's mechanism of action indicates that START may help transfer phospholipids between parasite and RBC membranes after invasion that are essential for growth and development of the young parasite.

44

Mitochondrial Sirtuin 4 regulates inflammation during *Salmonella* Typhimurium infection

Joanna Stepek[1], Nirmal Robinson[1, 2]

1. CECAD, University of Cologne, Cologne, Germany

2. Centre for Cancer Biology, University of South Australia and SA Pathology, Adelaide, South Australia, Australia

Sirtuins are NAD^+-dependent enzymes, which respond to changes in energy status by conferring post-translational modifications on their targets and altering their activities. Sirtuin 3, 4 and 5 have been reported to localize in the mitochondria and Sirtuin 1 is a nuclear regulator of mitochondrial biogenesis. We found that *S*. Typhimurium infection strikingly increases Sirtuin 4 (SIRT4) expression. SIRT4 is a multifunctional enzyme targeting the metabolic entry points of the TCA cycle, thereby inhibiting mitochondrial metabolism. We have deciphered that depletion of SIRT4 ameliorated mitochondrial morphology and improved their quality in the infected cells. Furthermore, loss of SIRT4 resulted in reduced inflammation and cell death, which are the hallmarks of *S*. Typhimurium-induced pathogenicity. Mechanistically, we deciphered that SIRT4 modulates pathogen induced inflammation by regulating the pyruvate dehydrogenase complex and mitochondrial reactive oxygen species. Additionally, Sirt4 KO mice were also characterized by altered inflammatory response to infection. Taken together, our data suggest that SIRT4 plays a key role in *S*. Typhimurium-induced mitochondrial dysfunction and thereby controls mitochondrial quality and cell-intrinsic immune responses.

52

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

Rebecca Edgar[3, 1, 2], Ghizal Siddiqui[4], Kathryn Hjerrild[1], Tess R Malcolm[5], Natalie B Vinh[6], Chaille T Webb[5, 6], Clare Holmes[7], Christopher A MacRaild[4], Hope C Chernih[1, 2], Willy W Suen[7], Natalie A Counihan[1, 2], Darren J Creek[4], Peter J Scammells[4], Sheena McGowan[5, 8], Tania F de Koning-Ward[1, 2]

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

2. The Institute for Mental and Physical Health and Clinical Translation, Deakin University, Geelong, Victoria, Australia

3. Deakin University, St Albans Park, VIC, Australia

4. Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, Victoria, Australia

5. Biomedicine Discovery Institute and Department of Microbiology, , Monash University, Melbourne, Victoria, Australia

6. Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University , Melbourne, Victoria, Australia

7. CSIRO, CSIRO Australian Centre for Disease Preparedness, Geelong, Victoria, Australia

8. Centre to Impact AMR, Monash University, Melbourne, Victoria, Australia

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

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

51

Otitis-Prone children have reduced *Haemophilus influenzae* protein D-specific memory B-cells compared to non-otitis-prone children but no reduction in overall T follicular helper cell proportions.

Sharon L Clark[1, 2], Elke Seppanen[2], Christian Tjiam[2, 3], Lauren Bakaletz[4], Allan Cripps[5], Harvey Coates[1, 2], Shyan Vijayasekaran[1, 6], Peter Richmond[1, 2, 7], Ruth Thornton[2, 3]

1. School of Medicine, The University of Western Australia, Perth, WA, Australia

2. Wesfarmers Centre of Vaccines & Infectious Disease, Telethon Kids Institute, Perth, WA, Australia

3. Centre for Child Health Research, The University of Western Australia, Perth, WA, Australia

4. Centre for Microbial Pathogenesis, Abigail Wexner Research Institute at Nationwide Children's Hospital & the Ohio State University College of Medicine, Columbus, Ohio, USA

5. School of Medicine & Menzies Health Institute, Griffith University, Gold Coast, Queensland, Australia

6. Otolaryngology Head and Neck Surgery, Perth Children's Hospital, Perth, Western Australia, Australia

7. Departments of Immunology and General Paediatrics, Perth Children's Hospital, Perth, Western Australia, Australia

Chronic and recurrent middle ear infections (otitis media-OM) are predominantly caused by persistence of nontypeable *Haemophilus influenzae* (NTHi) biofilms and often result in children undergoing surgery. These children are considered otitis-prone and, in Australia, are more likely to be Aboriginal. Previously we have shown that Aboriginal otitis-prone children have reduced serum IgG titres specific for NTHi adhesin/biofilm antigens, Protein D (PD), rsPilA and ChimV4, compared to non-Aboriginal otitis-prone and non-otitis-prone children, which could contribute to OM susceptibility. We aimed to determine whether lower NTHi specific IgG levels were related to frequencies of global and/or antigen-specific B-cells and circulating follicular helper T (cTfh) cells.

Peripheral blood mononuclear cells (PBMC) from Aboriginal otitis-prone (n=15), non-Aboriginal otitis-prone (n=15) and non-otitis-prone (n=15) children were assessed using memory B cell ELISpot for numbers of spot forming cells against PD, rsPilA, ChimV4 and OMP26 (per $2x10^5$ PBMCS), following 5-day stimulation with R-848 and IL-2. Proportions of circulating sub-populations of B-cells (CD3-CD19+CD27±IgD±) and cTfh (Tfh; CD4+CD45RA-CCR7+CXCR5+PD-1+ICOS±) were assessed using flow cytometry. Group comparisons were made using Kruskal–Wallis tests.

Aboriginal and non-Aboriginal otitis-prone children had lower PD-specific memory B-cell numbers (median=29 vs 18, respectively) compared to non-otitis-prone children (median=67;P<0.006) and a trend towards lower memory B-cell numbers for rsPilA and ChimV4 (medians: Aboriginal otitis-prone =6.5, 8.5; non-Aboriginal otitis-prone=19, 27; non-otitis-prone=31.5, 36.5;P=0.099; P=0.078, respectively). No differences were observed between groups for OMP26-specific memory B-cells (median=164.9-182.5). Overall proportions of switched-memory (IgD-CD27+; median=8.03%-12.06%), unswitched-memory (IgD+CD27+; median=4.88%-7.47%) and naïve (IgD+CD27-; median=71.52%-78.54%) B-cells were not different between groups. Aboriginal (ICOS+=0.15%, ICOS-=0.1%) and non-Aboriginal otitis-prone children (ICOS+=0.16%, ICOS-=0.08%) had higher proportions of cTfh cells compared to non-otitis-prone children (ICOS+=0.05%, ICOS-=0.04%;P<0.047).

Otitis-prone children had lower numbers of NTHi adhesin/biofilm antigen-specific memory B-cells, consistent with previously observed differences in IgG titres. Proportions of circulating B-cell and cTfh cell populations were similar across groups. Further investigation is needed to examine proportions of antigen specific cTfh cells or perturbation in alternate pathways. Better understanding of drivers for the development of long-lived memory B-cells to NTHi antigens is needed for the rational design the antigenic composition of NTHi vaccines most likely able to prevent chronic otitis media.

53

Using single-cell RNA sequencing to understand tissue- and drug- specific $CD4^+$ T cell responses in experimental visceral leishmaniasis

Jessica Engel[1], Fabian de Labastida Rivera[1], Patrick Bunn[1], Hyun Jae Lee[2], Jack Na[1], Marcela de Lima Moreira[2], Ashraful Haque[2], Christian Engwerda[1]

1. QIMR Berghofer, Brisbane

2. Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne

Chronic infectious diseases are responsible for significant morbidity and mortality. They impose high costs on health systems and promote poverty. Visceral leishmaniasis (VL) is caused by the protozoan parasite *Leishmania donovani*. Like other chronic infectious diseases, such as malaria, tuberculosis (TB) and human immunodeficiency virus (HIV), VL is characterised by immune dysfunction that impairs critical $CD4^+$ T cell responses for parasite control, which in turn can promote persistent infection and associated pathology. $CD4^+$ T cells are integral to disease outcome. To distinguish $CD4^+$ T cell molecules mediating effective and ineffective anti-parasitic immunity, we examined tissue-specific $CD4^+$ T cell responses using single cell RNA sequencing in a model of experimental VL. In this model, the liver is a site of acute, resolving infection with highly effective anti-parasitic $CD4^+$ T cells (similar to the immune presentation of asymptomatic humans), while the spleen is a site of chronic infection and ineffective $CD4^+$ T cells (similar to the immune presentation in VL patients). To study and track $CD4^+$ T cell responses in experimental VL, we employed $CD4^+$ T cell receptor (TCR) transgenic cells that are specific for a *Leishmania* antigen (PEPCK). We also employed a *Leishmania*-specific MHC-class II I-A^b tetramer ($PEPCK_{335-351}$) to examine the entire complexity of the $CD4^+$ T cell response encompassing a spectrum of TCR specificities. To determine how drug treatment modulates $CD4^+$ T cell responses, a group of mice were also treated with a single dose of AmBisome (current treatment for VL patients). PEPCK and $PEPCK_{335-351}$-positive cells were sorted from the liver and spleens of control- or AmBisome- treated mice at various time points

throughout infection and subjected to single cell RNA sequencing using the 10x Genomics Chromium platform and the 5' single cell reagent kit. Analysis of this data set will be presented.

54

Monocytes as effectors of antibody-mediated functions in malaria infection

Jessica Horton[1], Jessica Loughland[2], Megan Soon[2], Daisy Mantila[3], Shazia Ruybal-Pesántez[4], Nicholas Dooley[2], Julianne Hamelink[2], Moses Laman[3], Ivo Mueller[4], Leanne Robinson[1], Michelle Boyle[2], James Beeson[1]

1. Burnet Institute, Melbourne, VIC, Australia

2. QIMR-Berghofer Medical Research Institute, Herston, QLD

3. Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea

4. The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

Monocytes are critical effector cells in malaria immunity and support parasite clearance via several mechanisms including antibody-mediated phagocytosis. While antibodies have long been established as vital to malaria immunity, a high antibody magnitude alone does not ensure clinical protection. An increasing body of evidence suggests antibody functional properties, such as complement fixation and the ability to bind Fcγ-receptors, are more meaningful measures of immunity acquired through natural exposure or vaccination. Defined correlates of malaria immunity have to date remained elusive, complicating analysis of vaccine efficacy in clinical trials, so the effects of these functional antibodies warrant further exploration. Monocytes are capable of phagocytosing blood stage malaria parasites (merozoites and infected red blood cells) opsonised with IgG and/or complement. Therefore, we investigated the surface expression of monocyte functional receptors in children in a malaria-endemic setting to better understand the interaction between antibodies, complement and monocytes in malaria immunity. Cells isolated from 80 participants in Papua New Guinea, comprising children with *Plasmodium falciparum* or *P. vivax* infection and children and adults without malaria infection, were analysed with spectral flow cytometry. While established markers of monocyte activation were not upregulated, we found that the expression of Fcγ-receptors, which enable phagocytosis of opsonized parasites, was increased on classical monocytes during infection. Key complement receptors were expressed by monocytes but were not upregulated during infection. *P. falciparum* and *P. vivax* infection modulated monocyte functional phenotypes in different ways. It was also evident that crucial functional markers on both monocytes and dendritic cells were differentially expressed in children compared to adults. This work reveals new insights on specific functional properties of monocytes relevant to their role as effectors of antibody-mediated parasite clearance in malaria infection and supports the investigation of Fcγ-receptor binding by antibodies and monocyte functions in ongoing vaccine studies.

55

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

Dawson Ling[2, 1], Madeline Dans[3], Greta Weiss[1], Zahra Razook[5, 4], Somya Mehra[4], Alyssa Barry[5, 4], Brendan Crabb AC[2, 1], Hayley Bullen[2, 1], Paul Gilson[2, 1]

1. Malaria Virulence and Drug Discovery Group, Burnet Institute, Melbourne, Victoria, Australia

2. Microbiology & Immunology, The University of Melbourne, Parkville, Victoria, Australia

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

4. Infectious Diseases Systems Epidemiology Group, Burnet Institute, Melbourne, Victoria, Australia

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

In 2020, *Plasmodium falciparum* (*Pf*) accounted for nearly all the 627,000 malaria-associated deaths globally (1). This rise in mortality is partly due to emerging drug resistance against all currently available antimalarials, underpinning the need for new antimalarials with novel mechanisms of action. Parasite invasion of erythrocytes represents an attractive novel drug target as it is responsible for parasite proliferation and symptomatic malaria. By screening the Medicines for Malaria Venture's (MMV) open-access compound library, the Pathogen Box, we discovered an invasion-specific compound, MMV687794 (2). MMV687794 does not affect the development of ring- and trophozoite-stage parasites but instead specifically inhibits invasion from schizonts, the mature form of the parasite containing the invasive daughter merozoites. Genomic analysis on MMV687794-resistant parasites unveiled mutations in an alpha/beta hydrolase enzyme we have termed ABH-83 that contains a lysophospholipase (LysoPL) motif. To validate ABH-83 as the target of MMV687794, these mutations were engineered into wild-type parasites using CRISPR/Cas9, which recapitulated the drug resistance phenotype. An epitope tag and a GlmS riboswitch were also introduced into these parasites to allow for the detection of ABH-83 within the parasite using epitope-specific antibodies. Furthermore, when activated by the sugar glucosamine, the riboswitch facilitates inducible transcriptional downregulation of ABH-83, consequently reducing ABH-83 expression within one lifecycle and enabling closer examination of the role/s of ABH-83. By conducting a time-course western blot series on the transgenic parasites, the LysoPL ABH-83 is most highly expressed in the schizont stage, concordant with a role in invasion. ABH-83 has also been visualised by microscopy at the surface of rhoptry organelles which secrete important invasion-related proteins during erythrocyte invasion. This data is consistent with previous live-cell microscopy analysis of MMV687794-treated schizonts, which displayed invasion defects exacerbated by increased drug treatment duration. Overall, these results suggest that ABH-83 is involved in rhoptry functioning and/or morphology normally required for efficient merozoite invasion of erythrocytes. Investigations are ongoing to determine how ABH-83-mediated lipid modifications within the rhoptry membrane regulate rhoptry functioning during erythrocyte invasion.

1. World Health Organization. World Malaria Report 2021. Geneva: World Health Organization; 2021.
2. Dans MG, Weiss GE, Wilson DW, Sleebs BE, Crabb BS, de Koning-Ward TF, et al. Screening the Medicines for Malaria Venture Pathogen Box for invasion and egress inhibitors of the blood stage of Plasmodium falciparum reveals several inhibitory compounds. bioRxiv. 2020:768648.

56

Assessing the feasibility, specificity and sensitivity of a serological testing and treatment approach to eradicate *P. vivax* in Cambodia

Costanza Tacoli[1, 2], Rhea Longley[1], Ramin Mazhari[1], Shazia Ruybal-Pesántez[1], Thomas Obadia[3], Michael White[4], Ivo Mueller[1], Jean Popovici[2]

1. Population Health and Immunity Division, Walter and Eliza Hall Institute of Medical Research (WEHI), Melbourne, Victoria, Australia

2. Malaria Research Unit, Institut Pasteur du Cambodge, Phnom Penh, Cambodia

3. STATS, Institut Pasteur, Paris, France

4. Infectious Disease Epidemiology and Analytics, Institut Pasteur, Paris, France

A broad range of *P. vivax* proteins can rapidly and effectively induce a robust and lasting IgG antibody response, even after low-density asymptomatic infections.[1] As these responses persist for months or even years after clearance of blood-stage parasites, such antibodies represent ideal biomarkers of both past exposure and concurrent infections. Given that almost all *P. vivax* hypnozoite carriers did experience a primary blood-stage infection in the preceding 6-9 months, antibody responses are thus also potential biomarkers of hypnozoite carriage. We have used these anti-vivax antibody response patterns to develop a panel of serological exposure markers (SEMs) to *P. vivax* infections that could promote the identification of people at risk of carrying clinically silent hypnozoites hence that should be targeted with anti-hypnozoite therapy.[2] In preliminary studies in Indonesia, serological testing using these markers identified 85% of people experiencing a relapse (Mueller, Noviyanti personal communication).

We assessed the specificity and sensitivity of these candidate antigen proteins in a Cambodian cohort and their application in field trials in Cambodia. The development and adoption of a serological testing and treatment approach (SeroTAT) where upon population screening only individuals testing positive for recent *P. vivax* exposure with a point-of contact test receive treatment, could provide a valid alternative to current mass-testing and treatment (MTAT) and mass drug administration (MDA) strategies and significantly reduce vivax prevalence and transmission rate globally.

1. Longley RJ, Sattabongktot J, Mueller I. Insights into the naturally acquired immune response to Plasmodium vivax malaria. Parasitol 143 (2), 154-70 (2016).
2. Longley RJ, White MT, Takashima E, et al. Development and validation of serological markers for detecting recent Plasmodium vivax infection. Nat Med 26, 741–9 (2020)

57

A novel recombinant *Lactococcus lactis* mucosal vaccine platform based on group A streptococcus pili

Catherine Tsai[1, 2], Sam Blanchett[3], Jacelyn Loh[1, 2], Kohtaro Fijihashi[4], Thomas Proft[1, 2]

1. Molecular Medicine and Pathology, University of Auckland, Auckland, New Zealand

2. Maurice Wilkins Centre, University of Auckland, Auckland, New Zealand

3. Division of Infection & Immunity, Faculty of Medical Sciences, University College London, London, United Kingdom

4. Institute of Medical Science, University of Tokyo, Tokyo, Japan

Well-defined synthetic peptides are attractive for vaccine development. However, they are usually poorly immunogenic and sensitive to proteolytic degradation, thus require conjugation to carrier proteins and/or addition of adjuvants. Lactic acid bacteria (LAB) have become promising vehicle for mucosal vaccines due to their safety profile and natural adjuvanticity. The choice of carrier and mode of presentation hugely affect the stability and immunogenicity of the antigen, thus determine effectiveness of the resulting vaccines. We propose that the group A streptococcus (GAS) pilus structure expressed on the surface of *Lactococcus lactis* can be an ideal carrier for antigenic peptides, and established a novel mucosal vaccine platform termed PilVax[1]. Pili (*sing.* pilus) are hair-like bacterial cell surface protrusions important for host cell adhesion. The GAS pili consist of covalently linked pilins that are structurally stable and highly immunogenic. We identified several regions within the backbone pilin that can be replaced with antigenic peptides. Expressing the peptides within the pilus structure allows for peptide amplification, stabilisation and enhanced immunogenicity. Intranasal immunisation of mice with the resulting recombinant *L. lactis* strain produced strong peptide-specific antibody responses in serum and bronchoalveolar fluid. A recently developed tuberculosis vaccine based on a dominant T-cell epitope generated both humoral and cellular immune responses in the immunised mice[2]. PilVax vaccination resulted in peptide-specific CD4+ T cells at levels similar to those resulting from BCG immunisation, as well as an unexpected increase in the numbers of CD3+CD4-CD8- (double negative [DN]) T cells in the lungs of vaccinated animals. These cells types were shown to be responsible for the cytokine production following stimulation with the cognate peptide. This presentation will also give an overview of other PilVax projects focusing on various infectious diseases, including gonorrhoea and influenza. Results from these ongoing studies demonstrate the suitability of developing PilVax into useful mucosal vaccines.

1. Wagachchi D, Tsai JC, Chalmers C, Blanchett S, Loh JMS, Proft T. PilVax - a novel peptide delivery platform for the development of mucosal vaccines. Sci Rep. 2018;8(1):2555.
2. Blanchett S, Tsai CJ, Sandford S, Loh JM, Huang L, Kirman JR, et al. Intranasal immunization with Ag85B peptide 25 displayed on Lactococcus lactis using the PilVax platform induces antigen-specific B- and T-cell responses. Immunol Cell Biol. 2021.

58

Salmonella Typhimurium induces cIAP1 degradation to promote death in macrophages

Madeleine Wemyss[1, 2], Rebecca Ambrose[1], Kate Lawlor[1], Jaclyn Pearson[1, 2]

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

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

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

Our research discovered that *S*. Typhimurium induces the degradation of cellular inhibitor of apoptosis protein 1 (cIAP1), an important host cell adaptor of inflammatory signalling and inhibitor of apoptotic cell death. Using an *in vitro* model of human infection in THP1 macrophage-like cells, we observed strong association between cIAP1 loss and increased cellular cytotoxicity, with corresponding caspase-8/-3 activation. Depletion of cIAP1 required functional *Salmonella* Pathogenicity Island 1 (SPI-1) T3SS effector translocation, and was not prevented by pan-caspase, proteasomal or lysosomal inhibitors. Anti-cIAP1 immunoblot detected a low molecular weight peptide following *S*. Typhimurium infection, suggesting that SPI-1 effector/s may cleave cIAP1 during infection. Current work combines molecular, *in vitro* and *in vivo* techniques to explore the cIAP cleavage mechanism and determine the responsible effector protein, with transfection screens suggesting several key candidates. This finding suggests a new role for *Salmonella* effector proteins in activating, rather than preventing, host cell death in macrophages, which we hypothesise may promote dissemination of the bacteria.

59

Doxycycline inhibits both apicoplast and mitochondrial translation in *Plasmodium falciparum*

Michaela S Bulloch[1], Emily M Crisafulli[1], Jenni Hayward[2], Giel van Dooren[2], Stuart A Ralph[1]

1. The University of Melbourne, Parkville, VIC, Australia

2. Division of Biomedical Science and Biochemistry, Australian National University, Canberra, Australian Capitol Territory, Australia

Doxycycline is a tetracycline-class antibiotic used for malarial prophylaxis and as a partner drug with quinine for treating malaria. Doxycycline's antimalarial mechanism of action has widely been accepted as a translation inhibitor, specifically blocking the prokaryotic 70S ribosomes of the *Plasmodium* apicoplast. At low concentrations (<5 μM) doxycycline exhibits a delayed death phenotype, typical of inhibitors of apicoplast housekeeping processes. In this process parasites are morphologically normal in their first growth cycle after treatment, dying only after one round of replication.

At higher concentrations (≥10 μM) doxycycline has schizonticidal activity in the first cycle via an unknown and apicoplast-independent mechanism. In other eukaryotes, doxycycline also inhibits mitochondrial translation. The mitochondrial genome of *Plasmodium falciparum* encodes only 3 proteins, each of them subunits of complexes III or IV of the electron transport chain. To investigate whether mitochondrial translation is a target at high doxycycline concentrations, we assessed protein abundance in drug treated parasites using mass spectrometry. Assessing steady state protein levels along with protein turnover using isotope-labelled amino acids we directly detected apicoplast encoded proteins for the first time and showed that these proteins decrease in abundance following doxycycline treatment. We detected no differences in mitochondrial translation via this method, although background mitochondrial protein abundance was already very low.

As an alternative assay for mitochondrial protein translation, we assayed the oxygen consumption of the electron transport chain for the mitochondrial encoded complexes. We detected severe perturbations to parasites' oxidative phosphorylation using a Seahorse assay. The reduced oxygen consumption rate following doxycycline treatment strongly suggests that *Plasmodium* mitochondrial translation is indeed a target of doxycycline. This disrupted electron transport chain phenotype was also replicated in Seahorse assays of the related protozoan parasite *Toxoplasma gondii*.

These studies confirm that the apicoplast ribosome is the major target in *Plasmodium* for antibiotics targeting protein translation, but indicates that at higher doxycycline concentrations, mitochondrial translation and thus mitochondrial electron transport are also impacted. This reveals a second target for these drugs in malaria treatment and insight into their strange dynamics of killing.

60

The Human Dendritic Cell Atlas: An integrated transcriptional tool to study human dendritic cell biology

Zahra Elahi[1], Paul Angel[1], Suzanne Butcher[1], Nadia Rajab[1], Jarny Choi[1], Yidi Deng[2], Justine Mintern[3], Kristen Radford[4], Christine Wells[1]

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

2. University of Melbourne, Melbourne, VIC, Australia

3. Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, VIC 3010, Australia

4. Mater Research Institute,The University of Queensland, Brisbane, Australia

Dendritic cells (DCs) are functionally diverse and are present in most adult tissues, but deep understanding of human DC biology is hampered by relatively small numbers of these in circulation and their short lifespan in human tissues. We built a transcriptional atlas of human DCs by combining samples from 14 expression profiling studies derived from 10 laboratories. We identified significant gene expression variation of DC subset-defining markers across tissue-type and upon viral or bacterial stimulation. We further highlight critical gaps between *in vitro*-derived DC subsets and their *in vivo* counterparts and provide evidence that monocytes or cord blood progenitor *in vitro*-differentiated DCs fail to capture the repertoire of primary DC subsets or behaviours. In constructing a reference DC atlas, we provide an important resource for the community wishing to identify and annotate tissue-specific DC subsets from single-cell datasets, or benchmark new *in vitro* models of DC biology.

61

GPR183 antagonism reduces macrophage infiltration in SARS-CoV-2 infection

Cheng Xiang Foo[1], Stacey Bartlett[1], Keng Chew[2], Minh Dao NGO[1], Helle Bielefeldt-Ohmann[2, 3], Buddhika Arachchige[4], Benjamin Matthews[4], Sarah Reed[4], Ran Wang[1], Christian Smith[1], Matt Sweet[3, 5], Lucy Burr[6], Kavita Bisht[1], Svetlana Shatunova[1], Jane Sinclair[2], Rhys Parry[2], YuanHao Yang[1], Alexander Khromykh[2, 3], Mette Rosenkilde[7], Kirsty Short[2, 3], Jean-Pierre Levesque[1], Katharina Ronacher[1, 3]

1. Mater Research, University of Queensland, Woolloongabba, QUEENSLAND, Australia

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

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

4. Mass Spectrometry Department, Centre for Clinical Research, The University of Queensland, Brisbane, Australia, Brisbane, Queensland, Australia

5. Institute for Molecular Bioscience (IMB), IMB Centre for Inflammation and Disease Research, The University of Queensland, Brisbane, QLD, Australia

6. Department of Respiratory Medicine,, Mater Adult Hospital, South Brisbane, Queensland, Australia

7. Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

Severe COVID-19 is often characterized by hyper-inflammation due to excessive immune cell infiltration to the site of infection and pro-inflammatory cytokine production. However, the immunological mechanisms underlying the excessive inflammation in the lung remain elusive. We recently discovered that oxidised cholesterols, so called oxysterols, are produced in the lung upon SARS-CoV-2 infection in mice. These oxysterols chemotactically attract infiltrating macrophages to the site of infection via the oxysterol-sensing receptor GPR183. We therefore hypothesised that GPR183 is a novel host target for therapeutic intervention to reduce macrophage-mediated hyperinflammation and disease severity in viral respiratory infections.

Here we demonstrate that either genetic deletion or pharmacological antagonism of GPR183 in mice reduced macrophage infiltration into the lungs after SARS-CoV-2 infection without impairing type I IFN responses. Further, we found that animals treated with the GPR183 antagonist had reduced expression of pro-inflammatory cytokines in the lung. Most strikingly, animals treated with the GPR183 antagonist had lower viral loads, lost significantly less weight, and were clinically less ill compared to vehicle-treated mice. Single-cell RNA Seq analysis of bronchoalveolar lavage samples from healthy controls and COVID-19 patients with moderate and severe disease revealed that macrophages are the primary producers of the oxysterol producing enzymes and that expression of these enzymes positively correlates with COVID-19 severity.

Together, we identified a novel chemo-attractant role for oxysterols in the lung and provide the first preclinical evidence for the benefits of inhibiting GPR183 activity to reduce severity of viral respiratory infections.

62

A novel chimeric virus for vaccine and diagnostic application for the Japanese encephalitis outbreak in Australia

Jessica J Harrison[1], Mahali Morgan[1], Gervais Habarugira[1], Henry De Malmanche[1], Helle Bielefeldt-Ohmann[1], Peter D Kirkland[2], Alexander A Khromykh[1], Roy A Hall[1], Jody Hobson-Peters[1]

1. University of Queensland, St Lucia, QLD, Australia

2. Department of Primary Industries, Elizabeth Macarthur Agriculture Institute, Menangle, New South Wales, Australia

Introduction: Mosquito-borne viruses continue to cause outbreaks of disease in Asia, Africa, Europe, the Americas and Australia, where an estimated 2 billion people are at risk of infection. Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus that is the leading cause of viral encephalitis in Southeast Asia and is responsible for an estimated 70,000 cases per year. This year, an unprecedented outbreak of JEV occurred in Australia, affecting humans, pigs and horses, with transmission and cases detected in most states of the country. In this study, we assessed the ability of novel, recombinant viruses to act as diagnostic and vaccine antigens for the Australian outbreak strain of JEV.

Methods: A chimeric flavivirus displaying the virion surface proteins of the Australian outbreak strain of JEV (Genotype 4) and containing the backbone genome of the insect-specific Binjari virus (BinJV), was constructed using the circular polymerase extension reaction method (CPER), recovered in a mosquito cell line and designated BinJ/JEV. The ability of the recombinant virus to serve as a diagnostic antigen was assessed in a range of diagnostic assays. The immunogenicity of the chimeric virus was also assessed by immunising mice.

Results: We showed that the BinJ/JEV virus grew to high titres in insect cells while remaining replication defective in vertebrate cells. Furthermore, we showed that the chimeric virus particles were antigenically indistinguishable from the parental wild-type JEV and behaved similarly in a range of diagnostic assays, including ELISA, IFA and neutralisation tests. Mice immunised with purified BinJ/JEV virions also generated an immune response to JEV after a single vaccination.

Conclusion: The chimeric BinJ/JEV described here represents a promising recombinant platform for developing a safe an effective vaccine and diagnostic assays for JEV, with scope for efficient large-scale production without the requirement for PC3 biocontainment.

63

Safety and immunogenicity of a 2+1 DTPa infant vaccination schedule in Australian infants.

Sonia McAlister[1, 2], Anita van den Biggelaar[1, 2], Ruth Thornton[1, 3], Matthew Cooper[4], Peter McIntyre[5], Peter Richmond[1, 2, 6], Nicholas Wood[5]

1. Wesfarmers Centre of Vaccines and Infectious Diseases, Telethon Kids Institute, The University of Western Australia, Perth, Australia

2. School of Medicine, The University of Western Australia, Perth, WA, Australia

3. Centre for Child Health Research, The University of Western Australia, Perth, WA, Australia

4. Telethon Kids Institute, The University of Western Australia, Perth, WA, Australia

5. National Centre for Immunisation Research and Surveillance, Sydney, NSW, Australia

6. Departments of Immunology and General Paediatrics, Perth Children's Hospital, Perth, WA, Australia

A 2+1 pertussis vaccination schedule administering DTPa-IPV-HBV/Hib at 6-weeks, 12-weeks, and 12-months of age may maximise early protection and improve antibody persistence into the second year of life. This may be equivalent to the current 3+1 schedule where DTPa-IPV-HBV/Hib is administered at 2, 4, 6 and 18 months. Both schedules include DTPa-IPV boosting at 4-years. We aimed to evaluate the 2+1 schedule in Australia.

Serum was collected pre- and 1-month post-primary and booster vaccinations; and during the second year of life from 82 infants. IgG-geometric mean concentrations (GMC) to pertussis-toxin (PT), pertactin (PRN), filamentous-hemagglutinin (FHA), tetanus-toxin (TT) and diphtheria-toxoid (DT) were measured using a DTPa-multiplexed bead-based immunoassay.

The 2+1 schedule was well tolerated and immunogenic with increasing DTPa-IgG-GMC observed after each subsequent dose, except for TT for which levels remained unchanged between the first two doses.

Post-hoc analyses showed that high baseline antibody titres in infants pre-vaccination (i.e. maternally-derived) influenced immunogenicity of subsequent infant vaccines. GEE modelling of infant PT antibody responses across the first 4 years of life based on serostatus at baseline showed an average reduction in PT-IgG by 40% (p=6.6e-05). However, all children responded well to the 4-year booster, achieving antibody levels associated with seropositivity/sero-protection for all DTPa antigens.

Comparing the 2+1 schedule to a historical control cohort vaccinated with a 3+1 schedule showed that after three doses, DTPa-IgG concentrations were higher in the 2+1 group, except for FHA-IgG. During the second year of life, PT, PRN and FHA-IgG antibodies remained higher in 2+1 compared to 3+1 vaccinated infants. By 4-years of age, similar DTPa-IgG concentrations were observed between groups for all antigens. Responses to a 4-year booster were comparable between schedules, except DT-IgG was higher in the 2+1 group. All children were seroprotected throughout the study (TT- and DT-IgG≥0.01IU/mL).

These results suggest a 2+1 DTPa-schedule provides immunity into the second year of life and may induce comparable protection to the current 3+1 schedule. Given the impact of maternal antibody on infant responses, future studies should determine the optimal 2+1 schedule based on current recommendations for repeated maternal pertussis immunisation.

64

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

Joseph Menassa[1], Christina Nedeva[1], Marcel Doerflinger[2], Andrew J Kueh[3], Marco J Herold[3], Mark Hulett[1], Hamsa Puthalakath[1]

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

2. Infection and Immunity Devision, Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC, Australia

3. Blood Call and Cancer Division, Walter and Eliza Hall Institute f Medical Research, Melbourne, VIC, Australia

Introduction:

Sepsis is a serious life-threatening condition (death rates up to 50%) characterised by an acute inflammatory response to an infection. Current treatment options focus on controlling this initial inflammatory response. Although these treatments alleviate the acute disease symptoms, they have not lowered the overall mortality rates. Recently it has been shown that this is due to a prolonged immunosuppressive phase that follows the acute phase of sepsis. Little is known about this secondary phase except for the apoptosis of immune cells.

Aims:

We sought to uncover the molecular details that trigger and control the immunosuppressive phase of sepsis.

Methodology:

We used a combination of whole-genome CRISPR screening, mice knockouts and studies using immune cells.

Results:

The CRISPR screening revealed a (TREM) family receptor TREML4 on myeloid cells as the molecular switch that triggered and regulated inflammation and immune cell death during sepsis. Importantly, genetic ablation of Treml4 in mice led to an overall increase in survival rate, both during the acute and chronic phases of sepsis. Adaptations to neutrophils in TREML4 ablated mice during sepsis were found to be the basis for increased survival during sepsis.

Conclusions:

We show for the first time that the receptor TREML4 is responsible for the high mortality rates during sepsis and that targeting this receptor represents a valid strategy for the design of new treatments for sepsis.

1. Nedeva, C., Menassa, J., et al. (2020). Nature Immunology, 21(12), 1585-1596.

65

MDA5 guards against infection by surveying cellular RNA homeostasis

Natalia Sampaio[1, 3, 2], Liden J Gearing[1, 2], Antonio G Dias Junior[3], Lise Chauveau[4], Valerie Odon[3], Chiara Cursi[3], Alice Mayer[3], Madara Ratnadiwakara[5], Minna-Liisa Anko[6], Paul J Hertzog[1, 2], Jan Rehwinkel[3]

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

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

3. Medical Research Council Human Immunology Unit, Medical Research Council Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK

4. RNA viruses and metabolism team, Institut de Recherche en Infectiologie de Montpellier , Montpellier, France

5. Centre for Cancer Research, Hudson Institute of Medical Research, Clayton, VIC, Australia

6. Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland

MDA5 is an innate immune RNA sensor that detects a range of viruses. MDA5's RNA agonists are not well defined. We used individual-nucleotide resolution crosslinking and immunoprecipitation (iCLIP) to study its ligands. Surprisingly, upon infection with SARS-CoV-2 or encephalomyocarditis virus (EMCV), MDA5 bound overwhelmingly to cellular RNAs. Many binding sites were intronic and proximal to *Alu* elements. MDA5-bound RNA was enriched in Poly(A) and Poly(U) motifs, some of which may form double-stranded RNA. In SARS-CoV-2 and EMCV-infected cells, cytoplasmic levels of intron-containing unspliced transcripts were increased, suggesting dysregulation of splicing. Concomitantly, MDA5 iCLIP peaks were enriched in introns accumulating in the cytoplasm of infected cells. Moreover, rescue of splicing abrogated MDA5 activation. Finally, when depleted of viral RNA, RNA extracted from infected cells still stimulated MDA5. Taken together, MDA5 surveys RNA processing fidelity and detects splicing perturbation during infection, establishing a paradigm of innate immune 'guarding' for RNA sensors.

101

Strategic and scientific contributions of human challenge trials for vaccine development: facts versus fantasy

Yara N Abo[1], Andrew Steer[1], James McCarthy[2], Zeb Jamrozik[3], Meta Roestenberg[4], Josh Osowicki[1]

1. Murdoch Children's Research Institute, Melbourne, VIC, Australia

2. Victorian Infectious Diseases Service, Royal Melbourne Hospital, Melbourne, Australia

3. Population Health, University of Oxford, Oxford, UK

4. Controlled Human Infections Center, Leiden University Medical Center, The Netherlands

Introduction

Fast-tracked pandemic vaccine development has offered hope for accelerated development across a range of vaccines. Human challenge studies model an encounter between human hosts and pathogens by deliberately exposing selected volunteers to a well-characterised pathogen under controlled conditions. Human challenge trials have been proposed as a means to expedite vaccine development. They can provide early proof-of-concept for vaccine efficacy using fewer volunteers and resources, and allow the most promising candidates to advance to further phases. Human challenge can also provide correlate of protection data for use in future vaccine efficacy testing and give insights into the impact of pre-existing immunity on vaccine responses.

Methods:

We identify human challenge trials contributing to development of vaccines for 22 different pathogens, to discuss limitations, barriers, opportunities, and pitfalls for efforts to realise their full scientific and strategic potential.

Results:

Eleven vaccine candidates for 7 pathogens tested in human challenge trials have reached phase III and/or IV studies. Vaccine efficacy results in healthy adult participants have translated well to phase II field trials, with notable exceptions (e.g., influenza T-cell vaccines). Additional vaccine candidates tested in human challenge are currently in late phase trials or are soon to be tested in phase II human challenge trials. Human challenge trials have helped to down-select >40 vaccine candidates for 8 pathogens. Correlates of protection have been identified in cholera and influenza human challenge vaccine trials, and human challenge vaccine trials have provided valuable insight into correlates of protection for further enteric and respiratory pathogens as well as malaria. Limitations of human challenge trials are discussed, as well as mitigation strategies, based on findings from trials in this review.

Conclusion:

Human challenge trials have successfully contributed to advancement of vaccines. A focussed pathogen- and product-specific use case should be applied to maximise the contribution of human challenge research to vaccine development.

102

Development of a *Lactobacillus* therapeutic approach for HIV prevention in women

Grace Androga[1], Jenna Wilson[1], David Tyssen[1], Celia Mehou-Loko[2], Monalisa T Manhanzva[2], Andrea G Abrahams[2], Nina Radzey[2], Rushil Harryparsad[2], Bahiah Meyer[2], Eric Hunter[3], Anna Hearps[1, 4], Jo-Ann S Passmore[2, 5, 6], Hilton Humphries[6, 7], Pamela Gumbi[7], Linda-Gail Bekker[2, 8], Heather B Jaspan[2, 9], Gilda Tachedjian[1, 10], Lindi Masson[1, 2, 4, 6]

1. 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. Department of Pathology and Laboratory Medicine, Emory University School of Medicine; Emory National Primate Research Center; Emory Center for AIDS Research, Atlanta, GA, US

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

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

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

7. School of Medicine, School of Life Sciences, University of KwaZulu-Natal, Durban, South Africa

8. Desmond Tutu HIV Centre, University of Cape Town, Cape Town, South Africa

9. Seattle Children's Research Institute , University of Washington, WA, US

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

Human Immunodeficiency Virus (HIV) remains among the world's most serious healthcare challenges, with young African women at extreme risk of infection. Previous studies have shown that optimal *Lactobacillus* spp. in the female genital tract (FGT) protect against HIV acquisition. This is thought to be partly mediated by the production of lactic acid and regulation of inflammatory responses. The aim of this study was to characterise the HIV inhibitory properties of *Lactobacillus* isolates towards the development of live biotherapeutics to reduce infection risk in women.

Lactobacillus spp. were isolated from cervicovaginal specimens of women with optimal microbiota who were enrolled in our National Institutes of Health-funded study in South Africa. Isolates were identified by 16S rRNA gene sequencing and 50 morphologically unique isolates were selected for further analysis. The HIV-1 inhibitory activities of vaginal *Lactobacillus* isolates were assayed using TZM-bl indicator cells and the transmitted founder strain generated from a full length infectious molecular clone of HIV-1 subtype C (Z3576F_TF). The production of nine cytokines by vaginal epithelial cells in response to lactobacilli was measured using Luminex and lactic acid production and pH modifications were evaluated using an enzyme assay and pH meter, respectively.

Overall, *Lactobacillus* isolates produced greater amounts of D-lactic acid (median = 2.04g/L), which has been found to be more protective against bacterial infections, compared to L-lactic acid (median = 0.80 g/L), which is more protective against viral infections. *L.*

jensenii and *L. crispatus* produced the most D-lactic acid while *L. salivarius* produced the most L-lactic acid. The immunomodulatory properties of individual *Lactobacillus* strains were found to be highly diverse. 40 out of 50 lactobacilli isolates analysed were able to suppress HIV-1 virus infectivity, with *L. crispatus* (p= 0.0154) and *L. vaginalis* (p= 0.0029) species demonstrating significant suppression.

L. crispatus isolates produced the most lactic acid, caused the most significant reduction in culture pH, induced the lowest inflammatory cytokine responses and showed the most HIV-1 inhibitory effects. These isolates will be further characterised by whole genome sequencing towards the development of a potential live biotherapeutic HIV preventative.

103

Elucidating the contribution of regulatory T cells to the prevention of Cutaneous Squamous Cell Carcinoma

Shoaib Anwaar[1], Jazmina-Libertad Gonzalez-Cruz[1], James Wells[1]

1. The University of Queensland Diamantina Institute, Brisbane, Australia

Cutaneous squamous cell carcinoma (cSCC) is one of the most prevalent cancers in Caucasian populations, and its aggressive form poses a high risk of metastasis resulting in increased rates of mortality and morbidity. Recurrent and chronic exposure to ultraviolet (UV) radiation from the sun plays a crucial role in the initiation, development, and perpetuation of cSCC. Accumulating evidence suggests that regulatory T (Treg) cells are associated with UV-induced immunosuppression, however, their direct contribution to the establishment of cSCC remains elusive. When mice were exposed to 5 consecutive days of UVB (dose rate 150mJ/cm^2), they showed reduced ear swelling in response to ovalbumin challenge in a contact hypersensitivity assay, suggesting that functional antigen-specific Tregs were induced. Ear swelling responses did not show signs of suppression however, when mice were treated with an anti-CTLA-4 antibody following the cessation of UV treatment. Phenotypically, the expression of Foxp3, FR4, GITR, CTLA-4 and TIGIT on Tregs in the inguinal lymph nodes and spleen of UV-exposed mice did not differ from those in non-UV-exposed mice. Following the treatment of mice with UVB 5 days per week for different time periods (2w, 4w, 6w, 8w), it was determined that only UVB treatment for eight weeks consistently allowed the establishment and growth of tumours following the adoptive transfer of cSCC tumour fragments from donor mice. We aim to target Tregs in these tumour models in our future studies to determine whether Treg depletion or manipulation will reverse the capacity of UV to enable cSCC tumour establishment. Overall, this study will examine the plausibility of Treg manipulation as a preventative strategy to prevent UV-induced cSCC tumour establishment.

104

Characterising macrophage proteomic signatures of tuberculosis infection

George Ashdown[1], Toby Dite[1], Marc Pellegrini[1], Anna Coussens[1]

1. WEHI, Parkville, VIC, Australia

The pathogen causing tuberculosis disease, *Mycobacterium tuberculosis* (Mtb) is the second leading cause of death from a single infectious agent globally, behind COVID-19. Mtb infected 10 million and killed 1.5 million people in 2020. Part of Mtb's success is due to the bacteria's ability to modulate its macrophage host. Detecting infected individuals is challenging due to some 90 % of individuals being infected but clinically silent (e.g. latent infection), while Mtb's intracellular nature makes detecting host cells equally challenging. As such new targets are needed for disease detection and elimination.

Most cellular processes, including host-pathogen interactions during Mtb invasion, infection, and survival, are controlled by proteins. Here, the promising but underrepresented sub-field of plasma membrane proteomics is exploited. It was hypothesized that the surface of the host macrophage itself may be modulated during Mtb infection.

Using the virulent Mtb strain H37Rv to infect primary human macrophages, differences in plasma membrane proteomic signatures were quantified compared to controls. This is, to our knowledge, the first time the plasma membrane proteome has been quantified using label-free mass spectrometry.

These protein hits will now be taken forward to better characterise Mtb driven changes to host cells and may lead to biomarkers of Mtb infection, including detection of clinically silent or 'latent' infection.

105

Activation of the NLRP3 inflammasome by Hendra Virus C-protein

Kristian T Barry[1], Maggie Lam[1], Michelle Tate[1], Ashley Mansell[1]

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

Hendra virus is an emerging virus with a geographically broad host reservoir capable of infecting a variety of hosts. In humans, Hendra virus causes overt inflammatory disease of the lung and nervous system driven in part by the inflammatory cytokine IL-1β. There are currently no therapeutic options available for patients who contract Hendra virus, and very little of how it induces inflammatory disease is currently known. Recent studies have identified viral aggregating proteins as drivers of inflammation in Influenza A virus and SARS-Cov-2 virus. In this study, we sought to identify mechanisms of inflammation during Hendra virus infection and the possibility of another viral aggregating protein driving inflammatory disease. We have shown that a peptide analogue of Hendra virus C protein is able to form protein aggregates and activate the NLRP3 inflammasome through phagocytic uptake into cells *in vitro* using both temporal inhibition and gene deletion. Treatment of cells with the NLRP3 inhibitor MCC950 ameliorated IL-1β secretion responses, highlighting a possible therapeutic angle to reduce inflammation during Hendra virus infections. These results demonstrate a possible contributor of the detrimental immunopathology identified in Hendra virus infections, and critically identify a possible therapeutic strategy to alleviate inflammatory disease in infected patients.

106

Characterization of a novel Transcriptional Start Site in human myeloid cells that generates unique NRG1 isoforms

Miguel MAB Berrocal[1], Ricardo De Paoli-Iseppi[1], Marija Dinevska[1], Sefi Prawer[1], Samuel Widodo[1], Nadia Rajab[1], Matthew Watt[1], Theo Mantamadiotis[1], Michael Clark[1], Christine Wells[1]

1. University of Melbourne, CARLTON, VIC, Australia

Macrophages are homeostatic controllers of most human tissues, controlling cell behavior and tissue architecture through morphogens, cytokines, and growth factors. Macrophages are a primary source of the growth factor Neuregulin-1 (NRG1), which has pleiotropic roles in proliferation and differentiation of the stem cell niche in different tissues, and has been implicated in gut, brain and muscle development and repair. NRG1 presents a variety of isoforms that differ in structure and function; however, it is not yet understood which isoform or combination of isoforms are expressed by macrophages. Here we report a new class of NRG1 that initiates from a unique Transcriptional Start Site (TSS) and appears to be exclusively used by cells of the myeloid lineage, designated NRG1-VII according to the nomenclature conventions of this locus. Long-read sequencing identified up to 9 different transcripts that arise from the use of the novel TSS, some of which show major structural differences from one another due to use of alternative stop codons and 3'UTRs. Expression of NRG1-VII isoforms was confirmed in different myeloid cells, confirming that this is the major TSS in this lineage. There is no evidence that any other cell type outside of this lineage can generate said isoforms. NRG1-VII expression appears to be regulated by monocyte maturation and macrophage differentiation. A subset of macrophages in tissues express NRG1, however the role of these variants in tissue homeostasis or repair is not yet determined.

107

Features of a novel sheep model of ALI/ARDS induced by pulmonary instillation of live *Streptococcus pneumoniae*

Rob Bischof[1, 2], Habtamu Derseh[2], Paris Papagianis[2], Andrew Davies[3], Sylvie Bischof[2], Andrew Greenhill[2], Els Meeusen[1], David Piedrafita[1, 2]

1. Allergenix Pty Ltd, Clayton, VIC, Australia

2. Institute of Innovation, Science and Sustainability, Federation University Australia, Berwick and Churchill, VIC, Australia

3. Biomedicine Discovery Institute, Monash University Peninsula Campus, Frankston, VIC, Australia

Background Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), often the outcome of pneumonia and/or sepsis, are serious conditions that can lead to acute respiratory failure. ARDS is characterised by severe tissue inflammation and pulmonary oedema leading to arterial hypoxaemia. While there has been some advance in understanding the pathophysiology of ARDS, treatment options are limited. This work details a novel large animal model of ALI/ARDS intended for therapeutic efficacy studies.

Methods ALI/ARDS was induced in several sheep by pulmonary inoculation of up to 10^{10} CFU *Streptococcus pneumoniae* (log-phase culture), delivered as a bolus instillation or via nebulisation. Prior to bacterial inoculation, cannulas were surgically placed for the collection of venous and arterial blood samples to assess inflammation and monitor changes in arterial blood gases over time. In some experiments, ventilation under general anaesthesia was included to provide tighter control of breathing parameters. Bronchoalveolar lavage (BAL) was performed at the beginning and end of the experiment (48/72h post-bacterial inoculation), with post-mortem lung tissue samples collected for histopathological analyses.

Results There was a decline in PaO_2 levels, indicative of mild-moderate ARDS, within 4-6h following *S. pneumoniae* inoculation. Due to respiratory compensation, more severe ARDS (PaO_2/FiO_2 <150) could only be maintained under controlled ventilation but could be recovered with increased O_2 delivery. Neutrophils were elevated in blood and BAL post-inoculation, together with cytokines IL-6, IL-8 and TNF. At post-mortem, tissue sections taken from *S. pneumoniae* infected lungs showed marked infiltration of neutrophils into the alveolar and interstitial spaces, and changes suggesting widespread tissue damage (increased hyaline membrane deposits, proteinaceous debris and alveolar septal thickening). Injury scores were significantly elevated in infected sheep compared to controls.

Conclusions ALI/ARDS induced in sheep by pulmonary *S. pneumoniae* inoculation resulted in extensive acute lung inflammation, lung injury and bronchopneumonia that was maintained through the period of experimentation (up to 72h). This included a marked decline in blood gases reflecting a state of moderate to severe ARDS, which could be sustained under controlled ventilation. This model provides unique opportunities for non-clinical investigations into emerging therapies for ARDS.

108

Title: Understanding $CD4^+$ T cell mediated immune regulation in malaria: Assessing the immunomodulatory potential of Ruxolitinib during controlled human malaria infection.

Luzia Bukali[1], Fabian de Labastida Rivera[1], Jessica Engel[1], Dean W Andrew[2], Megan S.F Soon[2], Nicholas L Dooley[2], Jessica R Loughland[2], Julianne Hamelink[2], Teija Frame[1], Mayimuna Nalubega[2], Reena Mukhiya[2], Bridget Barber[3], Michelle J Boyle[2], Christian R Engwerda[1]

1. Immunology and Infection, QIMR Berghofer Medical Research Institute, Bowen Hills, Queensland, Australia

2. Human Malaria Immunology, QIMR Berghofer Medical Research Institute, Bowen Hills, Queensland, Australia

3. Clinical Malaria , QIMR Berghofer Medical Research Institute, Bowen Hills, Queensland, Australia

Malaria is a globally important parasitic disease, particularly in Sub-Saharan Africa., It caused approximately 600,000 deaths in 2021 alone, and pregnant women and children under 5 years old are especially vulnerable to infection and severe disease. Repeated exposure to parasites leads to the development of protective immunity that is characterised by an improved ability to control disease-mediated pathology and the production of protective anti-parasitic antibodies. $CD4^+$ T cells, including $CXCR3^+$ IFNγ producing Th1 cells are important for mediating parasite control following infection via the production of pro-inflammatory mediators. However, these cells and their products can also damage tissue. Type I IFNs regulate these responses by driving the differentiation of Th1 cells into IL-10-producing

type I regulatory (Tr1) cells, which are important for controlling inflammatory responses. However, the establishment of these immunoregulatory networks is thought to lead to poor parasite control and delayed development of immunity.

Ruxolitinib is a JAK1/2 protein inhibitor that is FDA approved for the treatment of myeloproliferative disorders and type I interferonopathies. We hypothesize that ruxolitinib can transiently block the anti-inflammatory pathways driven by type I IFN signalling and enhance anti-parasitic immune responses during controlled human malaria infection (CHMI). To test the immune boosting potential of ruxolitinib, a double blind phase Ib clinical study was designed in which participants were inoculated with blood-stage *Plasmodium falciparum*, randomized in a 1:1 ratio and administered the anti-malarial drug artemether/lumefantrine in combination with ruxolitinib or a placebo drug. Preliminary results from the analysis of $CD4^+$ T cells in the clinical trial will be presented.

109

Antimalarial compounds the 2-Anilino 4-Amino substituted quinazolines, are irresistible and promiscuous.

Hayley Bullen[1], Molly Parkyn-Schneider[1], Trent Ashton[2], Paul Sanders[1], Ghizal Siddiqui[3], Amanda Depaoli[3], Brendan Crabb AC[1], Darren Creek[3], Brad Sleebs[2], Paul Gilson[1]

1. Burnet Institute, Melbourne, VIC, Australia

2. Walter and Eliza Hall Institute, Parkville

3. Monash Institute of Pharmaceutical Sciences, Parkville

Malaria causes significant global morbidity and mortality, and while antimalarial treatments are available, resistance has developed to nearly all frontline medicines. New antimalarial compounds therefore need to be discovered but a major challenge to this is the propensity of the parasites to rapidly develop resistance to new antimalarials. We therefore need to prioritise new compounds that are refractory to resistance. Here we report our development of a series of 2-anilino quinazoline compounds that are rapidly acting against the asexual blood stage of *Plasmodium falciparum*, the parasite responsible for the majority of morbidity and mortality associated with malaria disease. These compounds are also effective against drug resistant parasites and target multiple species and stages of the malaria parasite's lifecycle. The 2-anilino quinazolines are also highly effective in mouse models of malaria via an oral route. When developing novel compounds, it is also important to consider the biological mechanism of action. This has proven impossible with the 2-anilino quinazolines as they are refractory to the selection of genetic resistance mutations in their target proteins. Therefore, to understand how these compounds are killing malaria parasites, we have had to employ alternative proteomic, metabolomic and biochemical approaches to define their mechanism of action. Thus far we have found that the 2-anilino quinazolines are likely promiscuous and do not have a single protein target responsible for their mechanism of action. We show that they bind several functionally important parasite proteins including PNP, RACK1 and 14-3-3, however none of these appear to be solely responsible for the quinazoline's mechanism of action.

110

Identification of sulfone-containing compounds that impair dengue virus infectious particle production

Thomas D Burton[1], Nicholas S Eyre[1]

1. Flinders Medical Centre, Flinders University, Adelaide, SA, Australia

Dengue virus (DENV) is the most common mosquito-borne viral disease and is responsible for a major public health burden in tropical and subtropical regions around the world. Despite this, there are no approved antiviral therapeutics available. We sought to discover novel inhibitors of DENV non-structural protein 1 (NS1), using a Nanoluciferase-based thermal shift assay in conjunction with a Nanoluciferase-tagged dengue reporter virus (DENV2-NS1-NLuc) in a high-throughput compound screen of 3,378 drug-like compounds. While we were unable to unambiguously identify NS1-targeting compounds, 'hit' compound validation studies revealed a collection of structurally related compounds which inhibit DENV infection in a hepatoma cell culture model. Following testing of 40 structurally related analogues, we identified a top hit (PubChem CID: 50839998) which had minimal impact on viral RNA replication and cell viability but inhibited infectious particle production at low micromolar concentrations. Examination of the impact of this compound on viral protein localization profiles by confocal microscopy revealed dose-dependent reductions in the abundance of the viral Envelope (E) protein, consistent with the observed inhibition of infectious virus production. Further investigation into the mechanism of action of this compound is warranted to determine its exact molecular target(s), while testing of a wider range of structural analogues may enable identification of related compounds with greater efficacy and lower cytotoxicity.

111

An integrated atlas of innate immune cell responses to activating stimuli reveals time-, dose- and ligand-dependent axes of inflammation.

Suzanne Butcher[1], Linden J Gearing[2, 3], Paul J Hertzog[2, 3], Jarny Choi[1], Christine Wells[1]

1. Stem Cell Systems, Department of Anatomy and Physiology, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville, Victoria, Australia

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

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

Current models of the innate immune response have been constructed from decades of studying individual pathogens and cell types in isolation. Though useful, the broad response described by these models means the nuances of pathogen-specific programs are lost to summarisation, and cell-specific aspects of inflammation are under-explored. Whilst the dynamics of cell- and pathogen-specific responses are difficult to compare simultaneously in a dish, we can use computational biology to combine large-scale expression studies into atlases, which allow the major axes of convergence and variation amongst activation states to be identified.

We have compiled an Atlas of Activation from 29 studies describing monocytes, macrophages, and dendritic cells stimulated with over 65 extrinsic and intrinsic stimuli. Our Atlas is an evolving tool that allows users to explore variation within innate responses from multiple perspectives. Sample derivation method, cell type, predicted receptor, and stimulus can be explored at a range of resolutions, from individual receptors and ligands, through to broad categories of treatment such as bacteria, fungi, and viruses. This combined view of experimental factors and cellular diversity aims to characterise relationships between biological and experimental factors, and signalling outcomes, which may help identify new regulators of inflammatory signalling.

In time, our Atlas of Activation will become available for users to explore at www.stemformatics.org, where it will complement our existing transcriptional atlases. Users will be able to project their own expression data onto the Atlas, allowing them to define activation states within their data, and to benchmark the behaviour of their cellular models of immune activation against a broad, highly curated cohort of data reflecting the breadth of pathogenic challenge to the innate immune system.

112

Development of a multiplex bead-based assay with novel autoantigens to investigate autoantibodies in Acute Rheumatic Fever

Lauren Carlton[1, 2], Reuben McGregor[1, 2], Alana Whitcombe[1, 2], Natalie Lorenz[1, 2], Julie Bennett[3], Michael Baker[3, 2], Uri Laserson[4], Timothy O'Donnell[4], Nikki Moreland[1, 2]

1. School of Medical Sciences, The University of Auckland, Auckland, New Zealand

2. Maurice Wilkins Centre, The University of Auckland, Auckland, New Zealand

3. Department of Public Health, University of Otago, Wellington, New Zealand

4. Icahn School of Medicine at Mount Sinai, New York, The United States of America

Acute Rheumatic Fever (ARF) is a serious post-infectious sequela of a Group A Streptococcus (GAS) infection that can progress to rheumatic heart disease. ARF rates remain unacceptably high in Indigenous children in Australia and Māori and Pacific children in New Zealand, yet pathogenesis is poorly understood. During a GAS infection, immune dysregulation can result in the generation of immune cells and antibodies that bind to human proteins and contribute to ARF disease. The current dogma is that mimicry exists between GAS and human heart proteins providing a basis for cross-reactivity, but few studies have applied contemporary antibody profiling technologies to ARF. In this project, two high-throughput techniques were employed to identify novel human antigens that are reactive in ARF patient sera compared with matched healthy controls and GAS pharyngitis. These being Human Protein Arrays, containing between 9,000-16,000 full-length proteins and Phage Immuno-Precipitation Sequencing (PhIP-Seq), which displays the entire human proteome as 250,000 peptides 90 amino acids in length on the surface of phage molecules. From these two approaches, four autoantigens found in heart and connective tissue (PTPN2, DMD, a myosin associated protein and collagen 1) were selected for orthogonal validation by ELISA. For all four autoantigens, serum reactivity was significantly elevated in an expanded ARF cohort. Peptides corresponding to the specific epitopes of the myosin associated protein and collagen 1 detected by PhIP-Seq have also been synthesised for validating by ELISA. The microarray and ELISA techniques were restricted to measuring total IgG only, however Luminex bead-based assays allow for simultaneous detection of antibody isotypes and subclasses in a multi-plex format. To further explore the autoantibody features associated ARF, the autoantigens have been coupled to Luminex beads as both recombinant proteins, as well as long peptides, and this multiple autoantigen bead-based assay is undergoing optimisation.

113

Establishing human small intestinal monolayers to study EPEC infection

Eva Chan[1, 2], Cristina Giogha[1, 2], Paul Hertzog[1, 2], Elizabeth Hartland[1, 2]

1. Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, 27-31 Wright Street, Clayton, VIC 3168, Australia

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

The gastrointestinal tract is lined by a single layer of epithelial cells that forms a barrier to the external environment. With regular exposure to the external environment, the epithelial barrier is exposed to a number of stressors. An example of this is the introduction of gastrointestinal pathogens, such as enteropathogenic *Escherichia coli* (EPEC). This bacterium can disrupt the intestinal epithelial lining leading to symptoms such as diarrhoea, fever, vomiting, and in extreme cases death. This is possible due to the bacterial effector proteins that are injected into the host cell resulting in the remodelling of host cell cytoskeleton and changes to host cell signalling pathways.

Such findings have been possible due to the use of immortalised cell lines in the laboratory. However, immortalised cell lines do not recapitulate the epithelial cell heterogeneity that is exhibited in the human gastrointestinal tract. Therefore, to better understand the effect of EPEC infection on human intestinal epithelial cells, the organoid culturing system will be utilised. A protocol has been established to grow these cells as a monolayer to allow access to the apical cell surface by EPEC, where immunofluorescent staining and imaging has been used to visualise the interaction. By establishing this model, this will provide further insight to host cell response and epithelial cell function following infection.

114

Functional antibody responses to *P. falciparum* transmission stage antigens following experimental malaria infection

Jo-Anne Chan[1, 2, 3], Shirley Lu[1], Linda Reiling[1, 3], D. Herbert Opi[1, 2, 3], Damien Drew[4], Zuleima Pava[5], Michelle Boyle[1, 5], Michael Theisen[6], James McCarthy[4, 5], Bridget Barber[5], James Beeson[1, 2, 6]

1. Burnet Institute, Melbourne, VIC, Australia

2. Department of Medicine University of Melbourne, Melbourne

3. Department of Immunology, Monash University, Clayton, VIC, Australia

4. Walter & Eliza Hall Institute, Melbourne

5. QIMR-Berghofer, Herston, QLD

6. Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark

Background: Although there has been major progress in reducing the global malaria burden, this decline has recently plateaued. There are still over 200 million cases annually, exacerbated by the impact of COVID-19. An effective vaccine would be a key weapon to eliminate malaria by interfering with the rapid spread of parasites throughout a population. As such, the development of transmission-blocking vaccines is a primary goal of WHO and Gates Foundation, highlighted in the Malaria Vaccine Roadmap. Transmission-blocking vaccines are designed to target an essential biological bottleneck in the malaria life cycle by generating antibodies that can block parasite transmission from humans to mosquitoes. When a mosquito feeds on an infected person, whole blood containing antibodies and transmission stage parasites is ingested. Antibodies of the right functional properties that specifically target gametocytes can inhibit parasite development within the mosquito and prevent their subsequent transmission to humans. However, major knowledge gaps in our understanding of how antibodies block transmission represents a critical roadblock to vaccine development.

Methods: Here, we assessed antibody responses in samples from a unique experimental *P. falciparum* infection of malaria-naïve Australian adults designed to induce transmission stage parasites. This innovative approach enables the study of immunity upon a single defined infection without the influence of prior malaria exposure. We measured serum antibody levels and functional antibody mechanisms (complement fixation and Fc receptor binding) at multiple time points concurrent with existing clinical data on mosquito transmission collected during the study.

Results/Conclusions: Using recombinant transmission stage antigens that we expressed, we detected high levels of IgG and IgM in the majority of serum samples tested at each time point. However, there was little cytophillic subclasses, IgG1 and IgG3 measured towards these key antigens which resulted in limited functional antibody activity observed. Further, we performed mathematical modelling to determine whether antibody levels correlated with mosquito transmission data. Our findings have major implications to further understand how the acquired human immune response potentially interrupt the transmission of malaria and accelerate the development of transmission-blocking vaccines crucial for malaria elimination.

115

Smith-specific TCR-Tregs successfully treat lupus nephritis in a humanised model of disease

Rachel MY Cheong[1], Peter J Eggenhuizen[1], Janet Chang[1], Boaz Ng[1], Ting Tian[1], Kylie Loh[1], Sarah Snelgrove[1], Julie Monk[1], Chanjuan Shen[1], Jia Xi Chung[1], Alberta Hoi[1], Eric Morand[1], Joshua D Ooi[1]

1. School of Clinical Sciences, Monash University, Melbourne

Regulatory T cells (Tregs) play an important role in maintaining immune system homeostasis. In particular, antigen-specific Tregs have been shown to be able to specifically and potently suppress antigen autoreactivity, suggesting there is potential for the development of antigen-specific Tregs to treat autoimmune diseases. One such autoimmune disease is systemic lupus erythematosus (SLE), a chronic inflammatory disease with heterogenous manifestations including lupus nephritis (LN). LN is one of the more severe manifestations of SLE associated with high morbidity and mortality – risk factors for development of LN include the presence of anti-Smith (Sm) autoantibodies as well as the HLA phenotypes DR3 and DR15, with the majority of patients typed as DR15. Due to the strong association of LN with a specific autoantigen and HLA DR15 phenotype, it is a good candidate disease for the development of antigen-specific Tregs for treatment. We first identified DR15-restricted Sm T cell epitopes using a physical affinity binding assay. High-affinity DR15-restricted Sm-specific T cell receptors were identified using single cell sequencing, then transduced onto primary SLE patient Tregs using lentiviral vectors. In both *in vitro* co-cultures and *in vivo* NSG mouse models of disease using SLE patient PBMCs, our lentivirally-transduced Sm-specific Tregs were significantly better at suppressing Sm-autoreactivity as measured by cell proliferation and pro-inflammatory cytokines, and nephritis as measured by proteinuria and glomerular segmental necrosis. These results demonstrate that Sm-Treg therapy is a promising treatment for patients with LN with future potential for use in a clinical setting.

116

Neutrophils show distinct functional phenotypes in response to malaria infection

Sandra Chishimba[1, 2], Dean Andrew[3], Jessica Loughland[3], Shirley Lu[2], Lee Yeoh[2], Nicholas Dooley[3], Rebecca Webster[3], Gaoqian Feng[2], Bridget Barber[3], Raffi Gugasyan[2], Stephen J Rogerson[4], Michelle J Boyle[3], James G Beeson[2]

1. Medicine at Royal Melbourne Hospital, University of Melbourne, Melbourne, Victoria, Australia

2. Life Sciences, Burnet Institute, Melbourne, Victoria, Australia

3. QIMR Berghofer Medical Institute , Brisbane, QLD, Australia

4. Medicine at Royal Melbourne Hospital, Peter Doherty Institute, University of Melbourne, Melbourne, Victoria, Australia

Neutrophils, a heterogenous cell group, play an essential role in clearing many infectious pathogens. Mounting evidence suggests that neutrophils may play a central role in antibody-mediated naturally acquired and vaccine-induced immunity to malaria. Currently, there is very little known about the phenotypes and functions of neutrophils in human malaria infection. We investigated neutrophil phenotypes in *in vitro* models of direct neutrophil-malaria parasite interactions and a clinical trial of experimental human malaria. We examined Fcg-receptors and adhesion molecule expression involved in parasite killing and clearance. Flow cytometry analysis revealed no change in

neutrophil phenotype in direct contact with un-opsonized malaria parasites. However, phagocytosis of antibody-opsonized parasites leads to distinct neutrophil functional phenotypes, characterized by changes in specific Fcg-receptor and adhesion molecule expression. These neutrophil phenotypes are different to typically activated phenotypes seen in bacterial infections and sepsis. In experimental human malaria, neutrophils showed early phenotypic and functional changes even at low parasitemia prior to clinical presentation. These data suggest an early influence of systemic mediators and/or cellular interactions on neutrophil functions. Furthermore, RNA-seq analysis revealed an upregulation of gene clusters relevant to neutrophil functional properties for clearance and killing of malaria parasites. Overall, our work identifies novel neutrophil phenotypes and functional changes in response to malaria parasites. This knowledge will be valuable to inform vaccine designs that maximize protective functions and understand why some vaccines fail in malaria-endemic populations.

117

Treating influenza A exacerbations – the case for pirfenidone over glucocorticoids

Julia Chitty[2, 1], Bruce Montgomery[3], Phillip Bardin[1], Jane Bourke[2, 1], Belinda Thomas[1]

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

2. Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

3. Avalyn Pharma, Seattle, WA, USA

Introduction/Aim: Patients with respiratory diseases are more susceptible to viral infection and develop more severe symptoms, associated with immunosuppression by elevated transforming growth factor-beta (TGFb). Glucocorticosteroids (GCS) effectively reduce inflammation when inhaled prophylactically, or taken orally for exacerbations, but can also cause further immunosuppression. Oral pirfenidone (PFD), an anti-fibrotic used to treat patients with pulmonary fibrosis, has been shown to reduce TGFb-enhanced influenza A virus (IAV) infection in mice (Thomas *et al*, Respirology, 2021). The current aim was to determine if administration of inhaled PFD prior to infection, or with the addition of oral PFD post-infection, is as effective at reducing IAV-induced inflammation as GCS, without causing immunosuppression.

Methods: Mice were treated with i.n. vehicle (control), PFD (13.3 mg/kg) or GCS (1 mg/kg) daily, starting 2 days prior to infection with IAV (10^2 PFU, HKx31, n=6-10). Separate mice were treated daily pre- and post-infection (i.n. then oral) with vehicle (i/o control), PFD (i/o PFD) or GCS (i/o GCS). Mice were culled 3 days post-infection to measure viral load, inflammation and immune responses in BALF and lung tissue.

Results: Inhaled PFD, but not GCS, reduced viral load ($p<0.05$). Both PFD and GCS reduced RANTES, while PFD, but not GCS, reduced IL-6, TNFα and KC. IAV-induced Inflammatory cells, notably macrophages and neutrophils, were further increased by GCS ($p<0.001$) but not by PFD. Preliminary results suggest that i/o PFD reduced the severity of IAV infection, while i/o GCS increased severity.

Conclusion: Treatment with either inhaled or i/o PFD afforded greater protection against TGFβ-enhanced viral infection and inflammation than GCS. These positive findings support the repurposing of PFD beyond its current use in IPF to include other respiratory diseases, offering superior protection from worse disease outcomes associated with viral exacerbations.

118

Functional and immunological characterisation of merozoite surface proteins 4 and 5 in *Plasmodium knowlesi*

Jill Chmielewski[1], Isabelle Henshall[2], Danny Wilson[3, 1]

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

2. Bernard Nocht Institute for Tropical Medicine, Hamburg, Germany

3. Burnet Institute, Melbourne, Victoria, Australia

Malaria is a parasitic disease caused predominantly by two species in humans: *Plasmodium falciparum* and *P. vivax*. These parasites have a complicated multi-host lifecycle, in humans this includes a continuous asexual replication within the blood which results in clinical disease. This begins when the invasive form of parasite, called a merozoite, attaches to and infects an erythrocyte in which they replicate to form the daughter parasites. There is still much to understand regarding merozoite cell-entry biology, particularly for *P. vivax* which is yet to be adapted to *in vitro* culture. Merozoite surface proteins (MSPs) are proposed to play a role in attachment of merozoites to erythrocytes and have long been considered as potential vaccine targets. However, the function of most MSPs has yet to be defined. Here, I applied targeted gene editing to investigate MSP4 and 5 function in the *in vitro* culturable *P. falciparum* and *P. knowlesi*, a close relative of *P. vivax*. These proteins likely arose from a gene duplication event as their chromosomal locus is highly conserved and there is structural similarity, hence they may have a linked or complementary biological role. CRISPR-Cas9 gene-editing revealed that *P. knowlesi* MSP5 is refractory to gene deletion, but it could be functionally replaced by *P. vivax* MSP5. Proceeding to conditional knock-down of MSP5 protein expression revealed a severe cell-entry defect. Conversely, deletion of *P. knowlesi* MSP4 had no phenotype. We, and others, found the opposite for *P. falciparum* where MSP4 is essential but MSP5 is dispensable. This study provides a range of gene-edited lines to investigate MSP4 and MSP5 function and immunological importance and emphasizes that vaccine candidates must be considered individually for the two most prominent human malarias, while promoting MSP5 as a potential vaccine candidate for *P. vivax*.

119

Clade-specific gastrointestinal microbiota modulates epithelial endoplasmic reticulum stress response

Michelle Chonwerawong[1, 2], Gemma L DAdamo[1, 2], Vanessa Rossetto Marcelino[1, 2], Linden J Gearing[1, 2], Jodee A Gould[1, 3], Emily L Rutten[1, 2], Trevor J Wilson[3], Tamblyn Thomason[1, 2], Emily L Gulliver[1, 2], Manal Alshareef[1, 2], Kitty McCaffrey[1, 2], Paul J Hertzog[1, 2], Edward M Giles[1, 4], 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. MHTP Medical Genomics Facility, Hudson Institute of Medical Research, Clayton, VIC, Australia

4. 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 that recapitulates the nature and complexity of host state driven by these diverse bacteria remains to be established. Applying our novel site-specific bacterial culturing from patient intestinal mucosal biopsies, we have found key bacterial subclades that associated with host state in matched bacterial metagenomic and host gene transcriptional analysis. To experimentally validate these findings *in vitro*, mucosal bacterial isolates were co-cultured with intestinal epithelial cells which resulted in a clade-specific cell cytotoxic phenotype and transcription of host genes belonging to the unfolded protein response (UPR) and endoplasmic reticulum (ER) stress pathways. This host cell phenotype was subsequently observed following bacterial co-culture in Transwell assays and similarly in response to conditioned media from epithelial cells that were treated with candidate bacterial isolates. However, epithelial cell cytotoxicity and transcription of ER stress and UPR genes were abrogated upon treatment with heat-treated bacterial lysates. This indicates that there may be specific microbiota effector proteins or metabolites that function as mediators of epithelial cell signalling pathways important in regulating cellular stress and intestinal inflammation. A comprehensive understanding of bacterial-host cell interactions will provide key insights toward harnessing gut bacterial candidates for novel microbiome-based therapeutics that target disorders with a complex pathophysiology such as inflammatory bowel disease.

120

Understanding how Autotransporter Proteins Promote Bacterial Disease

Kaitlin Clarke[1], Jason Paxman[1], Begoña Heras[1]

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

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

Despite their abundance and important role in bacterial diseases, ATs are poorly understood and, until recently, there was no adequate classification system to describe the functional classes of the protein family. We addressed this by developing a phylogenetics-based classification system drawing on insights from our own research and the published literature. For the first time, all members of the AT family are classified into groups according their molecular structure and function [1].

This new classification provides new insights and information to further characterise AT protein mechanisms in disease. Using this system, we have directed our attention toward characterising relatively unknown ATs from the pathogen *Bordetella pertussis* (whooping cough). Using a combination of structural biology (Australian Synchrotron) along with other experimental techniques, we are uncovering the molecular mechanisms of these ATs and their role in promoting whooping cough. In other research, we have used our new AT classification system to identify AT functions for use in medical applications. Currently, I am re-purposing the AT toxins to create the first AT platform for the intracellular delivery of therapeutics into human tissue. Such a medical innovation would be highly beneficial to medicine, as 30% of all human therapeutics are peptide/protein based, which cannot cross human cell membranes.

121

Helicobacter pylori extracellular vesicles contain urease and catalase which promote bacterial survival

Nina Colon[1], Liam Gubbels[1], Richard L Ferrero[1, 2, 3]

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

2. Biomedicine Discovery Institute, Department of Microbiology, Monash University, Clayton, Victoria, Australia

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

Extracellular vesicles (EVs) are released during bacterial growth and contain various cargo involved in pathogenesis. Proteomic studies showed that the gastric pathogen *Helicobacter pylori* produces EVs containing urease (UreA, UreB) and catalase (KatA), known to be essential for its survival and colonisation in the stomach. Nevertheless, little is known regarding the functional properties of these enzymes in EVs. We hypothesised that functionally active urease and catalase within *H. pylori* EVs may promote bacterial survival. To address this hypothesis, we first characterised the urease and catalase activities of *H. pylori* wild-type (WT) EVs and corresponding sonicated preparations of the whole bacteria. Western blotting confirmed the presence of UreA, UreB, and KatA subunits within sonicates and EVs. Bacterial sonicates and EVs had: urease activities of 0.618 ± 0.084 and 0.45 ± 0.099 µmol/min/mg protein, respectively (p=0.270); and catalase activities of 770.64 ± 10.51 and 729.36 ± 13.76 µmol/min/mg protein, respectively (p=0.076). These results indicate that EV-associated urease and catalase activities are comparable to those of the whole bacteria. Following this finding, we aimed to determine the impact of the EV-associated enzymes on bacterial survival under environmental stresses. *H. pylori ureA* and *ureB* mutant bacteria were challenged for one hour at pH 3.5 with and without EVs isolated from an *H. pylori* WT strain. Bacteria treated with WT EVs (100 µg/mL) showed 2.7- and 2.5-fold increases in survival of *ureA* and *ureB* mutants, respectively, compared to non-treated bacteria. Similarly, the survival of *katA* mutant bacteria in 10 mM H2O2 after 1 hour was restored to that of the WT in the presence of WT EVs (40 µg/mL). Together, these data highlight that *H. pylori* EVs contain functionally active enzymes which may contribute to bacterial survival *in vivo.*

122

Investigating and inhibiting DsbD, an essential enzyme in Neisserial pathogens

Taylor Cunliffe[1], Tony Wang[1], Pramod Subedi[1], Jason Paxman[1], Begoña Heras[1]

1. Biochemistry & Chemistry, La Trobe Institute of Molecular Science, La Trobe University, Melbourne, Victoria, Australia

Neisseria meningitidis and *N. gonorrhoeae* are causative agents of meningococcal disease and gonorrhoea respectively. These pathogens, particularly *N. gonorrhoeae* are recognised by the World Health Organization (WHO) as urgent threats to global health due to their increasing resistance to antimicrobials. New approaches are urgently needed to combat these pathogens.

Disulphide bonds are an important structural feature that proffers stability and function to many proteins. The process of disulphide bond catalysis is mediated by Dsb proteins in the periplasm of Gram-negative bacteria. A member of the Dsb family, DsbD is an enzyme essential for Neisserial viability rendering it an attractive target for antimicrobial development.

DsbD acts as an electron transport hub in the bacterial plasma membrane; transferring electrons sequentially and unidirectionally from the cytoplasm to target virulence substrates. Understanding the mechanism behind this unidirectional transfer is key to understanding Neisserial pathogenesis and ultimately to developing specific inhibitors. Our structure of the n-terminal domain of *Neisseria* DsbD revealed a flexible 'Phen-cap loop' postulated to control unidirectional electron flow (Smith et al., 2018).

In the present study, mutations have been introduced into key amino acids hypothesised to underpin the flexibility of the Phen-cap loop to determine their function. The structures of the mutant proteins were solved utilising X-ray crystallography to assess the effect of the mutations in the positioning of the Phen-cap loop. Overall, through structural and biochemical studies this work identified key amino acids that modulate the positioning of the Phen-cap loop, and ultimately regulate the reactivity of DsbD.

Further, the mechanistic understanding of DsbD has been utilised in a collaboration with Oracle Cloud Infrastructure to identify inhibitors in silico using cloud-based computing introducing a new method of inhibitor development.

1. Smith RP, Mohanty B, Mowlaboccus S, Paxman JJ, Williams ML, Headey SJ, Wang G, Subedi P, Doak BC, Kahler CM, Scanlon MJ, Heras, B. (2018). Structural and biochemical insights into the disulfide reductase mechanism of DsbD, an essential enzyme for neisserial pathogens. J Biol Chem, 293(43), 16559-16571.

123

Decoding resident immune cell communication

Caleb Dawson[1, 2], Julie Tellier[1, 2], Raymond Yip[1, 2], Andrew Kueh[1, 2], Marco Herold[1, 2], Jane Visvader[1, 2], Stephen Nutt[1, 2]

1. WEHI, Parkville, VIC, Australia

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

Immune cells regulate breast development, function and cancer, but little is known about the mechanisms involved. Broadly, we know that duct growth and tumorigenesis are negatively regulated by T cells, while mast cells, eosinophils and macrophages promote morphogenesis and remodelling. Macrophages also reduce the effectiveness of cancer treatments and plasma cells provide protective IgA antibodies to breastfeeding infants. We want to determine how these functions are carried out and how breast tissue instructs these immune cells in their unique roles.

We previously used 3D imaging and intravital imaging to discover a unique population of 'ductal' macrophages that survey the entire mammary epithelium with fine dendrites. We have also revealed the location of plasma cells that arrive in the breast during lactation. We are now implementing spatial transcriptomics to reveal how neighbouring cells communicate and influence immune cell function. We are also developing methods for gene editing of the mammary epithelium and stromal cells to determine the role of individual niche signals. Finally, novel mouse models for targeted breast immune cell ablation are helping us to define breast immune cell function more accurately. Through these efforts, we hope to discover new avenues for immunotherapeutic treatment of breast diseases like inflammation and cancer.

124

Chlamydia pneumoniae can infect human glial cells and modulate metabolism-related gene expressions

Ali Delbaz[3, 1, 2], Todd Shelper[3, 1, 2], Souptik Basu[3, 1, 2], Linh Nguyen[3, 1, 2], Amy McEwen[3, 1, 2], Kyle Mathew Hatton-Jones[3, 2], James Sinclair[3, 2], Nicholas West[3, 2], James St John[3, 1, 2], Jenny Ekberg[3, 1, 2]

1. Clem Jones Centre for Neurobiology and Stem Cell Research, Griffith University, Southport, QLD, Australia

2. Menzies Health Institute Queensland, Griffith University, Southport, QLD, Australia

3. Griffith University, Southport, QLD, Australia

Neurodegenerative diseases are the leading cause of disability-adjusted-life-years (DALYs) globally. The infectious hypothesis has been proposed as one of the underlying causes of certain neurodegenerative diseases such as Alzheimer's disease (AD). Respiratory pathogens including intracellular bacteria *Chlamydia pneumoniae* may infiltrate the central nervous system (CNS) via the nasal cavity and initiate a cascade of host inflammatory responses over the lifetime of a patient. This hypothesis is supported by several studies which have found the presence of pathogens more commonly in the CNS of AD patients.

Beside the well described characteristics of profuse neurofibrillary tangles and Aβ deposits, metabolic dysfunction is a core feature of different types of neurogenerative diseases, including AD. Thus, early detection of the metabolic changes in glial cells (crucial support cells in the brain), may reflect the role of infection determinants in contribution to the neurodegenerative diseases such as AD.

Objectives: To determine whether *C.pneumoniae* could infect human glial cells and alter metabolism-related genes expressions.

Methods: Infected human glial cells (Astrocytes and microglia) after 24h, 48h and 72h were imaged using widefield microscope. CellProfiler was used to determine infection by detecting the presence of inclusion bodies. Nanostring metabolism gene panel (for humans)

was used to study 770 metabolism-related genes from these infected human glial cells. Data analyses was performed using web-based Rosalind bioinformatic, eClustvis tool and Morpheus software.

Results: *C.pneumoniae* can infect human glial cells which is comparable to our previously established results on mouse models . We have also showed that bacterial infection was able to regulate the genes related to host cell metabolism such as lipid, amino acids, and carbohydrates at transcription level in different stages of acute infection.

Conclusion: This study shows that *C.pneumoniae* can infect human CNS glial cells, and that the bacteria can alter the host metabolism-related genes.

125

Cumulative vaccine take of RV3-BB rotavirus vaccine observed in Indonesian infants regardless of histo-blood group antigen status

Celeste M Donato[1, 2, 3], Amanda Handley[1, 4], Sean G Byars[5], Nada Bogdanovic-Sakran[1], Eleanor Lyons[1], Darren Suryawijaya Ong[1], Daniel Pavlic[1], Julie E Bines[1, 2, 6]

1. Enteric Diseases, Murdoch Children's Research Institute, Parkville, Victoria, Australia

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

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

4. Medicines Development for Global Health, South Bank, VIC, Australia

5. University of Melbourne, Parkville, VIC, Australia

6. Department of Gastroenterology and Clinical Nutrition, Royal Children's Hospital, Parkville, Victoria, Australia

Background: A disparity in vaccine effectiveness between high- and low-income settings has been observed for live-oral rotavirus vaccines administered to infants. Population differences in histo-blood group antigen (HBGA) status (determined by polymorphisms of the FUT2 and FUT3 genes) could be one factor contributing to this disparity due to human rotavirus strains binding to HBGAs in a strain dependent manner.

The RV3-BB rotavirus vaccine is comprised of a naturally attenuated human neonatal G3P[6] rotavirus strain. A phase IIb, randomized, double-blind, placebo-controlled trial of oral RV3-BB rotavirus vaccine was conducted in infants in Central Java and Yogyakarta, Indonesia. The aim of this study was to determine whether Lewis and secretor status impacted vaccine take.

Methods: Total DNA was extracted from infant stool samples (n= 164), the FUT2 and FUT3 genes amplified by PCR and sequenced on a NovaSeq SP Lane (Illumina). The single nucleotide polymorphisms (SNPs) identified in the FUT2 and FUT3 genes were analysed to infer the Lewis and secretor status of each participant. Cumulative vaccine take was defined as a serum immune response of anti-rotavirus immunoglobulin A (IgA) or serum neutralizing antibodies 28 days following dose administration or as detection of RV3-BB virus excretion in stool.

Results: Combined Lewis and secretor phenotype could be determined for 147/164 participants, with 94 designated as Lewis positive secretors, 1 designated as a Lewis positive non-secretor and 31 designated as Lewis positive weak secretors. A further 16 participants were designated as Lewis negative secretors and 5 were designated as Lewis negative weak secretors. Cumulative vaccine take was not significantly associated with either participant secretor status (RR=1.00, 95%CI=0.94-1.06, p=0.97) or Lewis phenotype (RR=1.03, 95%CI=0.94-1.14, p=0.33).

Conclusions: The G3P[6] human neonatal RV3-BB vaccine produced positive cumulative vaccine take, regardless of participant HBGA status in Indonesian infants.

126

Novel strategy to identify monocytes subsets contributing to increased COVID-19 disease severity in older individuals

Salimeh Ebrahimnezhaddarzi[1], Jingling Zhou[2], Alistair Deller-blue[1], Hans Kek[1], Irene Boo[1], James McMahon[4, 3], Bradley J Gardiner[4], Anna Coldham[4], Gilda Tachedjian[1, 3, 5], Heidi Drummer[1, 3, 5], Anthony Jaworowski[1, 4], Anna C Hearps[1, 4]

1. Disease Elimination Program , Burnet Institute, Melbourne, VIC, Australia

2. Emerging Infections Program, School of Health and Biomedical Sciences, RMIT University, Melbourne, Victoria, Australia

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

4. Department of Infectious Diseases, The Alfred and Monash University, Melbourne, Victoria, Australia

5. Department of Microbiology, Monash University, Melbourne, VIC, Australia

Introduction: Coronavirus disease-19 (COVID-19) can cause substantial morbidity and mortality, particularly in older individuals. In this study, we investigated whether an altered inflammatory status of monocyte subpopulations in older people may contribute to severe COVID-19.

Methods: PBMC samples were obtained from the Alfred Hospital COVID-19 Biobank in two groups of younger (n=21, median age 30 years [range 21-40]) and older (n=14, median age 69 years [61-96]) individuals within 8 days of diagnosis. Control individuals were SARS-CoV-2 negative of comparable age and sex. Monocytes were assessed for immunophenotype and intracellular levels of pro-inflammatory cytokines TNF-α, IL-6 and IL-1β measured in unstimulated cells and following LPS treatment. A novel panel of cell surface markers which are stably expressed on stimulated monocytes (CD86, CD33, HLA-DR, CCR2 and CD64) was applied to identify monocyte subsets.

Results: In addition to the traditional classical, intermediate and non-classical subsets, the novel phenotyping panel identified a new subpopulation of monocytes which expressed lower levels of HLA-DR, CD33 and CD64 and produced less pro-inflammatory cytokines than classical and intermediate monocytes. The proportion of this novel subpopulation was higher in COVID-19+ individuals than in age-matched controls in both stimulated and unstimulated conditions ($p<0.0001$ and $p<0.02$ respectively). Monocytes from older COVID-19+ individuals had lower levels of TNF compared to older controls at baseline ($p=0.005$ in intermediate monocytes). Monocytes from both younger and older COVID19+ individuals produced less of the pro-inflammatory cytokines IL-6, TNF and IL-1β when stimulated *ex vivo* as compared to controls.

Discussion & Conclusion: We identified a unique subpopulation of monocytes which are expanded in COVID19. Further, monocytes from COVID19+ individuals showed impaired inflammatory responses to stimulation, indicating a form of tolerance which was more pronounced in older individuals. Understanding altered inflammatory responses in COVID19 may illuminate the mechanisms contributing to severe hyper-inflammatory disease.

127

The *Helicobacter pylori* virulence factor, Tipa, is carried by bacterial extracellular vesicles to the nuclear compartment of host cells.

Jack Emery[1, 2], Kate Friesen[2], Variya Nemidkanam[3, 2], Maxine Yap[2], Nina Colon[2], Georgie Wray-McCann[2], David McGee[4], Natalia Castano Rodriguez[5], Dongmei Tong[2], Caroline Skene[2], Laurent Terradot[6], Richard Ferrero[1, 2, 7]

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. Department of Microbiology and Immunology, Louisiana State University Health Sciences Center, New Orleans, Louisiana, USA

5. School of Biotechnology and Biomolecular Science, University of New South Wales, Kensington, NSW, Australia

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

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

The bacterium *Helicobacter pylori* tightly regulates the host immune response to dampen inflammation and promote persistence. Excessive inflammatory responses to the bacterium, however, promote gastric carcinogenesis. Several *H. pylori* proteins have been associated with an increased risk of gastric cancer. One of these proteins, tumour necrosis factor-a-inducing protein (Tipa), when produced in its recombinant form was reported to traffic to the host cell nucleus, leading to tumour necrosis factor (TNF) gene expression and carcinogenic effects. Although Tipa is secreted by the bacterium, we showed in proteomic studies that it is also released by bacterial extracellular vesicles (BEVs); these membrane "blebs" are highly efficient at entering and subverting host cell functions. We hypothesised that *H. pylori* BEVs carry Tipa to the nucleus, resulting in perturbed nuclear processes that promote carcinogenesis. To address this hypothesis, we generated *H. pylori tipA* mutants and complemented *tipA*/*tipA*+ strains. By immunoblotting, we confirmed that *H. pylori* BEVs harbour Tipa. In addition, the quantity of Tipa secreted into culture supernatants varied between *H. pylori* strains, but was not associated with disease outcome. Gastric epithelial cells (AGS) were treated with *H. pylori* OMVs or recombinant Tipa (rTipa). By immunoblotting and confocal microscopy, we showed that BEV-associated Tipa is present within the cytoplasm at 4 hours post-treatment and accumulates in the nuclear compartment by 18 hours. We then tested the ability of Tipa to induce pro-inflammatory signalling in AGS and THP-1-derived macrophages. Consistent with previous work, rTipa induced TNF production in THP-1 cells. Conversely, BEVs from *tipA* bacteria induced significantly less TNF than those from wild-type (WT) or *tipA*/*tipA*+ bacteria ($p < 0.0001$), as well as significantly less IL-8 production in AGS ($p = 0.036$) and THP-1 cells ($p = 0.018$). Taken together, we propose that BEV-associated Tipa may dampen pro-inflammatory responses but promote carcinogenesis in *H. pylori* infection.

128

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

Vik Ven Eng[1, 2], Jaclyn Pearson[1, 3, 2]

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

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

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

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

Using *Citrobacter rodentium* – the model organism for EPEC, evaluation of disease in a panel of RIPK knockout mice revealed that compound deletion of RIPK1, RIPK3 and caspase-8 greatly heightened the diarrhoeal severity and bacterial burden experienced at peak of infection. Notably, only RIPK3 played a significant role in moderating colonic pathology and bacterial burden in a manner independent from necroptosis. While RIPK1 kinase activity was shown to be dispensable for mediating protection, RIPK1 does contribute to immunity through a separate yet undefined mechanism. More interestingly, flow cytometry analysis of RIPK3-deficient mice found a marked reduction in the T-helper 1 (Th1), Th17 and T-regulatory cell populations in the colonic lamina propria. This loss of RIPK3 also correlated with an altered colonic expression of *Cd59a* and *Clca1* that are involved in the regulation of leukocyte function. Dysregulation of these key immune responses is proposed to contribute to host decline upon EPEC infection.

Together, our results describe an association between RIPK1/3 (innate immunity) and T cell responses (adaptive immunity) that is involved in host mucosal protection against enteric bacteria. Further characterisation of the underlying signaling pathways will be important to inform future management and treatment of serious gastrointestinal diseases.

129

Mapping of critical dengue virus-host interactions by APEX proximity labelling to reveal future antiviral drug targets.

Siena Centofanti[1], Alex Colella[1], Timothy Chataway[1], Nicholas Eyre[1]

1. College of Medicine and Public Health, Flinders University, Bedford Park, SA, Australia

Dengue virus (DENV) is a rapidly-spreading mosquito-borne (+)RNA virus that is currently endemic in over 100 countries and responsible for an estimated 390 million yearly infections. Despite an urgent need, safe and effective DENV vaccines or antiviral therapies are not yet available. The DENV non-structural protein 1 (NS1) plays critical roles in viral RNA replication, infectious virus particle production and viral pathogenesis and has emerged as a major target in the development of vaccines and antivirals. Towards the development of antivirals targeting NS1-host factor interactions, we have adopted an APEX2 proximity labelling-coupled quantitative proteomics approach to map the proteomic composition of the DENV NS1 microenvironment in infected cells. The engineered ascorbate peroxidase tag, APEX2, catalyses the conversion of biotin phenol into biotin-phenoxyl radicals that covalently tag proximal endogenous proteins. We have generated and validated an APEX2-tagged dengue reporter virus (DENV2-NS1-APEX2) to enable the biotinylation of NS1-proximal endogenous proteins, optimised the biotinylation reactions and the purification of biotinylated proteins, and acquired preliminary mass spectrometry data identifying these proteins. It is hoped that the characterization of novel DENV NS1-host protein interactions that are essential to the viral replication cycle will identify targets for future antiviral drug development and yield insights into the mechanisms underlying the essential functions of this highly enigmatic protein.

130

Identification of key residues in dengue virus NS1 protein that are essential for its secretion

Brandon E.K. Tan[1], Michael R Beard[1], Nicholas S Eyre[2]

1. University of Adelaide, Adelaide, SA, Australia

2. College of Medicine and Public Health, Flinders University, Bedford Park, SA, Australia

Dengue virus (DENV) is a Flavivirus of the Flaviviridae family of (+) RNA viruses that causes significant morbidity and mortality in tropical and sub-tropical areas worldwide. A major determinant of the vascular leakage that is associated with severe DENV infections is the viral non-structural protein NS1. In addition to its essential roles in viral RNA replication and infectious virus production, NS1 is secreted from infected cells as a hexameric lipoparticle and it is this secreted form of the protein that can induce vascular leakage via induction of inflammatory cytokine production and endothelial glycocalyx disruption. Despite the importance of NS1 secretion in DENV pathogenesis, the exact features of NS1 that are critical to its secretion from infected cells are not fully characterised. Here, we employed random point mutagenesis and luminescent peptide (HiBiT)-tagged NS1 expression constructs to identify NS1 residues that are critical to its secretion. Amongst 10 identified point mutations, several mutations within the β-ladder domain of NS1 (V220D, A248V, T283A, C313S and R336S) heavily impaired its secretion by >90%. Follow-up studies on two of these mutants, V220D and A248V revealed they did not support viral RNA replication nor infectious virus production. Subsequent analyses of these mutants by confocal microscopy and immunoblotting in the context of a non-structural protein (NS1-NS5) expression system indicated that these mutations may disrupt NS1 processing or maturation events that are required for mutiple NS1 functions, including its secretion. We propose that disruption of NS1 secretion may represent a viable target of future antiviral drug and attenuated vaccine development and that our mutagenesis and luminescent detection approaches can be exploited towards these goals.

131

Do anti-LPS antibodies to patient microbiota associate with worse outcomes in Crohn's disease?

Wenkang Feng[1], Naiqi Yao[1], Lauren Schooth[1], Jinjie Teh[1], Timothy Wells[1], Mark Morrison[1]

1. UQDI, University of Queensland, Brisbane, QLD, Australia

Crohn's disease (CD) is a complex, chronic, relapsing inflammatory bowel disease (IBD) and typically causes abdominal pain, diarrhoea and sometimes life-threatening complications in patients. This life-long condition significantly reduces patient's quality of life and has both a large health and economic burden. Genetic, environmental, and immune responses all play a critical role in the pathogenesis of the disease, but it is believed that gut microbiota dysbiosis is the main trigger of CD onset and is linked to the relapsing nature of CD. Many exacerbations of CD have associated blooms in gram negative bacterial species such as *Proteobacteria.* Additionally, both IgG and IgA titres have been shown to increase in the gut during exacerbations.

Antibody usually protects against bacterial disease, however we have identified a specific antibody towards gram negative bacteria that actually protects these strains from immune killing. This 'cloaking antibody' (cAb) is specific for the O-antigen of lipopolysaccharide, and when in high titres prevents complement-mediated killing of the bacteria. Presence of these antibodies has been associated with worse outcomes in respiratory diseases.

To determine whether cAbs exist in patients with CD, we investigated 78 serum samples from patients in the 'post-operative CD endoscopic recurrence (POCER)' study. To do this, we extracted LPS from six representative gram-negative microbiota-associated bacteria. The titre of IgG and IgA against these extracted LPS samples was determined via ELISA. Up to 40% of the patients displayed high titres of anti-LPS IgG to at least one of the extracts examined. We demonstrated that these antibodies could inhibit normal serum-mediated killing of these bacterial strains. Patients at with high titres of anti-LPS IgG at baseline associated with worse baseline endoscopic scores. These antibodies may be a potential biomarker for worse outcomes in CD.

132

The m6A Writer in *Plasmodium falciparum* regulates the expression of specific genes by targeted degradation of mRNA

Lakvin Fernando[1], Amy Distiller[2], Shengjie Jin[2], Emma McHugh[1], Stuart Ralph[1]

1. Department of Biochemistry and Pharmacology, Bio21 Institute/University of Melbourne, Parkville, VIC

2. University of Melbourne, Melbourne, VIC

The phylogenetic group Apicomplexans include some of the most important protist parasites that play an important role in human health. Despite their global impact, they are understudied, which allows these parasites to continue to burden resource poor communities. Many of these parasites have a complex lifecycle such as the parasite that causes malaria, *Plasmodium falciparum*. This requires the parasite to rapidly adapt to many environments.

The parasite achieves this through dynamic regulation of gene expression as seen through transcriptomics studies. Precise regulation of gene expression is also critical within each developmental stage e.g., the cascading pattern of gene expression in the pathogenic, intraerythrocytic development cycle (IDC). The mechanisms of regulating gene expression within the IDC are poorly understood. The number of transcription factors in the genome of *Plasmodium falciparum* is one of the lowest among studied organisms. Therefore, it is apparent that there are other mechanisms of regulation that play an essential role.

One such mechanism that has gained attention is post-transcriptional (epitranscriptional) modification of mRNA, particularly the N6-methyladenosine (m6A) modification of adenosine catalysed by the evolutionarily conserved m6A writer complex. It is believed that m6A enrichment affects mRNA stability leading to faster degradation of transcripts, resulting in lower abundance and decreased translation. Studied in this field have been limited by the limited availability of inducible knockdown systems for this essential complex.

In this study we adapt knock-sideways to conditionally knockdown components of the m6A writer and study the resulting phenotypes. We report that the m6A writer is essential concurring with the work of Baumgarten *et al.* (2019). Knockdown of the m6A results in an increase in total RNA and in the specific transcripts. These changes were also observed at the protein level using proteomics studies. We further analyse the effect m6A writer knockdown has on the half-life of specific mRNA that is predicted to be m6A enriched by the m6A writer.

These findings support the hypothesis that the m6A regulates the expression of specific genes by targeted degradation of mRNA, thus reducing translation. Further studies are needed to identify the mechanisms of targeted mRNA degradation.

133

Characterising the role of NKG7 in $CD4^+$ and $CD8^+$ T cells during malaria

Teija Frame[2, 1], Christian Engwerda[1]

1. QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia

2. University of Queensland, Brisbane, QLD, Australia

Malaria has had an overwhelming impact on human lives and remains one of the most serious life-threatening infectious diseases in the world. Malaria is caused by infection with protozoan parasites from the genus *Plasmodium* and can present with a wide clinical spectrum. During *Plasmodium* infection there are multiple immune responses that are triggered to protect tissues and control parasite growth. These responses involve the activation of and interactions between many different immune cells. We recently discovered a molecule called NK cell granule protein 7 (NKG7) was an important mediator of inflammation in infectious diseases and cancer. NKG7 was first characterised in 1993 and has been shown to be expressed in different immune cell populations and implicated in both pro-inflammatory and anti-inflammatory responses. In many transcriptional studies, NKG7 was identified as a biomarker in various clinical and experimental contexts. However, its function in health and disease remains poorly characterised. This study reports on the role of NKG7 in $CD4^+$ and $CD8^+$ T cell responses during malaria. We identified key anti-parasitic roles for NKG7 in the generation and function of $IFN\gamma^+$ $Tbet^+$ T helper 1 (Th1) cells, $IFN\gamma^+$ $IL\text{-}10^+$ T regulatory 1 (Tr1) cells and $CXCR5^+$ $PD\text{-}1^+$ T follicular helper (Tfh) cells. Furthermore, we discovered a critical role for NKG7 in the generation of cytotoxic $CD8^+$ T cells that mediate neurological damage in a pre-clinical model of cerebral malaria. Together, these findings provide a better understanding of the role of NKG7 in inflammation and support the therapeutic targeting of NKG7 for treatment of chronic infectious diseases.

134

Murine double hit model for neonatal cardiopulmonary diseases: bronchopulmonary dysplasia (BPD) and pulmonary hypertension associated with BPD

Steven P Garrick[1, 2], Philip J Berger[1, 2], Marcel F Nold[1, 3, 2], Claudia A Nold-Petry[1, 2]

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

2. Department of Paediatrics, Monash University, Melbourne, VIC, Australia

3. Monash Newborn, Monash Children's Hospital, Melbourne, VIC, Australia

Background: Bronchopulmonary dysplasia (BPD) and pulmonary hypertension associated with BPD (BPD-PH) are severe cardiopulmonary morbidities faced by the preterm infant. These diseases are multifactorial in origin and underpinned by a rise in pulmonary inflammation. As there are no targeted anti-inflammatory therapies available there remains a major unmet need. Most rodent models of BPD induce disease with only postnatal hyperoxia, mimicking the increased fraction of inspired oxygen (FiO_2) often required in preterm infants. However, antenatal inflammation is a major driver of preterm birth and subsequent early life cardiopulmonary disease pathophysiology. Method: To account for the multifactorial origins of early life cardiopulmonary disease, we established a double hit model, combining antenatal systemic inflammation followed by postnatal hyperoxia. On embryonic day 14, pups are exposed to systemic maternal inflammation via a single intraperitoneal injection of 150 µg/kg of lipopolysaccharide to the dam. Within 24 h after birth, pups, and dams are randomized and exposed to gas with either an FiO_2 of 0.21 (room air) or 0.65 (hyperoxia 65%) for 28 days. Results: The combination of antenatal LPS and postnatal hyperoxia causes substantial damage to pulmonary architecture, including the development of large dysmorphic alveoli and a reduction in the number of small blood vessels. These clinically relevant structural changes translate to impaired gas exchange, increased pulmonary vascular resistance, and increased pulmonary blood pressure like what can be observed in BPD and

BPD-PH. Conclusions: We developed a clinically relevant murine model of disease by combining two key inciting stimuli to accurately reflect the multifactorial pathogenesis of human early life cardiopulmonary disease. The disease severity induced by the murine double hit model is ameliorated by (1) the administration of daily low dose anakinra (recombinant IL-1 receptor antagonist) to wildtype mice or (2) the use of mice deficient in the central transducer of type 2 signalling (STAT6). Thereby establishing IL-1 and type 2 immune responses crucial in murine BPD and BPD-PH.

135

Distribution of phenotypic antimicrobial resistance in the gastrointestinal microbiome.

Emily L Gulliver[1, 2], Jodee Gould[1, 2], Emily L Rutten[1, 2], Patricia Khoo[1, 2], Sean Solari[1, 2], Tamblyn Thomason[1, 2], Margarette Williamson[3, 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. Infection and Immunity Program, Monash Biomedicine Discovery Institute and Department of Microbiology, Monash University, Clayton, Vic, Australia

In 2019, 4.95 million deaths were associated with antimicrobial resistance (AMR) worldwide. The ESKAPE pathogens, comprised of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*. are the primary bacterial contributors. The human microbiome has been identified as a source of acquired resistance in ESKAPE pathogens, in particular the gastrointestinal microbiome, which is host to the highest abundance and diversity of bacteria within the human body. Previously, a high proportion of the bacteria within the gastrointestinal microbiome were unculturable. Consequently, the only method of identifying antimicrobial resistance in gastrointestinal microbiota was through metagenomics. Although metagenomics can identify any known AMR genes, it cannot identify novel AMR genes or determine their expression and functionality within the cell. To overcome these limitations, our study utilised novel culturing on YCFA media in anaerobic conditions, which has been shown to allow for 96% of the gastrointestinal microbiota to be cultured. 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, identity determine through 16s rRNA sequencing and resistance to each of the antimicrobials was confirmed in broth. High levels of antimicrobial resistance were determined, with 47.5% of isolates resistant Amoxicillin, 6.3% to Amoxicillin-Clavulanic acid, 44.3% to Cefalexin, 28.8% to Ciprofloxacin, 38.5% to Clindamycin and 25.5% to Doxycycline. Coupled with whole genome sequencing of resistant isolates and comparative genomics, this project aims to identify both known and novel AMR genes, and phenotypically validate this resistance, to demonstrate the diversity of AMR within the human gastrointestinal microbiota. This will allow for identification of the genetic elements responsible for phenotypic AMR within the microbiome, providing a greater understanding for how the microbiome may be spreading AMR.

136

Does type I interferon blockade improve B cell immunity in malaria?

Julianne Hamelink[1], Dean Andrew[1], Jessica Loughland[1], Nick Dooley[1], Megan Soon[1], Luzia Bukali[2], Reena Mukhiya[1], Teija Frame[2], Fabian Rivera[2], Jessica Engel[2], Rebecca Webster[3], Christopher Engwerda[2], Bridget Barber[3], Michelle Boyle[1]

1. Human Malaria Immunology, Queensland Institute of Medical Research, Herston, Queensland, Australia

2. Immunology and Infection, Queensland Institute of Medical Research, Herston, Queensland, Australia

3. Clinical Malaria, Queensland Institute of Medical Research, Herston, Queensland, Australia

Malaria caused >600,000 deaths in 2020. Although it is hoped that Mosquirix – the only licensed malaria vaccine – will reduce this mortality, Mosquirix is only 36% efficacious in children overall and 20% efficacious in children with the highest malaria exposure. Children represent 77**%** of malaria deaths, meaning this most vulnerable population receives the least protection from the vaccine. New strategies to boost vaccine efficacy are therefore needed.

One potential strategy to boost vaccine efficacy is blockade of type I interferon pathways. We have previously shown that type I interferon pathways are rapidly upregulated during malaria, driving the development of tolerogenic immune responses. It is thought that these tolerised responses can impair memory B cell and antibody responses, altering disease outcomes. It is therefore possible that blockade of type I interferon pathways may prevent such immunoregulation, thus boosting anti-parasite immunity.

To test this theory, we are conducting a phase 1b clinical trial of the drug Ruxolitinib – a JAK1/2 inhibitor that blocks type 1 interferon signaling – using the induced blood stage malaria model. Participants (n=26) will be inoculated with *P. falciparum* parasites, then treated with the anti-malarial artemether-lumefantrine 8 days later. Participants will also receive either Ruxolitinib treatment or placebo. On day 90 of the trial, participants will be re-infected with *P. falciparum* parasites, allowing us to determine if Ruxolitinib therapy improves the development of anti-malarial immunity.

To study B cell responses throughout the trial, antibody type, titre and activity will be assessed via ELISA and functional antibody assays, whilst variance in B cell populations will be analysed using flow cytometry, mass spectrometry and scRNAseq. Together these analyses will determine if blockade of type I interferon signaling reduces parasite driven immunoregulation, thus allowing protective B cell responses to develop. As the trial is ongoing, preliminary data will be presented.

137

COVID-19 in Brazil: Antibody Fc-dependent Mechanisms in Natural and Vaccine-Induced Immunity

Alexander Harris[2, 1], Liriye (Lydia) Kurtovic[2, 1], Shirley Lu[2], D. Herbert Opi[2, 1, 3], Heidi Drummer[2], Bruce Wines[2, 3], Mark Hogarth[2], Clarissa Valim[4], Luís Cristóvão Porto[5, 6], James Beeson[2, 1, 3]

1. Department of Immunology, Monash University, Melbourne, Victoria, Australia

2. Burnet Institute, Black Rock, VICTORIA, Australia

3. Department of Medicine, Doherty Institute University of Melbourne, Melbourne, Victoria, Australia

4. Boston University School of Public Health, Boston University, Boston, Massachusetts, USA

5. Histocompatibility and Cryopreservation Laboratory, Institute of Biology Roberto Alcantara Gomes, UERJ, Rio de Janeiro, Brazil

6. Clinical Pathology Service, Polyclinic Piquet Carneiro, UERJ, Rio de Janeiro, Brazil, Polyclinic Piquet Carneiro, UERJ, Rio de Janeiro, Brazil

Brazil has experienced a large COVID-19 burden, recording high levels of SARS-CoV-2 infections and deaths prior to the widespread deployment of vaccines. Natural infection and vaccination induce protective antibodies that can neutralise the virus or mediate other arms of the immune system by binding to Fcγ receptors (FcγR) on immune cells, facilitating phagocytosis and cell-killing, or by fixing the complement system. However, the induction and maintenance of Fc-dependent responses induced following natural infection and/or vaccination, especially in Brazil and with some vaccines, needs to be better defined.

In cohort studies in Brazil, we aimed to determine the magnitude and duration of FcγR-binding and complement-fixing antibodies targeting SARS-CoV-2 spike protein, elicited through natural infection or vaccination with the AstraZeneca or Sinovac vaccines, which have been widely deployed in Brazil. We also quantified the influence of prior SARS-CoV-2 infection on vaccine-induced antibody Fc-mediated functional activities. Serum samples were collected across multiple time points from Brazilian adults attending the UERJ Hospital with a SARS-CoV-2 infection (n=200) or for vaccination (n=222) with either the AstraZeneca or Sinovac vaccines (46.8% had a prior infection). Samples were tested for antibody FcγRI, FcγRIIa, and FcγRIIIa-binding, complement-fixation activity, and IgG against the SARS-CoV-2 spike protein.

AstraZeneca vaccination generally induced higher Fc-mediated functional responses compared to Sinovac vaccination and natural infection, which were comparable. Induction of complement-fixing antibodies was generally low. Vaccine-induced responses decayed substantially by 150 days. Vaccinees who had experienced SARS-CoV-2 infection prior to vaccination had substantially higher Fc-mediated functional antibodies than SARS-CoV-2 naïve adults. A greater magnitude of antibodies with Fc-mediated activities are induced by AstraZeneca vaccination in those who have previously experienced SARS-CoV-2 infection,

but antibodies decayed quickly over time. These findings are important in informing future vaccine design and optimal booster policy.

139

Antibody Protection of *Pseudomonas aeruginosa* Isolates from Bloodstream Infections

Sarah M Hickson[1], Johannes K Hoehensteiger[1], Von V L Torres[2], Wenkang Feng[1], Kate L McCarthy[3], Timothy J Wells[1]

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

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

3. UQ Centre for Clinical Research, University of Queensland, Brisbane, QLD, Australia

Pseudomonas aeruginosa is a frequent pathogen isolated from bacterial bloodstream infection (BSI) and can be problematic to treat due to the rising incidence of multi-drug resistance. Resistance to serum-killing by complement is a vital trait for Gram-negative bacteria like *P. aeruginosa* to survive in the bloodstream. Despite this, serum sensitive bacteria are commonly isolated from patients with active infection. Antibody-mediated protection from serum killing by 'cloaking antibodies (cAb)' has been described for multiple Gram-negative bacteria, however no studies have investigated the relevance of these in *P. aeruginosa* BSI. In this study, a cohort of 100 *P. aeruginosa* BSI patients in Brisbane, Australia was investigated for clinical relevance of cAb. Overall, 36% of patients produced high titres of cAbs specific for their cognate isolates. These cAbs are only clinically relevant if the patients isolate is sensitive to healthy control serum (HCS) killing. We found that 34% of patients had bacterial blood isolates that were sensitive to HCS with 41% (n=14) of these patients having cAbs that could inhibit the HCS-killing of their cognate isolate. Therefore, cAbs can protect almost half of HCS-sensitive *P. aeruginosa* isolates and allow these strains to survive in the bloodstream. Patients with cAb were significantly associated with non-neutropenic patients with no comorbidities. Whether induction of cAbs during hospital-stay is a risk factor for development of BSI is yet to be determined.

140

Investigating the role of PlpD in *Pseudomonas aeruginosa* infections

Johannes K. Hoehensteiger[1], Von V. L. Torres[2], Jessica L. Rooke[2], Ian R. Henderson[2], Timothy J. Wells[1]

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

2. Institute of Molecular Biosciences, The University of Queensland, Brisbane, Australia

Pseudomonas aeruginosa is a gram-negative, rod-shaped bacterial pathogen that, rising incidents of antibiotic resistance, poses a serious threat to patients in hospital care. It's large repertoire of virulence factors combined with its metabolic versatility enables *P. aeruginosa* to quickly establish infections and disseminate throughout the host. Proteins secreted by the type 5 secretion system (T5SS) are often found to be virulence factors which play a role in pathogenicity of other gram-negative infections. One of the lesser studied members of this secretion system is PlpD; the first discovered protein of a new T5SS subfamily. PlpD is a patatin-like phospholipase with affinity towards phospholipids commonly found in eukaryotic membranes, pulmonary surfactant, and lipids involved in eukaryotic signalling pathways. Despite initial interest in its biochemistry, the exact function of PlpD and its potential role in *P. aeruginosa* infections remains unknown.

In this study, a cohort of 164 *P. aeruginosa* isolates from chronic and acute infections along with matched serum was collected in Brisbane, Australia. Genomic analysis found the *plpD* gene was present in 83.9% of acute strains and 96.5% of chronic strains. To determine possible expression of PlpD during infections, titres of anti-PlpD antibodies were measured via ELISA and found that patient sera extracted

from chronic infections contained significantly higher titres of anti-PlpD antibodies than sera from acute infections. 7.4% of patients with acute and 20% of patients with chronic *P. aeruginosa* infections produced high titres of antibody against PlpD, indicating that this protein is expressed in some infections. In future, the impact of PlpD expression during infections will be investigated further and its impact on infections *in vivo* will be determined.

141

Developing a reproducible and defined protocol to differentiate human induced pluripotent stem cells into colon epithelium as a model to study colonic innate immunity and inflammatory and infectious diseases

Hani Hosseini Far[1, 2], Eva Chan[1, 2], Paul 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 Science, Faculty of Medicine, Nursing and Health Sciences, Monash University, Clayton, VIC, Australia

The adult colonic epithelium performs distinct physiological functions such as presenting a mucosal barrier to microorganisms and regulating the immune response to pathogens. Although there are various reports using animal models and cell lines to study colonic immunity and inflammatory and infectious diseases, they cannot thoroughly recapitulate the human colon *in vivo*. This issue has been addressed by developing methods to generate *in vitro* primary colon organoids from human colon tissue samples. Certain limitations such as limited supply can be overcome by generating colonic tissues from induced pluripotent stem cells (iPSC). Some studies have been reported so far that sought to develop effective protocols for deriving colonic organoids from human iPSC (Crespo et al., 2017; Múnera et al., 2017). With the aim of modelling human large intestine, we set up a stepwise, defined, serum-free and 38-day protocol for the progressive generation of definitive endoderm, mid-hind gut endoderm, hind gut endoderm, and subsequently, colon organoids through the modifications of some recently-published papers. A cocktail of embryonic stage-specific growth factors was applied to induce signalling pathways which were required to be modulated in each step. Flow cytometry analysis at day 3 of differentiation confirmed that around 100% of iPSCs were converted into definitive endoderm which is the main step in the endoderm differentiation. Extensive immunohistochemical analysis also proved that the 38-day iPSC-derived colon organoids mimic human colon epithelium through the expression of colon-specific markers (SATB2 and MUC5B), gut-specific markers (MUC2, CHGA and CDX2), junction and polarity-related markers (Zo-1, Villin and E-cadherin). iPSC-derived gut epithelium also secreted interferon epsilon, an epithelial cytokine under study in our lab in the control of intestinal infections. We are currently characterising the pattern recognition receptor pathway responses of iPSC-derived colonic epithelium to micro-organisms and synthetic innate immune stimulating ligands. In conclusion, our study provides an efficient method for differentiating human colon organoids from iPSCs, which have the advantage of relatively unlimited supply, ability to genetically manipulate (gene expression, etc), could study interactions with “matched” (e.g., immune) cell types, and can “capture” genetic of an individual such as modifications in “susceptibility genes”.

1. Crespo, M., Vilar, E., Tsai, S. Y., Chang, K., Amin, S., Srinivasan, T., . . . Chen, S. (2017). Colonic organoids derived from human induced pluripotent stem cells for modeling colorectal cancer and drug testing. Nat Med, 23(7), 878-884. doi:10.1038/nm.4355
2. Múnera, J. O., Sundaram, N., Rankin, S. A., Hill, D., Watson, C., Mahe, M., . . . Wells, J. M. (2017). Differentiation of Human Pluripotent Stem Cells into Colonic Organoids via Transient Activation of BMP Signaling. Cell Stem Cell, 21(1), 51-64.e56. doi:10.1016/j.stem.2017.05.020

142

The *Plasmodium* apicoplast is essential for the construction of glycophosphatidylinositol anchors needed for egress and invasion

Long K Huynh[1], Michaela S Bulloch[1], Kit Kennedy[1], Julie Ralton[1], Malcolm McConville[1], Stuart Ralph[1]

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

The apicoplast is a relict plastid of cyanobacterial origin found in most apicomplexan parasites, including *P. falciparum*, which can be selectively targeted by antibiotic-like drugs. Treatment of *P. falciparum* with apicoplast inhibitors causes a peculiar ‘delayed death’ phenotype, where treated parasites only arrest in the subsequent intraerythrocytic development cycle. Despite the widespread use of such drugs as malaria prophylactics (e.g. doxycycline), the molecular basis of delayed death has not been thoroughly described. Understanding these mechanisms will assist in informing appropriate clinical usage of apicoplast inhibitors. The apicoplast is responsible for the biosynthesis of isoprenoid precursors, which have multiple downstream fates in the parasite, including protein prenylation, ubiquinone and dolichols. The effect of protein prenylation and ubiquinone loss in the parasite has been previously described, however, the effect of dolichol loss has not been well-characterised. Dolichols are required for the biosynthesis of glycosylphosphatidylinositol (GPI) anchors, the predominant type of protein glycosylation present in *P. falciparum*. Many GPI-anchored proteins are thought to be essential for both parasite egress and reinvasion. We performed immunofluorescence assays on *P. falciparum* with inhibited apicoplasts but with exogenous rescue of their prenylation depletion. In these parasites, GPI-anchored proteins become mislocalised from their normal membrane association. These GPI-anchorless parasites also exhibited an egress defect and are unable to undergo proper segmentation and rupture the parasitophorous vacuole. Through flow cytometry-based invasion assays, we found that these GPI-anchorless parasites are unable to reinvade red blood cells. Our data indicates that apicoplast inhibitors cause a defect in GPI anchor biosynthesis that prevents egress and reinvasion of asexual-stage *P. falciparum*.

143

Defining the fine specificity of antibody responses to polymorphic and conserved epitopes of the lead malaria vaccine antigen: *Plasmodium falciparum* circumsporozoite protein

Alessia Hysa[1, 2], Liriye (Lydia) Kurtovic[1, 3], Herbert D Opi[1, 2, 3], Myo Naung[1, 4, 5], Alyssa E Barry[1, 4, 5], David Wetzel [6, 7], Michael Piontek [6], Jahit Scaralal[8, 9], Carlota Dobaño[9, 10], James Beeson[1, 3, 11]

1. Burnet Institute, Melbourne, Victoria, Australia

2. Department of Infectious Diseases , The University of Melbourne , Melbourne , Victoria, Australia

3. Department of Immunology and Pathology, Monash University , Melbourne , Victoria, Australia

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

5. Walter and Eliza Hall Institute, Parkville, Victoria, Australia

6. ARTES Biotechnology GmbH, Langenfeld, North Rhine-Westphalia, Germany

7. Laboratory of Plant and Process Design, Technical University of Dortmund, Dortmund, Germany

8. Centro de Investigação em Saúde de Manhiça, Maputo, Mozambique

9. Faculdade de Medicina , Universidade Eduardo Mondlane (UEM), Maputo, Mozambique

10. ISGlobal, Hospital Clínic Universitat de Barcelona, Barcelona, Catalonia, Spain

11. Department of Medicine , The University of Melbourne , Melbourne , Victoria , Australia

Malaria caused over 240 million infections resulting in over 600,000 deaths in 2020. In 2021, RTS,S became the first malaria vaccine recommended for susceptible young children; however, it confers modest efficacy (~30–50%). Developing highly effective vaccines requires understanding the specific epitopes within the vaccine antigen that mediate highly protective responses and how polymorphisms in the vaccine antigen impact efficacy.

RTS,S is based on the *P. falciparum* parasite circumsporozoite protein (*Pf*CSP) of one strain, 3D7. CSP includes an N-terminal, a central repeat composed of ~ 40 NANP and ~ 4 NVDP sequences, and highly polymorphic C-terminal regions. RTS,S comprises 19 NANP repeats and the entire C-terminal region. The central repeat region is intrinsically disordered forming epitopes that vary by the number of NANP and NVDP repeats. The C-terminal region is incredibly diverse between parasites and the 3D7 RTS,S strain represents less than 10% of African parasites. Furthermore, recent studies identified a highly conserved CSP epitope that is a target of potent neutralising antibodies but is excluded from the RTS,S construct.

We defined the fine specificity of RTS,S-induced antibodies to variable central repeat, polymorphic C-terminal and conserved epitopes of CSP. Our primary study population were children recruited in an RTS,S phase IIb clinical trial. We demonstrate that vaccine-induced antibody binding increases with the number of NANP repeats in a sequence; however, short NANP sequences may better represent the epitopes within CSP that mediate protection. Additionally, a proportion of children had promiscuous antibodies that cross-reacted with the highly conserved CSP epitope excluded from the vaccine construct. Lastly, RTS,S-induced antibodies appear to have reduced binding to diverse CSP alleles prevalent in African populations, which appears to contribute to low vaccine efficacy. Our findings reveal how targeting cross-reactive and conserved epitopes could achieve highly-effective next-generation malaria vaccines.

144

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

Sulaimon Idowu[1], Anna Walduck[1], Paul Bertrand[1]

1. RMIT University, Melbourne, VIC, Australia

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

Inflammatory Th1/17 responses are associated with both inflammatory gastritis which does not clear infection, and vaccine- induced reductions in colonization in animal models. An influx of CD4+ T cells is correlated with vaccine- induced protection. There is also evidence that gastric hormones play a role in the inflammatory response to *H. pylori* infection. Here we investigated the role of serotonin, also known as 5-Hydroxytryptamine (5-HT) in the response of gastric epithelia in and *in vitro* and a mouse model. In an *in vitro* model of AGS cells, qPCR analysis showed that 5-HT receptors HTR1A, HTR1B and the pro inflammatory chemokines IL-8 and CXCL8 were all upregulated, after co culture with *H. pylori* for 6h. In a mouse model, only HTR2B was upregulated after 3 days, at 3 weeks post infection Mip-1, Mip-2 and HTR1A were upregulated. Further we showed that vaccinated mice secreted higher levels of 5-HT in the antrum than controls. Flow cytometry analysis revealed that the expression of HTR1A was reduced on CD4+ T cells, Neutrophils and macrophages in vaccinated compared to control mice. Together these data support a role for 5-HT signaling in the inflammatory response to *H. pylori* infection, and that it may play a role in the protective response in vaccinated mice.

145

Modulation of ICAM-1 on vaginal epithelial cells by a microbiome bioactive and HIV transmission

Brianna Jesaveluk[1, 2], David Delgado Diaz[1, 2], David Tyssen[1], Joshua Hayward[1, 2], 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

The genital epithelium plays a critical role in regulating the sexual transmission of HIV. Expression of intercellular adhesion molecule-1 (ICAM-1) facilitates cell-to-cell virus transmission and is increased by inflammation. Women with *Lactobacillus* spp.-dominated vaginal microbiota have a decreased risk of HIV acquisition. Lactic acid (LA) is a key metabolite (or bioactive product) of *Lactobacillus* spp. with

antimicrobial and anti-inflammatory properties that is differentially produced by *Lactobacillus* spp. as L- and D- isomers. LA also acts directly on cervicovaginal epithelial cells to enhance barrier integrity. Here, we investigated whether LA could modulate the expression of ICAM-1 to influence HIV transmission through epithelial cells.

Immortalised ectocervical (Ect), endocervical (End) and vaginal (VK2) epithelial cells were cultured in transwells and treated apically for 1 h with 0.3% L-LA or D-LA (pH 3.9), L- or D-lactate (pH 7.0), or acidified media (pH 3.9, HCl adjusted) simultaneously with toll-like receptor (TLR) agonists poly I:C (PIC, TLR3), FSL-1 (TLR2/6), or PAM3CSK4 (TLR1/2). After 24 h incubation, expression of ICAM-1 mRNA was determined by RNASeq and surface protein expression by flow cytometry.

ICAM-1 mRNA was increased by PIC stimulation of Ect cells, which was reduced 2.2-fold in the presence of L-LA (n=3, FDR< 0.05). Stimulation of Ect cells with PIC significantly increased ICAM-1 protein expression by 1.6-fold (n=4, p< 0.05) compared to unstimulated cells. However, this increase was almost entirely abrogated by treatment with L- or D-LA (both p< 0.01), but not HCl-acidified media, or L- or D-lactate at a neutral pH (all p >0.05), indicating that inhibition of ICAM-1 upregulation is specifically mediated by the uncharged, low pH form of LA. Similar findings were observed for Ect cells treated with FSL-1 and PAM3CSK4, as well as TLR-stimulated End and VK2 cells.

Treatment of cervicovaginal epithelial cells with LA, a microbiome bioactive, specifically inhibits ICAM-1 upregulation associated with TLR stimulation, which may influence mucosal HIV transmission. These findings suggest a potential mechanism by which optimal *Lactobacillus*-dominated vaginal microbiome may protect against HIV transmission. Future studies will investigate direct cell-to-cell transmission of virus sequestered in epithelial cells to HIV target cells and the effect of LA treatment.

146

Lasting first impression: Ancestral imprinting restricts salivary antibody responses during Omicron breakthroughs

Kevin John Selva[1], Pradhipa Ramanathan[1], Ebene R Haycroft[1], Samantha K Davis[1], Ruth Purcell[1], Helen E Kent[1], Jennifer A Juno[1], Arnold Reynaldi[2], Deborah Cromer[2], Miles Davenport[2], Stephen Kent[1, 3], Amy W Chung[1]

1. Peter Doherty Institute for Infection and Immunity, Uni of Melbourne, Melbourne, VICTORIA, Australia

2. Kirby Institute, Uni of New South Wales, Kensington, NEW SOUTH WALES, Australia

3. Dept. of Infectious Diseases, Melbourne Sexual Health Centre, Alfred Hospital and Central Clinical School, Monash Uni, Melbourne, VICTORIA, Australia

Current COVID-19 intramuscular vaccination strategies stimulate good systemic responses that protect against severe disease, however, recurring breakthrough infections highlight the marked ability of SARS-CoV-2 variants of concern, especially Omicron, to escape neutralization. Furthermore, recent studies have observed limited mucosal immunity in COVID-vaccinated individuals, thus contributing to their increased susceptibility to Omicron variants. Here, we examined whether hybrid immunity following breakthrough infections may provide more robust systemic, and more importantly, mucosal humoral immunity to emerging SARS-CoV-2 variants.

A series of longitudinal paired mucosal (saliva) and systemic (plasma) samples were collected from individuals during the acute phase of Delta, Omicron BA.1 and BA.2 breakthrough infections. We profiled these samples for their SARS-CoV-2 variant-specific (Ancestral, Delta, Omicron BA.1, BA.2) antibody isotype responses (IgG, IgA) and their ability to mediate Fc-effector functions via engaging Fc-receptors.

Following Delta breakthrough infection, a surge in Delta-specific mucosal IgG (+4000%; p<0.01) mucosal were observed two weeks post-infection, mimicking the increase in plasma responses (+1800%; p<0.01). Conversely, smaller non-significant peaks in Omicron BA.1 (+330%) and BA.2 (+523%) IgG salivary responses were observed two weeks into their respective breakthrough infections. This disparity is also reflected in the enhanced ability of Delta-specific salivary antibodies to engage Fc-receptors (+1595%; p<0.01) as compared to the weak non-significant changes in Omicron-specific salivary Fc-responses following their respective breakthrough infections.

Furthermore, unlike the robust Delta-specific salivary IgA response (+709%; p<0.01) following Delta breakthroughs, only modest rises in BA.1-specific (+30%; p=0.01) and BA.2-specific (+80%; p<0.01) mucosal IgA responses were observed following the respective Omicron breakthroughs. These Omicron-specific IgA responses were several folds lower as compared to that raised against the ancestral spike (BA.1: 12-fold less; BA.2: 5-fold less), suggesting immune imprinting of ancestral spike.

Our findings suggest that acquiring a single Omicron breakthrough infection might not be sufficient in priming mucosal immunity against future Omicron infections and supports the case for mucosal vaccines and Omicron-specific boosters to better protect vulnerable populations.

1. Selva KJ, Davis SK, Haycroft ER, et al. Tear antibodies to SARS-CoV-2: implications for transmission. Clin Transl Immunology. 2021;10(11):e1354. Published 2021 Nov 1. doi:10.1002/cti2.1354

147

Characterisation of the DNA associated with bacterial membrane vesicles from the human gut microbiota

Ella L. Johnston[2, 1], Emily L. Gulliver[3, 4], Jodee A. Gould[3, 4], Samuel C. Forster[3, 4], Maria Kaparakis-Liaskos[2, 1]

1. Department of Microbiology, Anatomy, Physiology and Pharmacology, La Trobe University, Bundoora, VIC, Australia

2. Research Centre for Extracellular Vesicles, School of Molecular Sciences, La Trobe University, Bundoora, Victoria, Australia

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

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

Bacterial membrane vesicles (BMVs) are nanoparticles produced by all bacteria that are known to package various cargo, including protein, DNA and RNA. The main function of BMVs is to facilitate bacterial survival by sequestering antibiotics, bacteriophages and nutrients, and by mediating horizontal gene transfer. Although these functions of BMVs have been demonstrated using single bacterial

species, the DNA composition and functions of BMVs produced in a mixed microbial culture remain unknown. Therefore, we aimed to characterise the DNA content of BMVs produced by a mixed culture representative of the major bacterial phyla present in the human gut, and the effect of antibiotic treatment on their BMV DNA composition.

To do this, BMVs were isolated from cultures containing 95 microbiota strains grown in the presence or absence of antibiotics and the size and cargo composition of BMVs was determined. Using next generation DNA sequencing, we identified several bacterial species within the mixed microbial culture that produced BMVs containing partial bacteriophage and prophage DNA. We isolated BMVs from these single microbiota bacterial species and identified that of these, the opportunistic pathogen *Clostridium baratii* produced BMVs with high levels of DNA when grown in the presence or absence of antibiotics. We are currently investigating the composition of *C. baratii* BMVs to examine the contribution of antibiotic treatment to altering the production, morphology and DNA packaging within these BMVs.

To date, the production and composition of BMVs from a mixed microbial community in the presence or absence of antibiotics have not been investigated. These findings will provide insights into the biogenesis of BMVs within a mixed microbial community and the effect of antibiotics on their production and composition. Ultimately, these findings will broaden our limited understanding of the contribution of BMVs to mediating bacterial evolution and evasion of antibiotics in a mixed microbial setting.

148

An unbiased analysis of neonatal immune cell characteristics to discover novel biomarkers that predict the risk of neonatal sepsis

Srushti M Kasare[1, 2, 3], Simone S Schüller[1, 2, 3], Peter Richmond[2, 3, 4], Andrew Currie[2, 5], Tobias Strunk[1, 2, 3]

1. Neonatal Directorate, King Edward Memorial Hospital, Perth, Western Australia, Australia

2. Wesfarmers Centre of Vaccines and Infectious Diseases, Telethon Kids Institute, Perth, Western Australia, Australia

3. School of Medicine, University of Western Australia, Perth, Western Australia, Australia

4. Department of Immunology, Perth Children's Hospital, Perth, Western Australia, Australia

5. Medical, Molecular and Forensic Sciences, Murdoch University, Perth, Western Australia, Australia

Worldwide 15 million infants each year are born preterm and 1.3 million infants suffer from sepsis, a life-threatening bacterial bloodstream infection. Preterm infants are susceptible to sepsis and this is highly dependent on gestational age (GA) and most frequent during the neonatal period (28 days from birth). Infants who survive may suffer permanent disabilities due to organ damage resulting from either the infection itself or from the inflammatory responses. Despite established epidemiological and clinical risk factors for sepsis in preterm infants, the developmental maturation events of preterm immune system remain largely understudied.

One of the greatest challenges is to collect longitudinal samples from the neonates for immune cell analysis. The presentation will highlight results of a prospective observational study which recruited 129 very preterm infants (<30 weeks GA) of which 43 developed late-onset sepsis (LOS), and 20 healthy term infants born at King Edward Memorial Hospital, Perth, Australia. This study used seven colour flow cytometry to characterise peripheral blood innate cells (monocytes and dendritic) and adaptive T lymphocytes (regulatory T cells and δγT cells) at days of life 1, 7, 14, 21, and 28. The methods for flow cytometry data analysis included traditional manual gating and automated gating (clustering and dimensionality reduction) to overcome the risk of bias associated with traditional method.

The results from this study will provide vital information on the differences in characteristics of immune cells of preterm infants compared to term infants. Furthermore, the developmental trends of immune cells will be compared between sepsis and no-sepsis preterm infants. The findings will provide the foundation for larger studies for targeted immune therapeutics that will globally reduce the occurrence of sepsis in neonates.

1. Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. The Lancet. 2016;388(10063):3027-35.
2. Fleischmann C, Reichert F, Cassini A, Horner R, Harder T, Markwart R, et al. Global incidence and mortality of neonatal sepsis: a systematic review and meta-analysis. Archives of Disease in Childhood. 2021;106(8):745-52.
3. El Hassani SEM, Berkhout DJ, Niemarkt HJ, Mann S, De Boode WP, Cossey V, et al. Risk factors for late-onset sepsis in preterm infants: a multicenter case-control study. Neonatology. 2019;116(1):42-51.
4. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. Lancet Glob Health. 2019;7(1):e37-e46.
5. Amir E-aD, Davis KL, Tadmor MD, Simonds EF, Levine JH, Bendall SC, et al. viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. Nature biotechnology. 2013;31(6):545-52.
6. Yu N, Li X, Song W, Li D, Yu D, Zeng X, et al. CD4+ CD25+ CD127low/− T cells: a more specific Treg population in human peripheral blood. Inflammation. 2012;35(6):1773-80.
7. De Jong E, Strunk T, Burgner D, Lavoie PM, Currie A. The phenotype and function of preterm infant monocytes: implications for susceptibility to infection. Journal of leukocyte biology. 2017;102(3):645-56.

149

Exploiting rabies virus hijacking of neuronal synapse formation

Stephanie J Keating[1, 2], Vinod Sundaramoorthy[1, 2]

1. CSIRO, East Geelong, VIC, Australia

2. Deakin University, Geelong, VIC, Australia

Rabies (*lyssavirus*) is a deadly zoonotic virus that has the unique ability to infect the host nervous system and transfer across neural junctions known as synapses. This mode of transmission allows the virus to evade detection from the immune system and leads to irreversible damage to the nervous system. Although the virus has existed over several centuries, a lot is still unknown about the pathogenesis of the virus and the signaling pathways responsible for its trans-synaptic transmission. It is known that the rabies viral glycoprotein is essential in its movement across the synapse, but the neuronal proteins that are influenced by the glycoprotein to increase synapse formation is still unknown.

In this study we used advanced confocal microscopy to analyse the trans-synaptic transfer of the virus in high and low neuroinvasive strains. The highly invasive strain showed a more efficient trans-synaptic transmission of the virus, an increase in filipodia-like structures in the synaptic membrane, and an increase in synaptogenesis. We are now performing proteomic analyses to study and compare the viral glycoprotein in both strains and their associated interactomes. Super-resolution microscopy will then provide insight into the viral glycoprotein and its interacting partners at the synapse. This will allow us to identify what neuronal proteins may be responsible for the increase in synapse formation in the highly neuroinvasive strain.

This will provide novel insight into new signaling pathways and molecules that are involved in synaptogenesis. The identification of the neural proteins involved in trans-synaptic transmission of the virus could also provide valuable knowledge to develop future therapeutics for rabies treatment. This information could also be used to design next generation rabies inspired therapeutics to increase synapse formation in neurodegenerative and neurodevelopmental diseases that occur as a result of synapse degeneration or deformation.

150

HIV-Infected Macrophages Modulate Cell-Surface Ligands Relevant to NK Killing Over Time

Hans HK Kek[1, 2], Andy AP Poumbourios[1, 3], Anthony AJ Jaworowski[1, 4], Anna AH Hearps[1, 5]

1. Burnet Institute, Mount Waverley, VIC, Australia

2. Central Clinical School, Monash University, Melbourne, VIC, Australia

3. Department of Microbiology, Monash University, Melbourne, VIC, Australia

4. School of Health and Biomedical Sciences, RMIT University, Melbourne, VIC, Australia

5. Department of Infectious Diseases, Monash University, Melbourne, VIC, Australia

Introduction: HIV-infected macrophages form reservoirs in tissues of people living with HIV on antiretroviral therapy. The use of natural killer (NK) cell-mediated antibody-dependent cellular cytotoxicity (ADCC) is a promising approach to eliminating HIV-infected cells. ADCC requires antibody opsonisation of HIV envelope (Env) expressed on infected cells and is influenced by the interaction between ligands on the target cells with activating and inhibitory receptors expressed on NK cells. The nature and kinetics of HIV infection in macrophages is different to that in T cells, which may influence the expression of both HIV proteins and cellular HLAs, and subsequently NK cell recognition. However, it is unknown how expression of Env, or ligands that can modulate NK recognition (e.g. HLAs), changes during HIV infection in macrophages.

Methods: Monocyte-derived macrophages from HIV- blood donors were infected *in vitro* with HIV (BaL and AD8 strains) and synchronised using an HIV fusion inhibitor on day 3 post-infection. Productive infection (indicated by intracellular HIVp24 protein expression) and surface expression of Env/CD4/HLA-ABC/HLA-E on MDM were quantified using flow cytometry at various stages post-infection.

Results: Env was detected on the surface of HIV+ macrophages using the antibodies PGT151/NIH45-46 as early as 3 days post-infection and peaked at 7-10 days (mean=42%/26%, p=0.03 compared to p24 which did not plateau during this period). HIV+ MDM downregulated surface expression of CD4 and HLA-ABC relative to bystander (p24-) cells ($p<0.02$, days 3/7/10) but upregulated expression of HLA-C ($p<0.05$, days 3/7/10) and HLA-E. HIV-induced upregulation of HLA-C and HLA-E was specific to HIV+ macrophages and was not seen on T cells.

Discussion and Conclusions: Increased Env expression on HIV+ macrophages over time suggests higher susceptibility to antibody opsonisation and thus ADCC at later stages of infection. HLA downregulation on HIV+ MDM suggests susceptibility to NK cell recognition. However, specific upregulation of HLA-C/E in HIV+ macrophages may impair this response. Furthermore, increased HLA-C/E expression on macrophages could affect which types of NK cells recognise HIV-infected macrophages compared to T cells. Together these data indicate that HIV-infected macrophages are a dynamic reservoir that modulates ligands which may influence their ability to be targeted by NK cells.

151

Molecular regulation of Clec9A-mediated damage recognition and antigen presentation in dendritic cells

Kirsteen Tullett[1], Peck Szee Tan[1], Nicole Michael[1], Hae-Young Park[1], Ralf Schittenhelm[2], Anthony Purcell[1], Mireille H Lahoud[1]

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

2. Monash University, Clayton, VIC, Australia

Dendritic cells (DC) are sentinels of the immune system. DC use a variety of surface receptors to survey their environment and induce anti-viral immunity. The C-type lectin-like receptor, Clec9A is specifically expressed on cDC1, which excel at cross-presentation of exogenous antigen on MHC I to induce antigen-specific CD8+ T cell responses. Clec9A recognises actin filaments exposed on the surface of dead/damaged cells and plays a vital role in the processing of dead cell-derived antigens, mediating their delivery to the cross-presentation pathway. Clec9A is therefore particularly important for inducing cytotoxic T cells that are able to directly kill virally-infected and cancer cells.

We have identified Clec9A-regulatory pathways that control Clec9A fate and function in DC. We demonstrated the E3 ubiquitin ligase RNF41 negatively regulates Clec9A levels and function through ubiquitination. Interestingly, RNF41 ubiquitinates the extracellular domain of Clec9A to regulate its fate and function, in contrast with classical ubiquitination-mediated regulation of cell surface receptor fate via ubiquitination of cytoplasmic receptor domains. Indeed, knockdown of RNF41 in cDC1 results in enhanced cross presentation of dead cell antigen. We have further identified novel Clec9A-interacting proteins, including the ER-associated Erlin1 and Erlin2 proteins, implicated in receptor ubiquitination and membrane translocation, that negatively regulate Clec9A fate and function. Our research is currently focussing on the role of Clec9A regulatory pathways in the control of Clec9A, damage recognition, receptor trafficking and antigen cross-presentation.

Our findings provide important insights into the mechanisms regulating damage recogntion and cross-presentation of antigen from virally infected cells, which is key to understanding viral immunity and has implications for development of immune modulation approaches via Clec9A.

152

COVID-19 vaccine humoral responses in lymphopenic platelet donors

Annemarie Laumaea[1, 3, 2], Antoine Lewin[4], Debashree Chatterjee[1], Lorie Marchitto[1, 3], Shilei Ding[1], Gabrielle Gendron-Lepage[1], Guillaume Goyette[1], Marie-Ève Allard[5], Carl Simard[5], Tony Trembla[5], Josée Perreault[5], Ralf Duerr[6], Andrés Finzi[1, 3], Renée Bazin[5]

1. Centre de Recherche du CHUM, Montréal, Québec, Canada

2. Global Health and Diagnostics Development Laboratory, Burnet Insitute, Melbourne, VIC, Australia

3. Département de Microbiologie, Infectiologie et Immunologie, Université de Montréal, Montréal, Québec, Canada

4. Affaires Médicales et Innovation, Héma-Québec, Montréal, Québec, Canada

5. Affaires Médicales et Innovation, Héma-Québec, Québec, Canada

6. Microbiology, New York University School of Medicine, New York, USA

Background: Lymohopenia, in particular depressed levels of $CD4^+$T and B cells is an important consideration when assessing the effectiveness of vaccine responses. Blood donors, in particular frequent platelet donors (>20 donations annually) have been shown recently to exhibit severe plateletpheresis-associated lymphopenia; in particular $CD4^+$T but not B cell numbers are decreased. COVID-19 vaccination thereby provides a model to assess whether lymphopenic platelet donors present compromised humoral immune responses.

Methodology:43 plateletpheresis donors with a range of pre-vaccination $CD4^+$T cell counts (76-1537 cells/µl) who had received 2 doses of COVID-19 vaccination were recruited for the study. At least half of these individuals were frequent platelet donors and thus were likely to display low$CD4^+$T cell counts. In addition to baseline T cell measurements by flow cytometry, antibody binding assays to full-length Spike and the Receptor Binding Domain (RBD) were performed pre- and post-vaccination. Furthermore, pseudo-particle neutralization and antibody-dependent cellular cytotoxicity (ADCC) assays were conducted to measure antibody functionality.

Results: Participants were stratified into two groups: <400 CD4/µl (n=27) and ≥400 CD4/µl (n=16). Following the first dose, 79% seroconverted within the <400 CD4/µl group compared to 87% in the ≥400 CD4/µl group; all donors were seropositive post-second dose with significant increases in antibody levels. Importantly differences in $CD4^+$T cell levels minimally impacted neutralization, Spike recognition and IgG Fc-mediated effector functions.

Discussion: Overall, our results suggest that lymphopenic plateletpheresis donors do not exhibit significant immune dysfunction; they have retained the T and B cell functionality necessary for potent antibody responses after vaccination.

1. Laumaea A, Lewin A, Chatterjee D, Marchitto L, Ding S, Gendron-Lepage G, Goyette G, Allard M, Simard C, Tremblay T, Perrault J, Duerr R, Finzi A, Bazin R. 2022. COVID-19 vaccine humoral responses in frequent platelet donors with plateletpheresis-associated lymphopenia. Transfusion doi.org/10.1111/trf.17037

153

HIV-1 infected macrophages can be made susceptible to Antibody Dependent Cellular Cytotoxicity by small molecule CD4 mimetics

Annemarie Laumaea[1, 2, 3], Lorie Marchitto[1, 2], Shilei Ding[1], Guillaume Beaudoin-Bussières[1, 2], Jérémie Prévost[1, 2], Romain Gasser[1, 2], Debashree Chatterjee[1], Gabrielle Gendron-Lepage[1], Halima Medjahed[1], Hung-Ching Chen[4], Haitao Ding[5], John Kappes[5], Beatrice Hahn[4], Frank Kirchhoff[6], Jonathan Richard[1, 2], Ralf Duerr[7], Andrés Finzi[1, 2]

1. Centre de Recherche du CHUM, Montréal, Québec, Canada

2. Département de Microbiologie, Infectiologie et Immunologie, Université de Montréal, Montréal, Québec, Canada

3. Global Health and Diagnostics Development Laboratory, Burnet Insitute, Melbourne, VIC, Australia

4. University of Pennsylvania, Philadelphia, PA, USA

5. University of Alabama, Birmingham, Alabama, USA

6. Ulm University, Ulm, Germany

7. New York University, New York, USA

HIV evolved mechanisms to evade host immune responses allowing the persistence of the latent reservoir. Effective targeting of the HIV reservoir is the focus of intense cure research. HIV-1 envelope (Env) conformation determines the susceptibility of infected $CD4^+$ T cells to antibody-dependent cellular cytotoxicity (ADCC). Upon interaction with CD4, Env adopts more “open” conformations, exposing ADCC epitopes. HIV-1 limits Env-CD4 interaction and protects infected cells against ADCC by downregulating CD4 via Nef, Vpu, and Env. Limited data exist, however, of the role of these proteins in downmodulating CD4 on infected macrophages and how this impacts Env conformation. Furthermore, while small molecule CD4mimetic (CD4mc) have been shown to be effective at exposing ADCC epitopes on the surface of infected CD4+T cells, limited data exists as to the effectiveness of CD4mc for exposing vulnerable Env epitopes on HIV infected macrophages and its potential for targeting the myeloid reservoir. While Nef, Vpu, and Env are all required to efficiently downregulate CD4 on infected $CD4^+$ T cells, we show here that any one of these proteins is sufficient to downmodulate most CD4 from the surface of infected macrophages. Consistent with this finding, Nef and Vpu have a lesser impact on Env conformation and ADCC sensitivity in infected macrophages compared with $CD4^+$ T cells. However, treatment of infected macrophages with small CD4 mimetics exposes vulnerable CD4-induced Env epitopes and sensitizes them to ADCC.

1. https://www.sciencedirect.com/science/article/pii/S2211124722018873?via%3Dihub
2. Laumaea A, Marchitto L, Beaudoin Bussieres G, Ding S, Gasser R, Chatterjee D, Gendron-Lepage G, Medjahed H, Chen H, Smith III AB, Ding H, Kappes JC, Hahn BH, Kirchoff F, Richard J, Duerr R, Finzi A. 2023 Small CD4 mimetics sensitize HIV-1-infected macrophages to antibody-dependent cellular cytotoxicity. Cell Reports. 42(1):111983 https://doi.org/10.1016/j.celrep.2022.111983

154

The characterisation of cytolethal distending toxin associated with Campylobacter jejuni extracellular vesicles.

Lena Hoang My Le[1, 2], Bassam Elgamoudi[3], Frederic Poly[4], Le Ying[1, 2], Victoria Korolik[3], Richard Ferrero[1, 5, 2]

1. Biomedicine Discovery Institute, Department of Microbiology, Monash Univeristy, Clayton, Victoria, Australia

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

3. Institute for Glycomics, Griffith University, Gold Coast, Queensland, Australia

4. Enteric Diseases Department, Naval Medical Research Centre, Silver Springs, Maryland, USA

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

Cytolethal distending toxins (CDTs) are secreted by Gram-negative pathogens as both soluble proteins and associated with extracellular vesicles (BEVs). CDT production by the enteric pathogen *Campylobacter jejuni* has been associated with colorectal tumorigenesis. Despite CDT being a major virulence factor for *C. jejuni*, little is known about this toxin. To address this question, we generated *C. jejuni cdtA*, *cdtB* and *cdtC* mutants by double homologous recombination using suicide vectors harbouring a promoter-driven kanamycin resistance (Km^R) cassette. *C. jejuni cdtA* and *cdtC* mutant bacteria expressed wildtype (wt) levels of *cdtB/C* and *cdtA/B*, respectively, whereas the *cdtB* mutant expressed reduced levels of *cdtA/C*. Immunoblotting revealed that *C. jejuni cdtA* bacteria and BEVs did not contain CdtB/C subunits. Similar results were observed for a *C. jejuni cdtA* mutant constructed using a promoterless Km^R cassette. *C. jejuni cdtB* and *cdtC* bacteria and BEVs only contained CdtA. The loss of Cdt subunit production in the mutants was attributed to transcriptional and post-transcriptional modifications resulting from the mutagenesis procedure. Nanoparticle tracking analyses showed that *C. jejuni* wildtype (wt) and *cdt* mutants released similar numbers of BEVs with comparable morphologies and size distributions. As reported previously for *C. jejuni* bacteria, we found that the BEVs isolated from wt but not *cdt* mutants induced cell cycle arrest at the G2 phase in HCT-116 epithelial cells. “Surface shearing” with proteinase K and surface plasmon resonance showed that the majority of Cdt subunits are located inside BEVs, with small quantities detected on the surface. In conclusion, we have performed the first detailed characterisation of CDT release in *C. jejuni* BEVs. We suggest that BEV-associated CDT may be a new and important factor in *C. jejuni* gastroenteritis and associated diseases.

155

Recall, breadth and longevity of humoral immunity following Omicron BA.1 and BA.2 breakthrough infection

Wen Shi Lee[1], Hyon-Xhi Tan[1], Arnold Reynaldi[2], Robyn Esterbauer[1], Marios Koutsakos[1], Julie Nguyen[1], Thakshila Amaresana[1], Helen Kent[1], George Taiaroa[3], Paul Kinsella[3], Kwee Chin Liew[3], Deborah A Williamson[3], David Khoury[2], Miles P Davenport[2], Jennifer A Juno[1], Stephen J Kent[1], Adam K Wheatley[1]

1. Peter Doherty Institute of Infection and Immunology, Melbourne, VIC, Australia

2. Kirby Institute, University of New South Wales, Kensington, NSW, Australia

3. Victorian Infectious Diseases Reference Laboratory, Melbourne, VIC, Australia

Introduction:

SARS-CoV-2 breakthrough infections of fully vaccinated individuals are becoming increasingly common with the rise of highly transmissible and immune evasive Omicron variants. Upon antigen re-exposure, Spike-specific memory B cells (MBCs) are reactivated and differentiate into antibody-secreting cells to provide an anamnestic antibody response. It remains unclear how long immunity lasts following Omicron breakthrough infection and whether antibodies against neo-epitopes within Omicron spike are elicited.

Methods:

We recruited and longitudinally sampled 24 fully vaccinated individuals with PCR and whole genome sequence-confirmed Omicron infections (8 BA.1, 16 BA.2). We measured plasma neutralising activity using a live virus neutralisation assay and MBCs specific for ancestral and/or Omicron S using fluorescent S protein probes. We measured SARS-CoV-2 RNA levels within nasal swabs using RT-PCR.

Results:

In the early stages of infection (1-5 days post-symptom onset), plasma neutralising titres against BA.1 or BA.2 were low or undetectable, before rising from days 5-7 up to days 30-45. Importantly, the recall of antibody responses lagged behind nasal viral loads that peaked around 3-4 days post symptom onset. Neutralising antibody titres against the infecting strain were durable and dropped from a mean of 983.7 at 1 month to 498.4 at 7 months for BA.1 infected subjects and 1719.2 at 1 month to 1533.4 at 4 months for BA.2 infected subjects. Importantly, people who recovered from BA.1 and BA.2 infection gained neutralisation breadth against the more immune evasive BA.4 variant. Cross-reactive MBCs that recognised both ancestral and Omicron spike were robustly expanded and displayed an activated phenotype (CD21-CD27+) at early timepoints. In contrast, very low frequencies of MBCs that only recognised Omicron spike were detected. Consistent with this data, we did not detect a rise in neo-antibody responses against Omicron S that do not cross-react with ancestral S.

Conclusion:

We find that Omicron breakthrough infection primarily expands cross-reactive MBCs and antibodies, leading to the rapid rise and durable maintenance of Omicron neutralising antibodies. Understanding the breadth of antibodies and MBCs elicited by infection with distinct variants will be critical to informing vaccine design to maximise protection against future variants.

156

Investigating the mechanisms of action of the dengue virus inhibitor JNJ-A07

Roman V Lenchine[1], Nicholas S Eyre[1]

1. College of Medicine & Public Health, Flinders University, Oakland Park, South Australia, Australia

Dengue virus (DENV) is a Flavivirus that is primarily transmitted by Aedes aegypti mosquitoes and infects an estimated 390 million people worldwide, causing approximately 25,000 deaths per year. While most infections are asymptomatic or cause mild fever, some individuals develop severe dengue; a life-threatening disease characterised by vascular leakage due to endothelial dysfunction.

The DENV protein NS4B is a highly hydrophobic, non-structural transmembrane protein that is essential for viral RNA replication and immune evasion. NS4B is located in the virus-modified endoplasmic reticulum and has been shown to physically interact with other viral non-structural proteins such as NS1 and NS3, with these interactions being essential for DENV replication.

A recent study has described a highly potent DENV inhibitor (JNJ-A07) that blocks the interaction between NS3 and NS4B. While this inhibitor has a great therapeutic potential, it is unknown whether it affects interactions other than the disruption of the NS3:NS4B complex. It is important to determine the exact mechanism(s) of action of this drug and any unanticipated 'off-target' effects.

Here, we have investigated the efficacy of JNJ-A07 against DENV2 in Huh 7.5 hepatoma cells in time-of-addition studies using live cell imaging and a unique reporter virus that encodes a fluorescent protein insertion within NS1. These studies have revealed the dynamics of the antiviral effects of JNJ-A07 at high temporal resolution and have confirmed that its antiviral activity is greatest when it is applied during the early stages of infection. Furthermore, the inhibitor decreased the colocalization between NS4B and NS1, dsRNA and the viral Envelope protein. These effects on viral protein colocalization are currently being explored using a non-structural viral protein expression system that accurately reflects normal viral protein localization and membrane rearrangements. Proteomics studies will then seek to identify whether JNJ-A07 disrupts other NS4B interactions with viral and host cell factors. It is hoped that an improved understanding of the mechanism of action of JNJ-A07 will inform mechanisms of antiviral drug resistance and facilitate the development of analogous and complementary antiviral therapies that build upon the great promise of this potent, clinically relevant, and unique class of DENV inhibitors.

1. Kaptein, S.J.F., Goethals, O., Kiemel, D. et al. A pan-serotype dengue virus inhibitor targeting the NS3–NS4B interaction. Nature 598, 504–509 (2021). https://doi.org/10.1038/s41586-021-03990-6

157

Self-antigen-mediated T cell Immunity

Jia Jia Lim[1], Claerwen M. Jones[1], Tiing Jen Loh[1], Yi Tian Ting[1], Pirooz Zareie[1], Khai L. Loh[1], Nathan J. Felix[2], Anish Suri[2], Murray McKinnon[2], Frederik Stevenaert[3], Ravi K. Sharma[4], Lars Klareskog[4], Vivianne Malmstrom[4], Daniel G. Baker[2], Anthony W. Purcell[1], Hugh H. Reid[1], Nicole L. La Gruta[1], Jamie Rossjohn[1, 5]

1. Infection and Immunity Program, Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, VICTORIA, Australia

2. Janssen Research & Development, LLC, Horsham, Philadelphia, Pennsylvania, USA

3. Janssen Research & Development, Turnhoutseweg 30, BE-2340, Beerse, Belgium

4. Rheumatology Division, Department of Medicine, Karolinska Institute, Karolinska University Hospital 17177, Stockholm, Sweden

5. Institute of Infection and Immunity, Cardiff University, School of Medicine, Heath Park, , Cardiff CF14 4XN, UK

The autoreactive T cell repertoire driving disease activity in rheumatoid arthritis (RA) includes $CD4^+$ T cells that recognize HLA-DRB1 presenting post-translational modified self-antigens. Some HLA-DRB1 alleles have a shared susceptibility epitope associated with increased RA incidence. For example, certain post-translational modifications (PTM) of self-proteins via citrullination leads to the formation of neoantigens that can be presented by HLA-DR4 SE allomorphs. However, the interplay between the HLA molecule, post-translationally modified epitope and the responding T cell repertoire remain unclear.

To understand the molecular basis of the citrullinated self-epitope, HLA-DR4, and CD4 T cell, we synthesized citrullinated fibrinogen peptide, which is found abundantly in joint synovium of RA patient, and investigated structurally their impact on HLA-DR4 recognition. Using combinational techniques including transgenic mouse model, FACS analyses, biochemical analyses and X-ray crystallography, we analysed the $CD4^+$ TCR repertoire of HLA-DR4 presenting citrullinated epitope, affinities, and structure of multiple T cell receptors (TCRs) derived from humanized mice reactive with citrullinated peptides presented by HLA-DR4. TCR repertoire analysis revealed a citrullinated antigen-specific motif, conserved in both mice and humans. Crystal structures revealed duality function of shared epitope of HLA-DR4 in presenting citrullinated epitope, as well as direct contact with the TCR. This suggest that HLA-DRB1 alleles possess the shared epitope are contributed to the development of RA through both binding of stimulatory peptide epitopes and direct contact with a biased set of TCRs.

158

The molecular definition of potent *Plasmodium falciparum* invasion inhibitory epitopes against PTRAMP-CSS

Pailene S Lim[1], Stephen W Scally[1], Rainbow Chan[1], Wai-Hong Tham[1], Alan F Cowman[1]

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

Plasmodium falciparum is the causative agent of the most severe form of malaria and accounted for almost all the 619,000 reported malaria deaths in 2021 (World Health Organisation, 2022). Parasite invasion of human erythrocytes is a complex multi-step process that ends with parasite internalisation. An essential step in this process involves leading blood-stage vaccine candidates, the ligand PfRh5 which forms a complex with the Cysteine-Rich Protective Antigen (CyRPA) and the PfRh5-interacting protein (PfRipr; RCR) and binds to basigin on the host erythrocyte. Recently, we showed that a disulphide-linked heterodimer consisting of the *Plasmodium* Thrombospondin-Related Apical Merozoite Protein (PfPTRAMP) and the Cysteine rich Small-Secreted protein (PfCSS; PTRAMP-CSS) binds RCR to form a pentameric complex, PCRCR, which anchors the parasite to the erythrocyte membrane during invasion (Scally et al., 2022). Invasion inhibitory nanobodies to both PTRAMP and CSS were identified but only had moderate efficacy. To further the rational design of PTRAMP-CSS immunogens, potent inhibitory epitopes on PTRAMP and CSS need to be identified.

Previous efforts have isolated nanobodies that were raised against the monomeric proteins, and to extend this we immunised alpacas with the PTRAMP-CSS heterodimer to determine if these nanobodies have neutralising potential. We have screened > 20 nanobodies and have identified three potent inhibitory nanobodies against PTRAMP-CSS. Using X-ray crystallography, we have identified novel neutralising and non-neutralising epitopes on PTRAMP and CSS. Through understanding the molecular definition of potent neutralising epitopes on PTRAMP-CSS a roadmap is provided for structure-guided development of these proteins for a blood-stage malaria vaccine that aims to relieve the disease's current global burden.

159

Temporal kinetics of functional antibody responses following naturally acquired *Plasmodium vivax* malaria infections

Rhea Longley[1], Herbert Opi[2], Kael Schoffer[1], Yanie Tayipto[1], Jessica Brewster[1], Kaitlin Pekin[2], Macie Lamont[1], Benson Kiniboro[3], Leanne Robinson[2], Jetsumon Sattabongkot Prachumsri[4], James Beeson[2], Ivo Mueller[1]

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

2. Burnet Institute, Melbourne, VIC, Australia

3. Papua New Guinea Institute of Medical Research, Madang, PNG

4. Mahidol Vivax Research Unit, Mahidol University, Bangkok

Malaria infections due to *Plasmodium vivax* are a major challenge for elimination in the Asia-Pacific region. Despite progress in vaccine development for *Plasmodium falciparum*, there remains no advanced *P. vivax* vaccine candidates. Improving our knowledge of the targets and functional mechanisms of naturally acquired immunity to *P. vivax* could assist development of better *P. vivax*-specific vaccines. Antibodies play an important role in acquired immunity and can act in various ways including direct inhibition, activation of complement, and engagement with Fcγ-receptors (FcR). The major objective of this study was to characterise the acquisition and longevity of functional antibody responses following *P. vivax* infection.

We developed a multiplexed assay to measure functional antibody responses (complement fixing, FcR binding) against a panel of 30 *P. vivax* proteins simultaneously. We observed that both complement-fixing and FcR-binding responses were highest against the blood-stage antigens AMA1, RBP2b, MSP1-19, RAMA and MSP8. *P. vivax*-specific complement-fixing responses were higher in individuals with at least 2 *P. vivax* infections in the prior year in a Thai observational cohort (n=775) from a low-transmission setting and were weakly-moderately associated with age. Utilising a 9-month follow-up study following clinical *P. vivax* infections in Thailand (n=36), we observed that complement-fixing and FcR-binding responses were shorter lived than total IgG responses, declining significantly to background by 2-4 months post-infection. Temporal longevity of *P. vivax*-specific complement-fixing antibodies was remarkably similar in PNG children (n=33) following asymptomatic *P. vivax* infections.

These results demonstrate that *P. vivax*-specific functional antibody responses elicited following *P. vivax* infections are short-lived and suggests a focus of vaccine research should be inducing long-lived functional antibody responses. Future studies will assess downstream Fc-mediated effector functions such as phagocytosis and relate these to the FcR-binding antibodies measured.

160

Investigating the role of human γδ T cells in cytomegalovirus infection

Ellen J Mann[1], Martin Davey[2], Daniel Arsovski[1], Jamie Rossjohn[1]

1. Monash University, Clayton, VICTORIA, Australia

2. Warwick Medical School, The University of Warwick, Coventry, United Kingdom

γδ T cells have been implicated in the immune response to microbial infections. A sub population of γδ T cells that express an unusual Vδ1 chain are thought to play a role in anti-viral immunity, although specific antiviral mechanisms remain largely unknown. To better understand how Vδ1+ γδ T cells respond to viral infection and whether this response shapes their phenotype, we mapped the cytokine profile, changes in frequency and phenotype of Vδ1+ γδ T cells in lung transplant patients that developed acute Human Cytomegalovirus (HCMV) infection. Peripheral Blood Mononucleocyte (PBMC) samples were collected from 27 patients who underwent a lung transplant and contracted or reactivated latent HCMV. Using multiparameter immunphenotyping of longitudinal samples before HCMV infection and post-HCMV, we were able to determine the cytolytic capacity, phenotype alteration and activation of Vδ1+ γδ T upon viral infection or reactivation. Notably, we found an increase in CD45RA+, CD27- cells (effector phenotype) Vδ1+ γδ T across all samples post-HCMV infection, indicating a shift in Vδ1+ γδ T cell cytokine expression as a result of HCMV exposure. Further, the Vδ1+ γδ T population in patients experiencing an active HCMV infection, displayed a greater capacity for cytotoxicity, with an increase in granzyme expression, particularly granzyme B, as well as upregulation of NKG2D and CD94. In summary, HCMV drives Vδ1+ γδ T cell activation and subsequent phenotypic changes and our results affirm the potential of Vδ1+ γδ T cells to respond to HCMV infection.

161

The interaction between Dynamin 3 and Kelch 13 in Plasmodium falciparum

Richard R Marais[1], Emma E McHugh[1]

1. Biochemistry and Microbiology, Bio21 Institiute, Parkville, Victoria, Australia

Plasmodium falciparum is the deadliest species of malaria parasite that kills approximately 440,000 people every year. Artemisinin is the frontline antimalarial drug recommended by the WHO and makes a crucial contribution to global malaria control. Thus, emergent artemisinin-resistant strains of *falciparum* malaria pose a serious health threat. Partial artemisinin resistance manifests as a 24-48 h delay in parasite clearance, which allows the parasite time to further mutate and puts pressure on the partner drugs administered to protect artemisinin. Observed resistance mutations are often found in a protein called Kelch 13 (*Pf*K13). This protein is localised to a ring at the neck of the parasite cytostome, a membrane invagination the parasite uses to engulf the cytoplasmic contents of host red blood cells. By-products of haemoglobin digestion are required to activate artemisinin so, in healthy parasites, feeding directly activates the drug that will kill them. In *Pf*K13-mutants normal parasite feeding is diminished and the artemisinin-based cell death is delayed. In a related parasite, *Toxoplasma gondii*, *Tg*K13 associates with a Dynamin-like protein (*Tg*DrpC) that may mediate cytostome formation and we hypothesise that the same is true of *P. falciparum*. Using an inducible knockdown, we have shown that *P. falciparum* parasites die without the orthologous dynamin-like protein *Pf*DYN3, and that *Pf*DYN3 forms puncta at the parasite periphery that resemble PfK13 puncta. We are now in the process of co-transfecting a GFP-*Pf*K13 parasite line with a haemagglutinin (HA)-tagged *Pf*DYN3. This will be used to assess the relative localisation of *Pf*K13 and *Pf*DYN3. These experiments will interrogate whether *Pf*DYN3 is involved in the *Pf*K13-mediated parasite feeding process that is central to both parasite growth and artemisinin resistance.

162

Tuft cells regulate inflammation in the lung during influenza A virus infection

Jacqueline Marshall[1], Philip Hansbro[1], Gang Liu[1], Gabrielle Belz[2], Scott Byrne[3], Michael Buchert[4], Elinor Hortle[1], Ridhima Wadhwa[1], Shatarupa Das[1], Angelica Katsifis[1], Piyush Jha[1]

1. Centre for Inflammation, Centenary Institute, UTS, Sydney, NSW, Australia

2. The University of Queensland Diamantina Institute, Woolloongabba, Brisbane, Queensland, Australia

3. Westmead Institute for Medical Research, Centre for Immunology and Allergy Research, Sydney, New South Wales, Australia

4. Olivia Newton-John Cancer Research Institute, School of Cancer Medicine, La Trobe University, Melbourne, Australia

Tuft cells are rare, solitary, specialised epithelial cells found in most mucosal tissues. They have important roles in initiating and propagating immune responses in the gastrointestinal tract. However, the role of tuft cells in the respiratory tract has not been extensively studied, and their potential roles in respiratory inflammation during lung infection remains unclear. In this study, we investigate the role of airway tuft cells during inflammation in a mouse model of influenza A virus (IAV) infection. Double cortin-like kinase 1 (DCLK1) is a widely

used marker of murine tuft cells. We used a tamoxifen inducible DCLK1 deleter mouse to deplete DCLK1+ cells from C57BL/6 mice, then infected them with 33 p.f.u. of A/PR8 H1N1 IAV or sham. At day 7 post infection, DCLK1 deleter mice had significantly reduced numbers of total leukocytes in bronchoalveolar lavage fluid (BALF) compared to WT controls in cytospins. IAV infected DCLK1 deleter mice had no changes in neutrophil numbers in the lung tissues but significantly increased total numbers of neutrophils in the blood compared to WT controls by flow cytometry. Infected deleter mice showed an increase of IFN-Y and IL-6 and a decrease of IL-10 in BALF, and increased mRNA expression of IL-1b, IL-6, CXCL-1 and IFN-Y in the lung tissue after 7 days post infection compared to WT controls. Our data indicate that airway tuft cells contribute to inflammation in the lung during flu infection.

163

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

Christopher J McCrory[1], Jiyoti Verma[1], Ana Traven[1]

1. Monash University, Clayton, VICTORIA, Australia

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

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

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

164

PhiP-Seq reveals a global increase in autoantibodies in Acute Rheumatic Fever patients

Reuben McGregor[1, 2], Tim O'Donnell[3], Lauren Carlton[1], Julie Bennet[4], Michael Baker[4], Uri Laerson[3], Nicole J Moreland[1, 2]

1. University of Auckland, Auckland, AUCKLAND, New Zealand

2. Maurice Wilkins Centre and School of Medical Sciences, The University of Auckland, Auckland, New Zealand

3. Icahn School of Medicine, Mount Sinai, New York, USA

4. Department of Public Health, University of Otago, Wellington, New Zealand

Acute rheumatic fever (ARF) is a serious post-infectious condition that can develop after a Streptococcus A (StrepA) infection, which can result in permanent heart valve damage and rheumatic heart disease. The rates of ARF in Indigenous children in New Zealand and Australia are amongst the highest globally but disease mechanisms are poorly understood. The current hypothesis is based on molecular mimicry generating antibodies in response to StrepA infection, which cross-react with cardiac proteins such as myosin. Contemporary investigations of the broader autoantibody response in ARF are needed to both inform pathogenesis models and identify new biomarkers for the disease. In prior work we utilised high content protein arrays (Protoarray, 9000 proteins and HuProt Array, 16,000 proteins) to analyse ARF sera autoreactivity and showed broad yet heterogenous elevation of autoantibodies. To enable deeper exploration of ARF autoantibodies this study employed Phage Immunoprecipitation Sequencing (PhIP-seq) and a human autoantigen library comprised 259,345 overlapping 90-mer peptide sequences covering the human proteome. Sera from 52 ARF cases and 75 matched controls from the national Rheumatic Fever Risk Factor study were incubated with the library and next-generation sequencing of enriched clones was performed. PhIP-seq data was analysed using both conventional differential expression analysis, as well as more robust multivariate dimensionality reduction techniques in order to generate robust results. Both analyses confirmed a global increase in autoantigen reactivity in ARF patients compared with controls, as well as marked heterogeneity in the autoantibody fingerprint between ARF patients. This heterogeneity was reflected in the large proportion of private autoantibodies identified, with public autoantibodies (common between individuals) comprising just 1% of the enriched peptides. Filtering for peptides that were identified in both types of analysis, and were considered 'public', resulted in the identification of both historic and novel autoantigens with roles in heart structure and function. Orthogonal validation in optimised immunoassays indicates multiple autoantigens will need to be combined to capture the heterogeneity observed in future diagnostic tests.

165

Characterisation of an essential dynamin-like protein in the malaria parasite Plasmodium falciparum

Emma McHugh[1], Katie Fitzsimmons[1], Richard Marais[1], Stuart A Ralph[1]

1. University of Melbourne, Parkville, VIC, Australia

Feeding of *Plasmodium falciparum* on host red blood cell cytoplasm generates haemoglobin breakdown products required for activation of the antimalarial artemisinin. Resistance to artemisinin is mediated by mutations in the *Pf*Kelch13 protein, which is located at the constriction of the cytostome, the invagination through which host cytoplasm is uptaken into the parasite. Mutations in *Pf*Kelch13 decrease cytostomal haemoglobin uptake and result in decreased activation of artemisinin. In *Toxoplasma gondii*, an orthologue of *Pf*Kelch13 was shown to co-immunoprecipitate with a dynamin-like protein known as *Tg*DrpC. Dynamin-like proteins are involved in membrane constriction processes such as endocytosis and vesicle budding, and we therefore hypothesise that the orthologous *P. falciparum* dynamin-like protein *Pf*DYN3 (PF3D7_1218500) is involved in cytostomal feeding of *P. falciparum*. In order to investigate *Pf*DYN3, we used CRISPR to introduce an epitope tag and loxP sites that enable inducible disruption of the gene. Disruption of *Pf*DYN3 results in parasite death in the subsequent asexual cycle. Super-resolution fluorescence microscopy reveals that in early-stage trophozoites, *Pf*DYN3 is located in approximately three or four discrete puncta at the parasite periphery. Additionally, we have performed mass spectrometry on cross-linked *Pf*DYN3 co-immunoprecipitation eluates to elucidate interacting proteins.

166

Immune cell profiling of acute rheumatic fever

Francis M Middleton[1, 2], Reuben McGregor[1, 2], Ciara Ramiah[1], Anna Brooks[2, 3], Julie Bennett[1, 2, 4], Michael Serralha[5], Tim Barnett[5], Rachel Webb[1, 2, 6], Anna Ralph[7], Jonathan R Carapetis[5], Nikki J Moreland[1, 2]

1. Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand

2. Maurice Wilkins Centre, The University of Auckland, Auckland, New Zealand

3. School of Biological Sciences, The University of Auckland, Auckland, New Zealand

4. University of Otago, Auckland, New Zealand

5. Telethon Kids Institute, University of Western Australia, Perth, Australia

6. Kidz First Hospital, Counties Manukau Health District, Auckland, New Zealand

7. Global and Tropical Health, Menzies School of Health Research, Darwin, Australia

Acute Rheumatic Fever (ARF) is a serious autoimmune sequela that develops after a Group A Streptococcus (GAS) infection and is a major cause of health inequity in underserved Indigenous populations in Australia and New Zealand. Repeated ARF can result in chronic rheumatic heart disease (RHD) with significant global morbidity and mortality, particularly in low- and middle-income countries. The pathogenesis of ARF is poorly understood, which has contributed to a lack of specific biomarkers and effective immunomodulating treatments for the disease. Responding to these urgent needs, 'Searching for a Technology-Driven Acute Rheumatic Fever Test' (START) is a trans-Tasman study designed to identify a diagnostic signature for ARF by use of systems biology and serology technologies[(1)]. We will present preliminary results of 33-colour spectral flow cytometry profiling using peripheral blood mononuclear cells from START study participants. This provide the broadest characterization of immune cell populations in an ARF cohort to date (n=116). Age and ethnicity matched control groups, including infectious and inflammatory conditions that may present similarly to ARF, chronic RHD, and healthy subjects, will be used for rigorous identification of unique ARF-associated immunological traits (n=120). Automated clustering algorithms will be used to quantify changes in cell populations and phenotypes. This will provide insight into the role of immune cells in ARF pathogenesis, and has the potential to identify new therapeutic targets for immunomodulating intervention.

1. Ralph AP, Webb R, Moreland NJ, McGregor R, Bosco A, Broadhurst D, et al. Searching for a technology-driven acute rheumatic fever test: The START study protocol. BMJ open. 2021;11(9):e053720.

167

Trafficking of the RhopH complex in malaria parasites

Joyanta Modak[1, 2], Natalie Counihan[1, 2], Tania de Koning-Ward[1, 2]

1. School of Medicine, Deakin University, Geelong, VIC, Australia

2. The Institute for Mental and Physical Health and Clinical Translation, Deakin university, Geelong, VIC, Australia

Background: Malaria is a devastating parasitic disease that kills 400,000 people per year. Resistance to current antimalarials is rising, and therefore, it is an urgent need to better understand the biological functions of the essential blood-stage genes in *Plasmodium falciparum*, as these may serve as future drug targets. *P. falciparum* RhopH complex is essential for parasite survival and contains 3 proteins that localise to the host red blood cell (RBC) membrane that are involved in importing nutrients from the serum to support the rapid growth of the parasite. Currently, the mechanism by which the complex moves from the parasite to the RBC surface is unknown and is the focus of this study.

Methods: To determine the role of PTEX in trafficking RhopH complex, one of the PTEX components was conditionally knocked down using PfHSP101-HAglmS parasite line and the trafficking of the RhopH protein components was determined using immunofluorescence (IFA) techniques. Sequential solubilisation of parasite-infected RBCs was used to determine the localisation of the RhopH complex at different lifecycle stages. In parallel, split GFP technology was used to image RhopH components by fusing one component of GFP to a RhopH component, and the other component to a protein with a known localisation. When both components were in close proximity, an increase in fluorescence was used to infer localisation.

Results: IFA results showed that RhopH proteins reach the RBC surface after the knockdown of an essential PTEX component. Our results suggest that RhopH protein complex traffics to the RBC membrane via a PTEX-independent manner. Preliminary sequential solubilisation studies identify that RhopH components reside on the cytosolic side of the parasite vacuole membrane, and further

investigations are being done to confirm this. A stable parasite line containing RhopH components tagged with GFP1-10 has been created and GFP11 has been introduced into proteins known to localise in the parasite vacuole and exported to the RBC surface.

Conclusion: Using molecular and biochemical techniques, these studies will reveal how RhopH components traffic from the parasite to the RBC surface and will be an important tool for discovering how malaria parasites import nutrients into the host RBC.

168

Lipid droplets contribute to an antiviral bystander effect during infection

Ebony A Monson[1], Camille Braganca[1], Irumi Amarasinghe[1], William Phillips[2], Donna Whelan[2], Andrew Hill[3], Karla J Helbig[1]

1. Microbiology, Anatomy, Physiology and Pharmacology (MAPP), La Trobe University, Melbourne, VIC, Australia

2. La Trobe Institute for Molecular Science, La Trobe University, Bundoora, VIC, Australia

3. Institute for Health and Sport, Victoria University, Footscray, VIC, Australia

We have previously demonstrated that lipid droplets (LDs) are upregulated as a host response to early infection of multiple viruses (IAV, ZIKV, DENV and HSV-1), driving effective type I /III interferon responses. We have also discovered that important antiviral proteins localise to LDs, positioning them as important signalling platforms during this response. It's not known however if LDs may also contribute to the antiviral bystander effect seen during viral infection.

To explore this, stable cell lines fluorescently expressing the LD resident protein; ADRP in both mCherry and GFP were generated to assess LD transfer between cells. Co-culture of the two fluorescently tagged LD cell lines revealed transfer of LDs between cells occurs *in vitro*. Isolated fluorescent LDs from these cell lines were also very frequently taken up into the cell cytoplasm of naïve cells, using a dynamin-dependent mechanism of entry. To understand if LDs contribute to a bystander effect during viral infection, fluorescent LDs isolated from cells that had activated innate immune responses (via dsRNA viral mimic stimulation) were placed onto naïve cells prior to ZIKV infection. This treatment of LDs significantly increased type I and III interferon responses resulting in a 50% decrease in ZIKV replication. Utilising proteomics and super-resolution microscopy we identified multiple ESCRT pathway proteins known to be involved in the secretion of extracellular vesicles (EVs) present on LDs. Additionally, using cell lines fluorescently expressing GFP-labelled EVs, and mCherry-labelled LDs, we were able to demonstrate that LDs can also egress from cells packaged inside EVs *in vitro,* providing an alternate secretion mechanism.

We have shown that LDs play vital roles in facilitating the magnitude of the early antiviral immune response, in particular the production of interferon following viral infection. To our knowledge, this study is the first to examine the extracellular role of LDs, placing them as contributors to the antiviral state of bystander cells. This data represents a paradigm shift in our understanding of the molecular mechanisms coordinating an effective antiviral response by implicating LDs as a critical organelle in this response.

169

Targeting virus-STAT interactions to generate novel attenuated 'live' vaccines

Gregory W Moseley[1], Alamgir Hossain[2], Florence Larrous[3], Stephen Rawlinson[1], Jinyu Zhan[2], Asish Sethi[2], Youssef Ibrahim[2], Maria Aloi[1], Kim G Lieu[2], Yee-Foong Mok[2], Michael Griffin[2], Toyoyuki Ose[4], Herve Bourhy[3], Naoto Ito[5], Paul R Gooley[2]

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

2. Dept. of Biochemistry and Molecular Biology, Bio21 Institute, University of Melbourne, Parkville, VIC, Australia

3. Unit Lyssavirus Dynamics and Host Adaptation, Pasteur Institute, Paris, France

4. Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

5. Laboratory of Zoonotic Diseases, Gifu University, Gifu, Japan

Viruses exploit diverse molecular strategies to evade the host organism's immune responses, including through the function of viral proteins that act as antagonists of the antiviral interferon (IFN)-mediated innate immune system. A large number of viruses target members of a family of cellular immune signalling proteins called the signal transducers and activators of transcription (STATs). These include STAT1 and STAT2, which are critical factors in signalling by multiple cytokines, including type I antiviral IFNs. Recent work, including from our collaborative program (1-5), has shown that highly lethal viruses such including paramyxoviruses, lyssaviruses and filoviruses target several different members of the STAT family, and that this targeting can produce outcomes that are not only antagonistic, but also can manipulate signalling pathways, thereby shut-down antiviral outcomes while potentially enhancing or redirecting 'pro-viral' signalling (2-5). We also recently demonstrated that viral targeting of activated STATs not only acts to prevents the establishment of an antiviral state in virus-infected cells but has critical roles deactivating the pre-established antiviral state in cells previously activated by IFN, enabling invasion of non-infected cells during an active immune response (1). The latter is a critical element in viral spread within the infected host.

Based on these findings, and our progress in determining the structural interface of IFN-antagonists with STATs and with viral proteins critical to immune evasion and replication, we have generated new vaccine strains for lyssaviruses (6). These vaccine candidates are highly attenuated but induce potent protection of animals against pathogenic rabies virus challenge. Together, these data validate the targeting of STAT interactions for the design of attenuated virus vaccines/ Our novel vaccine strains have the potential for advancement into large animals experiments toward future application in campaigns to eliminate dog-mediated human rabies.

1. (1) PLOS Pathogens. 2022. 18(5): e1010533. https://doi.org/10.1371/journal.ppat.1010533 (2) PLOS Pathogens. 2021. 17(7): e1009729. https://doi.org/10.1371/journal.ppat.1009729 (3) PLOS Pathogens. 2021. 17(6): e1009636. https://doi.org/10.1371/journal.ppat.1009636 (4) PLOS Pathogens. 2020. 16(9):e1008767. doi.org/10.1371/journal.ppat.1008767 (5) Cell Reports 2019. 29: 1934-1945 (6) PCT/AU2019/050908 "Novel Viruses" (filed 27/8/2019)

170

Exploring the molecular signatures of *Staphylococcus aureus* transition into human serum using multi-omics analysis

Warasinee Mujchariyakul[1], Calum Walsh[1], Abdou Hachani[1], Tim Stinear[1], Benjamin Howden[1]

1. University of Melbourne, North Melbourne, VIC, Australia

Staphylococcus aureus is a common human commensal bacterium. However, these colonising bacteria can invade the bloodstream, causing devastating infections, aggravated by the widespread emergence of resistance to last-line antibiotics like vancomycin. Understanding how *S. aureus* infection occurs has become increasingly challenging. In this study, we integrated multi-omics data including transcriptomics, proteomics, and metabolomics to identify the molecular signatures of *S. aureus*transition into human serum. We focus on five sepsis isolates representing the most common lineages of *S. aureus* that are clinically important in Australia. Our preliminary results show that distinct *S. aureus* isolates share common and strain-specific responses to human serum. Interestingly, the up-regulation of genes involved in iron acquisition pathways was conserved across *S. aureus* lineages. We observed that staphylobilin-forming heme oxygenase (isdI) was up-regulated, at both transcriptional and translational levels, in *S. aureus* exposed to human serum. Consistent with our analyses, an isogenic *isdI* mutant showed growth defects in human serum when compared to *S. aureus* wild-type JE2, suggesting the importance of iron uptake for bacterial survival in human serum. For future analyses, multivariate data integration will be used to identify new potential conserved and strain-specific targets for further therapeutic treatments of sepsis.

171

Development and evaluation of novel immunotherapeutics for EBV lymphomas in preclinical models

Corey Smith[1], Katie Lineburg[1], Swathi Muralidhar[1], Rajiv Khanna[1]

1. QIMR Berghofer, Brisbane, QLD, Australia

Having survived millions of years of evolution amongst primates, Epstein Barr virus (EBV) is considered one of the most complicated viruses. It not only causes primary infectious mononucleosis but also has the ability to transform itself into an oncogenic virus to cause cancers like Nasopharyngeal carcinoma and Hodgkin's carcinoma. Recent evidence also strongly correlates EBV to various autoimmune diseases such as multiple sclerosis and systemic lupus erythematosus.

These diseases are usually treated with traditional approaches including ectomy and radio/chemotherapy, with the addition of monoclonal antibodies in some cases. Limited therapeutic progress has led to increased interest in the capacity for immunotherapy in the targeted treatment of EBV associated diseases. Strong evidence supporting the role of dysfunctional T cells in EBV cancers, has already led to the development of immunotherapies for EBV lymphomas. However, this approach warrants further studies with regard to testing the efficacy, safety and prolonged survival of infused immune cells for the targeted attack of virus/ virus transformed cells in vivo.

As a part of my PhD project in the Translational and Human Immunology lab, at QIMRB, we have developed and characterized a humanized murine model that enables us to replicate latent EBV cancer in vivo. Using this model, my project aims to profile the immune landscape and evaluate immunotherapies in vivo.

Additionally, this model provides us with a platform to investigate the underlying immunological, virological and serological parameters of EBV associated lymphomas and other EBV-associated autoimmune conditions. It provides a powerful resource for the characterization of viral trafficking, and furthermore histopathology analysis enables us to demonstrate the spatial configuration of immune cells in relation to EBV virus and its various strains. Importantly, this model allows us to examine the temporal activity of cytotoxic lymphocytes as a therapy for EBV-associated tumors in varying EBV strain landscape.

172

Elucidating Influenza Infections from the Nose to the Brain

Lynn Nazareth[1], Jasmina Luczo[1], Vinod Sundaramoorthy[2], Kim Halpin[1], John Bingham[1]

1. CSIRO, Geelong, VICTORIA, Australia

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

The shortest route to the brain is through the olfactory system. This consists of olfactory neurons (OSNs) embedded in epithelial cells. OSNs extend their axons into the lamina propria (LP) where they are wrapped by olfactory glia (OECs) and fibroblasts forming the olfactory nerve connecting to the brain. Highly pathogenic strains of influenza virus have been reported to target cells within the olfactory system disseminating into the CNS. While some strains of influenza are restricted within the olfactory system following experimental intranasal infection. However, exact mechanism of virus spread or lack thereof through the olfactory system is not well understood.

Here we infect murine olfactory epithelial cultures (containing OSNs and epithelial cells), olfactory LP cultures (containing OECs and fibroblasts) and forebrain (Fb) cultures (containing neurons and astrocytes) with a highly pathogenic strain of Influenza A virus (H5N1/VN1203/2004) and a low pathogenic strain- pandemic (pm) (H1N1/CA/07/2009). We then performed multicycle growth kinetic studies and confocal microscopy investigating influenza infection through the olfactory system and compared this to CNS host cell response.

Viral growth kinetic studies revealed strain specific differences, with robust replication seen for H5N1 compared to pmH1N1. We also observed cell specific differences. While pmH1N1 appeared to replicate successfully within Fb cultures this was restricted within olfactory cultures. On the other hand, both olfactory and forebrain neuron cultures were conducive for H5N1 replication, Using confocal microscopy, we were able to identify unique viral infection trends. H5N1 infection within olfactory epithelial cultures initial infection was observed mainly within non-neuronal (presumably epithelial cells) cells which appeared to spread to OSNs in later stages of infection (48 – 72 h). On the other hand, H5N1 infections was predominantly observed within OECs in the LP cultures with most infection resolved by 72 h. Furthermore, infection with H5N1 produced change in OEC morphology with increased cell area and tunnelling nanotubes (TNTs) seen between adjacent cells with influenza nucleoprotein within these structures. Similar changes were also observed in astrocytes, indicating potential viral manipulation for cell-cell transmission within glial cells.

Further molecular and microfluidic studies will be performed to investigate these differences in infection.

173

Mouse GBP1 is detrimental for the host during *Burkholderia thailandensis* infection

Garrett Ng[1, 2], Si Ming Man[3], Ian van Driel[4], Elizabeth Hartland[1, 2]

1. Centre for 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. Division of Immunity, Inflammation, and Infection, The John Curtin School of Medical Research, The Australian National University, Acton, Australian Capital Territory, Australia

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

Guanylate binding proteins (GBPs) are strongly induced by interferon-gamma (IFNγ) and bind a range of intracellular pathogens. GBPs are believed to mediate the release or binding of pathogen associated molecules to activate the inflammasome. Due to the rapid expansion of GBPs in mice (11 vs 7 in humans), individual murine GBPs are believed to have redundant and overlapping roles. Murine GBP1 arose from a duplication of murine GBP2, the direct homologue of human GBP2.

We initially investigated the relative contributions of IFNγ and mouse GBPs to *B. thailandensis* infection. Mice that lacked GBPs encoded on chromosome 3 (GBPΔchr3; GBP1, 2, 3, 5, 7) were highly susceptible to infection but mice that lacked several GBPs encoded on chromosome 5 (GBP4, 6, 8) were relatively similar to wildtype mice. GBP2 and GBP5 have been previously described to restrict *B. thailandensis* infection. Hence, we investigated if GBP1 had a role in infection. Surprisingly, *Gbp1*$^{-/-}$ mice had the opposite phenotype to GBPΔchr3 or *Gbp2*$^{-/-}$ mice, and were more resistant to infection.

In conclusion, murine GBP1 has a unique role in the response to infection despite high similarity to GBP2. We believe this is the first demonstration of a GBP being detrimental to the host during infection. The divergent roles of murine GBP1 and GBP2 in *B. thailandensis* infection could be exploited to understand the different roles of GBP1 and GBP2.

174

Robust SARS-CoV-2 T cell immunity towards COVID-19 vaccines in haematology patients of varying malignancies and immunosuppressive treatment

Oanh Nguyen[1], Louise Rowntree[1], Lily Allen[1], Brendon Chua[1], Lukasz Kedzierski[1], Chhay Lim[2], Masa Lasica[3], Surekha Tennakoon[2], Natalie Saunders[2], Megan Crane[2], Lynette Chee[2, 4], John Seymour[2], Mary Anderson[2], Ashley Whitechurch[2], Bridie Clemens[1], Wuji Zhang[1], So Young Chang[1], Jennifer Habel[1], Xiaoxiao Jia[1], Hayley McQuilten[1], Anastasia Minervina[5], Mikhail Pogorelyy[5], Priyanka Chaurasia[6], Jan Petersen[6], Tejas Menon[1], Luca Hensen[1], Jessica Neil[1], Francesca Mordant[1], Hyon-Xhi Tan[1], Adam Wheatley[1], Stephen Kent[1], Kanta Subbarao[7], Theo Karapanagiotidis[8], Suellen Nicholson[8], Florian Krammer[9], Grace Gibney[10], Fiona James[10], Janine Trevillyan[10], Jason Trubiano[10], Jeni Mitchell[4], Britt Christensen[4], Katherine Bond[4], Deborah Williamson[8], Jamie Rossjohn[6], Jeremy Chase Crawford[5], Paul Thomas[5], Karin Thursky[2], Monica Slavin[2], Constantine Tam[2, 3], Benjamin Teh[2], Katherine Kedzierska[1]

1. University of Melbourne, Melbourne

2. Peter MacCallum Cancer Centre, Melbourne

3. St Vincent's Hospital, Melbourne , Fitzroy

4. Royal Melbourne Hospital, Melbourne

5. St. Jude Children's Research Hospital, Memphis

6. Monash University, Clayton

7. WHO Collaborating Centre for Reference and Research on Influenza, Melbourne

8. Victorian Infectious Diseases Reference Laboratory, Melbourne

9. Icahn School of Medicine at Mount Sinai, New York

10. Austin Health, Heidelberg

Patients undergoing HSCT or CAR-T therapy and CLL patients are heavily immunocompromised and highly vulnerable to severe COVID-19 infection. These patients can respond poorly to vaccination and have perturbed immunity. We evaluated a breadth of immune responses in HSCT, post-CAR-T and CLL patients across three doses of COVID-19 vaccination in comparison to healthy individuals. Presence of seropositive RBD-IgG antibodies were low after 1st dose of BNT162b2 (27%, +3wk) and ChAdOx1 (26%, +12wk) vaccine, which increased to 75% and 59% at 1 month after 2nd dose, respectively. Third mRNA dose increased antibody responses to 85%, demonstrating that a subset of patients still had no antibodies after 3 doses of COVID-19 vaccination. Conversely, complete 100% seropositivity was observed in healthy controls following 1st, 2nd and booster dose with either vaccine. Two doses induced prototypical antibody-secreting cells (ASCs) and T follicular helper (Tfh) type-1 responses in healthy participants, while haematology patients showed prolonged presence of ASCs and skewed Tfh2/17 responses. Induction of spike-specific memory B-cells correlated with RBD-IgG antibodies, particularly in patients with non-B-cell malignancies. Importantly, vaccine-induced expansions of spike-specific (AIMS/ICS) and peptide-HLA tetramer-specific CD4^{+}/CD8^{+} T cell responses detected directly *ex vivo* and their TCR repertoires were robust across all patient disease groups, irrespective of B cell numbers, and comparable to healthy participants. RBD-IgG titres, but not tetramer^{+} T cell frequencies, positively correlated with B cell numbers. Vaccinated haematology patients with breakthrough infections had higher RBD-specific IgG antibody responses, while T cell responses were indistinguishable between infected and non-infected individuals in both patient and healthy groups. Overall, COVID-19 vaccination induces robust T-cell immunity in haematology patients of varying diseases and treatments, irrespective of their B-cell numbers and antibody response.

175

A novel approach to uncover cryptic cases of immunodeficiency

Tahereh Noori[1], Jesse Rudd-Schmidt[1], Alisa Kane[2], Katie Frith[3], Adrian Hodel[1], Paul Gray[3], Ilia Voskoboinik[1]

1. Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

2. Department of Immunology and HIV, St Vincent's Hospital, Sydney, NSW, Australia

3. Department of Immunology and Infectious Diseases, Sydney Children's Hospital, Sydney, NSW, Australia

Cytotoxic lymphocytes protect an organism against viral pathogens and cancer by killing infected and transformed cells, through perforin-mediated mechanism. Mutations in perforin (*PRF1*) itself or in the secretory machinery responsible for its release (*UNC13D*, *STX11*, and *STXBP2*) are catastrophic, and lead to fatal immune dysregulation, familial haemophagocytic lymphohistiocytosis (FHL).

Traditionally, FHL has been associated with infant patients. However, it is now apparent that many patients remain disease-free for years, and then present with highly variable and often unexpected symptoms. They remain undiagnosed for a long time and, instead of receiving curative stem cell transplantation, they are treated symptomatically leading to high risk of severe neurological impairment, organ failure and/or death.

While the pathogenicity of frame-shift/nonsense mutations is rarely in doubt, the effect of missense mutations on protein function can vary enormously. Yet, over the last two decades, the pathogenicity of missense mutations was almost invariably assumed, and invasive stem cell transplantation was considered without verified FHL diagnosis. Sadly, transplantation without genetically proven FHL results in a 40% increased mortality compared to patients with proven FHL. Therefore, early and accurate diagnosis of the disease is essential to determine the most appropriate treatment option.

Due to the diversity of genetic causes of FHL, there was no test available to directly assess the effect of mutations on cytotoxic lymphocyte function, leading to delayed/erroneous diagnoses.

To address this significant clinical problem, we developed a novel and rapid single-platform experimental approach for testing the function of missense mutations in all four genes associated with FHL. Not only did we uncover unique cryptic cases of FHL that enabled an accurate diagnosis/treatment of patients, but in some instances, we also demonstrated that previously reported mutations were unlikely to cause the disease.

In addition to diagnosing patients, our unique approach will be paramount for assessing the prognosis of asymptomatic siblings and to guide genetic counselling advice for prospective parents.

176

Development of an *in vivo* infection model for Genotype 4 Japanese encephalitis virus

Caitlin A O'Brien[1], Leonard Izzard[1], Andrea Certoma[1], Stacey Lynch[1], David T Williams[1]

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

Since early 2021, a new strain of Japanese encephalitis virus (JEV) has emerged in Australia. In 2022, this strain caused a widespread outbreak in Queensland, New South Wales, South Australia, and Victoria. The outbreak has impacted over 80 piggeries, with 32 confirmed human cases across the four affected states[1]. Reports of clinical disease in domestic pigs involved reproductive losses including an increased incidence of stillborn and mummified piglets, as well as neurological symptoms in newborn piglets. The causative strain of this outbreak has been identified as a member of the divergent Genotype IV, previously thought to be rarely sampled and geographically restricted to Indonesia[2]. JEV infection models in pigs have previously focused on the more widespread genotypes I – III, with existing vaccines and serological neutralisation tests primarily based on these genotypes. We have commenced a study to establish an infection model with Australian genotype IV Japanese encephalitis virus in grower pigs. In this study, we aim to characterise the replication dynamics and immune response in 12-week-old pigs by assessing clinical symptoms, viraemia, antibody response, routes of virus shedding and tissue tropism. We expect this work to provide valuable insight into the transmission, and pathogenesis of this novel outbreak strain in the main amplifier host of JEV, and to provide a baseline for future vaccine efficacy studies and serological diagnostic development.

1. 1 - Hutchinson, D., Kunasekaran, M., Chen, X., & Moa, A. (2022). Japanese Encephalitis Outbreak in South-Eastern Australia, 2022. Global Biosecurity, 4(1). DOI: http://doi.org/10.31646/gbio.159 2 - Kuwata, R., Torii, S., Shimoda, H., Supriyono, S., Phichitraslip, T., Prasertsincharoen, N....Maeda, K. (2020). Distribution of Japanese Encephalitis Virus, Japan and Southeast Asia, 2016–2018. Emerging Infectious Diseases, 26(1), 125-128. https://doi.org/10.3201/eid2601.190235.

177

Age, chorioamnionitis, and the innate immune system in preterm infants

Josephine C Owen[1, 2], Steven X Cho[1, 2], Ina Rudloff[1, 2], Merrin A Pang[1, 2], Jason C Lao[1, 2], Catriona A McLean[3, 4], Alex Veldman[1, 2, 5, 6], Claudia A Nold-Petry[1, 2], Marcel F Nold[1, 2, 7]

1. Department of Paediatrics, Monash University, Melbourne, Victoria, Australia

2. Ritchie Centre, Hudson Institute of Medical Research, Melbourne, Victoria, Australia

3. Department of Anatomical Pathology, Alfred Health, Melbourne, Victoria, Australia

4. Department of Medicine, Central Clinical School, Monash University, Melbourne, Victoria, Australia

5. Department of Pediatrics, Helios HSK, Wiesbaden, Germany

6. Department of Pediatric Cardiology, J. Liebig University, Gießen, Germany

7. Monash Newborn, Monash Children's Hospital, Melbourne, Victoria, Australia

Background: Trained immunity, also known as innate immune memory, describes the functional reprogramming of innate immune cells in response to prior stimulation. Trained immunity may lead to heightened immune responses to secondary, heterologous stimuli, but it is currently unknown whether trained immunity can develop in vulnerable premature neonates. Our research aims to examine the effect of

gestational and postnatal age on neonatal innate immune responses, and the development of innate immune memory after early-life exposures to challenges such as chorioamnionitis.

Methods: We collected blood from a population of 28 very/extremely premature neonates across five timepoints: cord blood and day 1, week 1, week 2, and at 12-16 weeks of postnatal age. Samples were then either vehicle-treated, or stimulated with 100ng/ml lipopolysaccharide, and we characterised monocyte and dendritic cell subtypes using flow cytometry, including analysis of the anti-inflammatory receptors CD85k and CD163, and of the immune activation receptor CD86.

Results: Both gestational and postnatal age significantly affected monocyte populations. At rest, the proportion of intermediate monocytes increased almost 1.5-fold from 25^{+0} weeks to 28^{+6} weeks corrected gestational age. Upon lipopolysaccharide stimulation, however, the proportion of classical monocytes increased by 1.7-fold. Expression of CD85k on monocyte and dendritic cell subtypes also increased by over 2-fold with increasing age. Abundance of CD85k on non-classical monocytes increased by 11.5-fold with increasing gestational age, and abundance of CD85k on dendritic cells increased by over 3-fold between birth and 12-16 weeks of postnatal age. Chorioamnionitis was associated with an acute reduction in CD86 expression by monocytes of up to 58.9% in cord blood and day 1 samples. However, at the week 1 and 2 timepoints, chorioamnionitis-exposed neonates demonstrated a 30% reduction in CD85k abundance on dendritic cells, and 25% reduction in CD163 abundance on intermediate monocytes. These longitudinal findings suggest the development of an innate immune memory after chorioamnionitis, resulting in increased pro-inflammatory responses to secondary stimulation with lipopolysaccharide.

Conclusion: These findings demonstrate a role of age, both gestational and postnatal, on the developing preterm innate immune system, and illuminate the possibility for the development of trained immunity in premature neonates in response to chorioamnionitis.

178

Investigating the molecular basis underlying death of human intestinal epithelial cells in the inflammatory environment

Jiyi Pang[1], James E Vince[1]

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

The integrity of intestinal epithelium is essential for maintaining gut homeostasis, and acts as both a microbiota barrier and a sensor to activate appropriate immune responses. Dysregulated and excessive programmed cell death of intestinal epithelial cells (IECs) is related to the pathogenesis of inflammatory bowel disease (IBD). However, how IEC death might be triggered, particularly from pro-inflammatory cytokines, remains unclear. The aim of this study is to understand how physiological ligands elevated in the inflamed intestine might cause IEC death and thereby contribute to IBD pathogenesis.

Human small intestine organoids were derived and differentiated into different intestinal cell types, and these were treated with inflammatory cytokines observed in IBD patients: tumor necrosis factor (TNF) and interferon-gamma (IFNγ). The combination of these two inflammatory ligands acted in concert to induce undifferentiated intestinal organoid death, as measured and quantified over time by Incucyte live cell analysis. However, organoids differentiated into an enterocyte-like phenotype were markedly more sensitive to death following TNF and IFNγ treatment. Mechanistically, TNF and IFNγ treatment of intestinal organoids revealed changes in the levels of several critical cell death regulators, and an increase in inducible nitric oxide synthase (iNOS) and its product nitric oxide, which we have recently implicated in executing an inflammatory cell death and COVID-19 disease severity. By delineating the molecular pathway by which excess inflammation can cause intestinal cell death in humans, new therapeutic targets for IBD might be uncovered.

179

BCL-2 and BCL-XL dynamically regulate LPS-primed macrophage survival in the absence of BCL2A1

Ashlyn P Pascoe[1], Garrett Z Ng[1], Timothy A Gottschalk[1], Mary Speir[1], Hazel Tye[1], Elizabeth L Hartland[1], James E Vince[2], Kate E Lawlor[1]

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

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

Intrinsic apoptosis is a cell death mechanism that is an important host response to combat pathogen infection. While traditionally thought to be immunologically silent, recent studies have demonstrated crosstalk between intrinsic apoptotic cell death signalling and inflammation via caspase-3/-7-dependent NLRP3 inflammasome activation. However, little is known about the regulation of intrinsic apoptosis during bacterial infection, and whether this inflammatory crosstalk is protective. Moreover, whether inducing cell death in infected macrophages, which act as a replicative niche for a growing number of antimicrobial resistant bacteria, can be harnessed to promote pathogen clearance remains unclear.

It is well established that bone marrow derived macrophages (BMDMs) rely on the BCL-2 family pro-survival proteins BCL-XL and MCL-1 for their survival. Our unpublished findings also reveal an essential role for the inducible, short-lived, BCL-2 family member BCL2A1 (A1) in delaying apoptosis upon Gram-negative bacterial LPS exposure and BCL-XL and MCL-1 targeting. Interestingly, we now find that targeting BCL-2, in conjunction with MCL-1 inhibition and A1 loss, also triggers late stage cell death and NLRP3 inflammasome activity in LPS-primed macrophages. Finally, we show the potential physiological relevance of A1 in limiting cell death and inflammation using the Gram-negative intracellular bacteria *Legionella pneumophila*. Collectively, our data suggest that pathogens may modulate BCL-2 family member expression and function to elude the host immune system.

180

Defining the mechanisms of action of antibodies against *Plasmodium vivax* malaria

Kaitlin Pekin[1, 2], Herbert Opi[1, 3, 4], Liriye (Lydia) Kurtovic[1, 3], Gaoqian Feng[1, 4], Daisy Mantila[5], Benishar Kombut[5], Maria Ome-Kaius[5], Chris King[6], James Kazura[6], Moses Laman[5], Ivo Mueller[7], Leanne Robinson[1, 7, 8], James Beeson[1, 2, 3, 4]

1. Burnet Institute, Melbourne, VIC, Australia

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

3. Department of Immunology, Monash University, Clayton, VIC, Australia

4. Department of Medicine, The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia

5. Papua New Guinea Institute of Medical Research, Mandang, Papua New Guinea

6. Case Western Reserve University, Cleveland, OH, USA

7. Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC, Australia

8. School of Public Health and Preventive Medicine, Monash University, Melbourne, VIC, Australia

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

In a cohort of children (n=37; ages 5-14), resident in a malaria-endemic region of PNG, we quantified functional antibody responses to four major *P. vivax* vaccine candidate antigens - AMA1, MSP3α and MSP1-19, and the two allelic forms of CSP. Specifically, we quantified IgG and cytophilic IgG subclasses, antibody-mediated complement fixation, Fcγ-receptor binding (types I, IIa and IIIa) and antibody-mediated opsonic phagocytosis of antigen-coated beads by THP-1 monocyte cells.

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

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

181

The role of gut macrophages in gut epithelial barrier formation

Sophia Piryaei[1], Hani Hosseini Far[2], Cristina Giogha[2], Eva Chan[2], Christine Wells[1]

1. The University of Melbourne, Parkville, VIC, Australia

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

The gut is an important organ that includes the most surface contact with the outside environment. This massive surface is covered by a layer of epithelial cells that creates a physical barrier to the environment, whilst absorbing nutrients. It is also a major site of commensal microbiota, which works together with gut epithelial and immune cells to maintain the integrity of this vital organ. Impaired inflammatory regulation of the intestine and bowel causes chronic inflammatory disorders such as inflammatory bowel disease (IBD). We currently lack human models that include relevant immune components, so we don't know how the different cell types in the gut epithelial interact together to form the healthy gut epithelial barrier. The purpose of this study is to investigate the contribution of macrophages to gut epithelial barrier function. We established two in vitro models: 1) Transwell coculture and 2) direct coculture of iPSC-derived macrophages and an epithelial cell line, to test the roles of secreted or contact-mediated factors respectively. The quality of the epithelial barrier is measured by examining polarisation and tight junction formation microscopically, using NF-κB and IRF reporters in the epithelial cells or macrophages to monitor for inflammatory pathway activation, and monitoring of barrier integrity using transepithelial electrical resistance (TEER) as well as dye exclusion assays. We hypothesise that introduction of macrophages to the gut epithelial models will improve the development and integrity of the gut epithelium.

182

TREML4 ablation in mice leads to the development of innate immune memory following polymicrobial sepsis

Christina Nedeva[1], Joseph Menassa[1], Corey Pollock[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 into extended immune suppression. Many clinical trials sought to modulate inflammation, but this had no effect on patient mortality. Recently, focus has shifted to addressing the immunosuppressive phase and allow the host's immune system to return to a functional state. Previous work by *Nedeva et al., 2020* identified triggering receptor expressed on myeloid cells (TREM) family receptor, TREML4 as the key regulator immune cell death and inflammation, following polymicrobial sepsis. Genetic ablation of TREML4 in mice using CRISPR demonstrated its role in regulating many cellular responses, especially apoptosis of innate immune cells, leading to a higher rate of survival in the acute and chronic phases of sepsis. This improved survival is due, in part, to long lasting epigenetic changes in the effector innate immune cells of TREML4 ablated mice. Neutrophils play an essential role in the early stages of many infections and recently been shown to play a major role in TREML4 ablated mice survival advantage. Therefore, epigenetic changes to the histones of neutrophils 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.

183

Enhanced stability of the SARS CoV-2 spike glycoprotein trimer following modification of an alanine cavity in the protein core

Christine Langer[1], Irene Boo[1], Tasnim Zakir[1], Rob J Center[1, 2], Anouschka Akerman[3], Vanessa Milogiannakis [3], Anupriya Aggarwal[3], Stuart G Turville[3], Heidi E Drummer[1, 4, 2], Andy Poumbourios[1, 4]

1. Burnet Institute, Melbourne, VIC, Australia

2. Department of Microbiology and Immunology, The University of Melbourne, Parkville, VIC

3. Kirby Institute, University of New South Wales, Kensington, NSW, Australia

4. Department of Microbiology, Monash University, Clayton, VIC

First-generation SARS CoV-2 vaccines that generate immune responses to ancestral Spike glycoprotein sequences have averted at least 14.4 million deaths, but their effectiveness against the recently emerged Omicron lineages is reduced. The updating of booster vaccines with variant Spike sequences is therefore likely required to maintain immunity as the pandemic continues to evolve. The Spike is a trimeric integral membrane protein with a membrane spanning sequence at its C-terminus. The Spike protein-based vaccine that is currently licensed for human use is produced by a complex process that reconstitutes the Spike in an artificial membrane. Alternatively, production of the Spike trimer as a soluble protein generally requires replacement of the membrane spanning sequence with a foreign often highly immunogenic trimerization motif that can complicate clinical advancement. We used systematic structure-directed mutagenesis coupled with functional studies to identify an alternative stabilization approach that negates the requirement for an external trimerization motif or membrane-spanning sequence. The replacement of 2 alanine residues that form a cavity in the core of the Spike trimer with bulkier hydrophobic residues resulted in increased Spike thermal stability. Thermostable Spike mutants retained major conserved neutralizing antibody epitopes and the ability to elicit broad and potent neutralizing antibody responses. One such mutation, referred to as VI, enabled the production of intrinsically stable Omicron variant Spike ectodomain trimers in the absence of an external trimerization motif. The VI mutation potentially enables a simplified method for producing a stable trimeric S ectodomain glycoprotein vaccine.

184

Adipose tissue-resident eosinophils are transcriptionally distinct from blood eosinophils: harnessing this knowledge to uncover anti-obesity targets

Manan Shah[1], Alexander Knights[1], Emily Vohralik[1], Annalise Psaila[1], Kate Quinlan[1]

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

Eosinophils are leukocytes with broad roles in tissue homeostasis and innate immunity. Eosinophils are important in host defense against helminth infection but are inappropriately activated in pathologies such as asthma and esophagitis. However, eosinophils also reside within normal healthy adipose tissue.

Adipose tissue-resident eosinophils play homeostatic roles and, along with other adipose tissue-resident immune cells, regulate the activation of beige adipocytes. Beige adipocytes residing within white adipose tissue burn fuels to generate heat, by a process called thermogenesis, and therefore may be able to be harnessed to reduce obesity by burning rather than storing excess fuels. We recently uncovered gene regulatory mechanisms in mice that allow adipose tissue-resident eosinophils to secrete molecules important for beige fat activation and prevention of weight gain.

Given the potential of adipose tissue-resident eosinophils to drive beige fat activation and weight loss, we sought to generate a better understanding of these cells. We performed bulk RNA-seq in mouse FACS-isolated adipose tissue-resident eosinophils, for the first time, and compared gene expression to blood eosinophils. We found a unique transcriptional landscape in adipose tissue-resident eosinophils that is distinct from blood eosinophils in circulation and also distinct from previously published transcriptomes of lung, colon and bone marrow eosinophils. Differential gene expression of surface receptors, chemokines and associated genes suggest that adipose tissue-resident eosinophils functionally adapt to their tissue niche. We also performed an analysis of transcription factors that may drive this adaptation which identified the importance of KLF family, Fos/Jun families and CEBP families in regulating the transcriptome of adipose tissue-resident eosinophils.

We are now working to further define the transcription factor network that drives this unique gene expression profile of adipose tissue-resident eosinophils. We are also testing whether novel adipose tissue-resident eosinophil secreted proteins, that we identified in our RNA-seq data, are able to induce beiging and energy expenditure and may present novel targets for obesity.

185

The *Plasmodium falciparum* artemisinin resistance protein Kelch 13 is required for formation of normal cytostomes and parasite feeding

Madel V Tutor[1], Gerald J Shami[1], Ghizal Siddiqui[2], Hamid Soleimaninejad[1], Darren J Creek[2], Leann M Tilley[1], Stuart A Ralph[1]

1. The University of Melbourne, Parkville, VIC, Australia

2. Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Vic, Australia

Resistance to the frontline antimalarial artemisinin is mediated by mutations in the Kelch13 (K13) gene in *Plasmodium falciparum*. The function of K13 is unknown. Using super-resolution microscopy we showed that K13 localises to a ring at the parasite periphery and under time lapse microscopy these rings co-localise with foci of host cytoplasm as they are taken up by the parasite. We localised K13 by electron microscopy to an electron dense collar surrounding the neck of the cytostome - a double membraned invagination that

allows the parasite to engulf large packages of host haemoglobin. This is the first molecular marker defined for this structure. We used a combination of 3D electron microscopic techniques (electron tomography and serial-block-face-scanning electron microscopy) to characterise *Plasmodium* parasites with aberrant K13. Inducible mis-localisation of K13 resulted in parasites that lack the electron dense cytostomal collar, and the parasite's tightly-constricted cytostomal neck is also lost, leading to aberrant cytostomal structures. Our data indicate that K13 is required for normal formation or stabilisation of the cytostome, and is likely a major component of the electron dense cytostomal ring itself. We show that these K13 mutant parasites have reduced uptake of haemoglobin, and consequently produce less of the haemoglobin breakdown products haem and haemozoin. Haem is required to activate artemisinin into its proteotoxic form, so a reduction in this process likely explains reduced artemisinin sensitivity. We hypothesise that K13 is required for formation of the normal cytostome used for feeding by intracellular *Plasmodium*, and that mutant K13 results in parasites with less efficient feeding, decreased activation of artemisinin, and ultimately, drug resistance. These data also suggest that artemisinin resistance is inextricably linked to less efficient feeding by the parasite, and suggest that further resistance through this mechanism could only come at the cost of substantially reduced parasite fitness,

186

Full length Immune Repertoire Sequencing enables Accurate Clonality Determination

Selva Kumari Ramasubramanian[1], Chen Song[1], Karen McKay[1], Pingfang Liu[1], Andrew Barry[1], Bradley W Langhorst[1], Eileen T Dimalanta[1]

1. New England Biolabs Australia, Melbourne, VIC, Australia

The study of complex immunological diseases and tumor microenvironments has progressed through recent developments on sequencing of the immune repertoire. Using this approach, the interrogation of disease progression is facilitated through analysis of millions of V(D)J combinations from B cell receptors (BCR) and T cell receptors (TCRs). One major challenge of immune repertoire sequencing is to accurately capture the structural and sequence complexities of antibodies and TCR genes. We have developed a method for accurate sequencing of full-length immune gene repertoires of B cells and T cells from both human and mouse samples. RNA was extracted from tissues and peripheral blood mononuclear cells (PBMCs) and used for reverse transcription, during which unique molecular identifiers (UMIs) were added to discretely barcode each mRNA molecule. BCR- and TCR-specific PCR primers were used to enrich full-length BCR and TCR sequences. We have implemented a data analysis pipeline to assemble the full length BCR/TCR transcripts and to collapse PCR copies of each mRNA fragment into a single consensus sequence using UMIs. UMI incorporation enables the absolute quantification of input RNA molecules and accurate ranking of antibody/TCR clone abundance. Furthermore, this method facilitates detection of distinct and shared clones in tissue and blood samples, allowing identification of disease-specific clones to evaluate immunotherapy effects. Our method accurately and sensitively detects target TCR clones down to 0.01%, enabling minimal residual disease (MRD) assessment.

Our immune repertoire sequencing approach allows accurate clonal determination for both BCR and TCR. This technique is applicable for a variety of applications including design of antibody chains for *in vitro* synthesis, investigation of T cell infiltration of tumor microenvironments, and monitoring of minimal residual disease in cancer patients.

187

Infectious Disease Research: It's time to think differently

Selva Kumari Ramasubramanian[1]

1. New England Biolabs Australia, Melbourne, VIC, Australia

Gaining a better understanding of infectious diseases, including their characterization, evolution, and transmission, continues to be a priority, both from an R&D standpoint and as a public health issue. The COVID-19 pandemic has demonstrated the need for a wide range of tools to research infectious diseases, develop diagnostics and vaccines, and conduct epidemiological and surveillance studies. Development of molecular diagnostics that robustly and accurately detect harmful micro-organisms, including viruses, are critical to identifying and preventing the spread of disease. Nucleic acid amplification is commonly used to identify the presence of specific sequences within pathogenic microbes. These techniques include qPCR and RT-qPCR for real time quantitation of nucleic acids and loop-mediated isothermal amplification (LAMP) for robust, single temperature amplification, which can be performed easily in the point-of-care setting, such as a clinic or in the field. Sequencing has also proven a useful tool in the detection of variants, aiding in the real-time monitoring of changing viral dynamics in an at-risk population. Epidemiological studies are important as they help to better characterize disease-causing agents, including viruses, as well as monitor evolution and spread of disease. NGS can be used to identify variants, which can then help track disease transmission, and inform public health decisions as well as drug and diagnostic development.

Recent experience has exposed the need for high quality reagents that are accessible at scale, often under more stringent conditions, and that are available in a variety of product formats. Many products from New England Biolabs have played a role in the global response to SARS-CoV-2, from supporting the development of COVID-19 diagnostics and vaccines to improving the speed and accuracy of Illumina® and Nanopore-based COVID-19 sample analysis. These products can also be utilized with other infectious diseases, such as influenza and malaria, in multiplex assays for simultaneous detection and differentiation.

188

Regulation of tissue-specific T_{RM} cell responses

Grace Rodrigues[1, 2], Jessica Runting[1, 2], Luke Gandolfo[3], Laura Mackay[4], Colby Zaph[1, 2]

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

2. Monash Biomedicine Discovery Institute, Clayton, VIC, Australia

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

4. Department of Microbiology and Immunology, Doherty Institute, Parkville, VIC, Australia

Tissue resident memory T (T_{RM}) cells are preferentially localised at the site of primary infection and in many cases are defined by the expression of the cell surface markers CD69 and CD103. Gene expression analysis has shown that T_{RM} cells in multiple tissues share a core transcriptome, characterised by the downregulation of a repertoire of pro-migratory genes, aiding retention. Although cytokines such

as TGF-β and IL-15 have been proposed to promote T_{RM} cell development, it is clear that there are tissue-specific determinants of T_{RM} cell development and maintenance that remain elusive. By developing a novel methodology to generate T_{RM}-like cells *in vitro*, we identify a critical role for retinoic acid (RA) and the transcriptional repressor Hypermethylated in Cancer 1 (HIC1) in the development of T_{RM}-like cells. Transcriptomic analysis revealed that activation of $CD8^+$ T cells in the presence of IL-2, IL-15, TGF-β and RA resulted in faithful expression and repression of the core T_{RM} cell transcriptome, and that HIC1 was critical in regulating repression of the core gene program. Critically, we find that RA, TGF-β and RA/TGF-β conditions induce unique gene signatures that have similarities to *bona fide* T_{RM} cells from the gut, liver or lung, providing a unique insight into the factors that potentially regulate tissue-specific T_{RM} cell development. As T_{RM} cells are key mediators of immunity in recurrent infections and cancer, but are capable of facilitating autoimmune disease when dysregulated, understanding how best to modulate these cells will be crucial in informing the design of novel therapeutics.

189

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

Sarah Rosli[1, 2], Christopher Harpur[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) is an infectious respiratory pathogen that is estimated to affect at least 1 billion people worldwide every year, with the young, old and immunocompromised being at most risk. Detection of IAV by the host's immune system aids viral clearance, however, hyperinflammation can lead to the development of fatal lung disease. Gasdermin D is an executioner of pyroptosis, a lytic form of cell death which results following NLRP3 inflammasome activation. Gasdermin D forms a transmembrane pore, which is thought 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 Gasdermin D has been implicated in many inflammatory diseases, its role in IAV infection is not well characterised. Here, we showed that mice lacking gasdermin D (*Gsdmd-/-*) are less susceptible to HKx31 H3N2 IAV infection, displaying significantly improved survival in comparison to wildtype controls. Further, bronchoalveolar lavage fluid presented with a significant reduction in total cellularity, which correlated with fewer numbers of infiltrating neutrophils at day 3 and 5 post-infection. This was accompanied with 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 at day 3. Together, these results suggest that gasdermin D limits the severity of IAV infection. Inhibition of gasdermin D may provide a novel host-targeted IAV therapeutic strategy, which limits the development of fatal lung disease.

190

Investigating host-pathogen interactions in Toxoplasma gondii infection

Ushma Ruparel[1], Marcel Doerflinger[1], Simona Seizova[2], Chris Tonkin[1]

1. Walter and Eliza Hall Institute of Medical Research, Parkville VIC 3052, VIC, Australia

2. Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, Nethergate, Dundee, Scotland

Toxoplasma gondii, an obligate intracellular parasite that causes toxoplasmosis, is responsible for substantial disease burden worldwide. Drugs against acute infection are limited by toxicity whilst chronic forms are completely drug resistant. During and after invasion of the parasite into the host cell, *T. gondii* relies heavily on the ability to export effector proteins into the host cell that manipulate signalling pathways to ensure parasite survival and long-term persistence. Host cell death pathways are an attractive avenue for the parasite to manipulate as programmed cell death is an important host defence mechanism against infection.

To investigate the role of programmed cell death pathways in *T. gondii* infection, we infected mice deficient for various combinations of mediators of programmed cell death pathways. We discovered that receptor interacting serine/threonine kinase 3 (RIPK3) and mixed lineage kinase domain like pseudokinase (Mlkl) (important for necroptosis), caspases-1, -11, and -12 (important for pyroptosis), and caspase-8 (important for apoptosis) are individually dispensable for control of *T. gondii* infection. However, combined loss of necroptosis and apoptosis resulted in reduced survival, suggesting that these pathways play an important role in mediating resistance to infection in the host. Loss of effector protein export rescued the survival of mice deficient in all pathways to a degree, but this was not an apoptosis- or pyroptosis-specific phenotype, implying that parasite protein export is not required to subvert host apoptotic pathways. The mechanism of how apoptosis is altered upon infection remains unknown.

191

Lipid nanoparticle-mediated delivery of RNA therapeutics to treat *Mycobacterium tuberculosis* infection

Jan Schaefer[1]

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

RNA therapeutics are becoming increasingly valuable in clinical applications but their efficient and targeted delivery to the organ and cell type of interest is crucial for clinical translation. Lipid nanoparticles (LNPs) have revolutionised the delivery of mRNA and other small molecules. Tuberculosis (TB) is the single biggest infectious killer worldwide, and current antimicrobial treatment paradigms are increasingly ineffective due to skyrocketing multi-drug resistance interventions need to target alveolar macrophages as these cells represent the major intracellular reservoir for *Mycobacterium tuberculosis* (Mtb). As proof of concept, we targeted delivery of mRNA coding for mCherry by encapsulating the coding sequence in lipid nanoparticles (LNPs) and we confirmed efficient mRNA transfection into mouse primary bone marrow-derived macrophages (pBMDMs) *in vitro*. These mRNA-LNPs were then administered to naive mice via intranasal instillation and after 7 h, mCherry expression was detected in the alveoli using IHC. The versatility of this delivery platform allows us to explore novel RNA-based treatment approaches for TB infection.

Using small-molecule IAP antagonists to degrade cellular inhibitors of apoptosis 1 and 2 (cIAP1/cIAP2) to induce apoptosis of Mtb-infected host cells provided proof-of-principle that this host-directed approach can lead to pathogen clearance *in vivo*. Building on this, we encapsulated cIAP1/2-specific siRNA into LNPs to generate a treatment platform for TB infection. These siRNA-LNPs induced efficient cIAP silencing in mouse pBMDMs, and induction of apoptosis in infected cells.

We combined this approach with another novel RNA-based treatment utilising mRNA that codes for a protective nanobody against a major Mtb virulence factor, ESAT-6. Recombinantly expressed ESAT-6 was used to immunise alpacas and raise ESAT-6-specific nanobodies. Of the resulting nanobody pool, ten ESAT-6 binding nanobodies were selected and their respective mRNA sequences will be encapsulated in LNPs to assess their effect on pathogen virulence in further tests.

With a robust RNA delivery platform suitable for clinical translation, we have a powerful tool at hand to target potent RNA therapeutics directly to the lung. The next step will be to use these LNPs to deliver cIAP1/2-specific siRNA or nanobody-encoding mRNA *in vivo* and assess their protective potential in a clinically relevant mouse model of TB.

192

Role of urokinase plasminogen activator (uPA) in *Helicobacter pylori*-mediated host responses

Modhusudon Shaha[1, 2], Rebecca Gorrell[1, 2], Joel Steele[3], Han Lee[3], Ralf Schittenhelm[3], Ruby Law[1], Benjamin Buckley[4], Marie Ranson[4], Terry Kwok-Schuelein[1, 2]

1. Biochemistry and Molecular Biology, Monash University, Melbourne, Vic, Australia

2. Microbiology, Monash University, Melbourne, Vic, Australia

3. Monash Proteomics and Metabolomics Facility, Monash University, Melbourne, Vic, Australia

4. School of Chemistry and Molecular Bioscience, University of Wollongong, Sydney, NSW, Australia

Helicobacter pylori is a human pathogen that causes gastric diseases such as chronic gastritis, peptic ulcer disease (PUD), and gastric cancer. *H. pylori* infection also enhances the invasion and metastasis of gastric cancer, but the molecular mechanism involved is unknown. In this study, we examined the molecular mechanism by which *H. pylori* exploits the urokinase-type plasminogen activator (uPA) proteolytic system for pathogenesis and metastasis-related host cell responses. uPA is a host serine protease that converts plasminogen to plasmin and plays important roles in tissue remodelling and cancer metastasis. Using a plasmin inhibitor and a novel uPA small-molecule inhibitor, we demonstrated that both uPA and plasmin activities contributed significantly to *H. pylori*-induced host cell motogenic responses. This effect was dependent on the *H. pylori* oncoprotein, CagA. To further elucidate the molecular mechanism involved, we examined the effect of the specific uPA inhibitor on the total secretome of *H. pylori*-infected epithelial cells using quantitative mass spectrometry. Our findings indicate that *H. pylori* broadly alters the abundance profile of a multitude of secreted plasmin substrates, including laminin, transforming growth factor β1, FAM3C, complement C3, and fibrinogen, in a manner dependent on uPA catalytic activity and CagA. Taken together, our findings suggest that *H. pylori*, through its oncoprotein, CagA, exploits the uPA-plasmin proteolytic cascade for dysregulation of the host extracellular proteome and processes involved in tissue remodelling and cancer metastasis. Whilst uncovering the therapeutic potential of the small-molecule specific uPA inhibitor for hindering *H. pylori* pathogenesis, this study has identified novel potential drug targets for the intervention of *H. pylori*-associated gastric disorders and gastric cancer metastasis.

193

Streptococcus pneumoniae potently kill mesothelial and mesothelioma cells in a strain dependent manner

Hui Juin Shak[1, 2, 3]

1. Pleural Medicine Unit, Institute for Respiratory Health, Perth, WA, Australia

2. School of Medicine, The University of Western Australia, Perth, WA, Australia

3. Respiratory Medicine, Sir Charles Gairdner Hospital, Perth, WA, Australia

Introduction & Aims: Mesothelioma is a fatal cancer and there is no cure for it. Chemotherapy treatment has shown only minor improvement in survival and current standard treatment is mainly palliative. The use of bacteria or their products presents an attractive approach in mesothelioma as there is strong evidence to suggest that bacterial infection in the pleura may increase survival in these patients. In this preliminary study, we aimed to assess the killing capacity of Streptococcus pneumoniae against mesothelioma cells.

Methods: Clinical S. pneumoniae strains (n= 10) collected from patients with invasive bacterial infection were co-cultured with patient-derived mesothelioma cells (n=9) and benign mesothelial cell line (MeT-5A). S. pneumoniae strains at concentration of 1×107 and 1×104 CFU/mL were added to confluent mesothelioma/mesothelial cells. Cell viability at 0, 4 and 8 hours post infection was assessed by flow cytometry using a Live/Dead cell viability assay.

Results: Our data show a high variability in the mesothelioma/mesothelial cells killing by clinical S. pneumoniae strains, where some strains have a high killing capacity towards some cells, but not the others. For example, on HAM cell line, one bacterial strain killed up to 83% cells while other strain only killed 9.2% of the cells at 4 hours, despite having the same starting inoculum. S. pneumoniae strains increase killing of the five mesothelioma cell lines by median of 1.9 to 9.44-fold over controls at 4 hours, and by 1.67 to 15.19-fold at 8 hours. The fold change varies significantly, between cell lines and bacterial strains.

Conclusion:

Understanding the mechanisms underlying the inter-strain variability may provide insight for future therapeutic approaches for mesothelioma.

194

BET inhibitors are therapeutic candidates in inflammasome-driven autoinflammatory diseases

Farzaneh Shojaee[1, 2], Sebastian Hughes[1, 2], Maryam Rashidi[1, 2], James Vince[1, 2]

1. The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia

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

Autoinflammatory disorders are characterized by innate immune cell-driven inflammatory episodes at disease-prone sites. The inflammasomes, cytosolic sensors of pathogen and host danger molecules, can contribute to a variety of hereditary and wide-spread autoinflammatory diseases via their pathogenic activation of interleukin-1b (IL-1b) and the pyroptotic cell death effector protein Gasdermin D (GSDMD). Although targeted cytokine therapies, in particular anti-IL-1b and anti-TNF, have been efficacious in some of autoinflammatory conditions, they lack efficacy in others, or have not been adequately tested. The development of small molecule inhibitors targeting the epigenetic regulators, Bromodomain and Extraterminal (BET) proteins, has identified BET proteins as key regulators of cell death and inflammation, although their roles in inflammasome signaling remain to be fully explored. In this study we investigated how BET proteins might modulate inflammasome-driven responses. Our findings identify how BET inhibitor targeting of specific bromodomains can potently shut down both inflammasome driven pyroptosis, as well as IL-1b activity. Using a range of gene targeted mice lacking TNF receptor signaling components (e.g. caspase-8, RIPK3 and death ligands) or the inflammasome machinery (e.g. IL-1b, NLRP3, GSDMD, caspase-1 and caspase-11) we delineate a unique inflammasome pathway perturbed by BET inhibitor treatment, and show that this contributes to disease severity in a model of the potentially lethal hyperinflammatory disease, hemophagocytic lymphohistiocytosis (HLH).

195

The role of BTN3A1 in regulating αβ T cell responses

Kezia Christilla Singgih[1], Tom Fulford[1], Caroline Soliman[1], Zheng Ruan[1, 2], Rachel Pascoe[1], Sharon Lewin[3, 4, 5], Dale Godfrey[1, 2], Adam Uldrich[1, 2]

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

2. Australian Research Council Centre of Excellence for Advanced Molecular Imaging, The University of Melbourne, Parkville, VIC, Australia

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

4. Department of Infectious Diseases, Alfred Hospital, Melbourne, VIC, Australia

5. Victorian Infectious Diseases Service, Royal Melbourne Hospital at the Peter Doherty Institute of Infection and Immunity, Melbourne, VIC, Australia

Butyrophilins (BTNs) are members of the immunoglobulin superfamily commonly expressed on peripheral blood mononuclear cells (PBMCs). These molecules have a well-understood role as activators of human γδ T cells. However, several murine studies suggest that BTNs can also negatively regulate αβ T cell function. A recent study described that BTN member 3A1 (BTN3A1) overexpressed on ovarian cancer tumours attenuated $CD4^+$ and $CD8^+$ αβ T cell responses in humans through interaction with CD45RO, suggesting that BTN3A1 might regulate anti-cancer αβ T cell responses. To further investigate the potential role of BTN3A1 in regulating $CD4^+$ and $CD8^+$ αβ T cell function, we engineered BTN3A1-overexpressing K562 antigen presenting cells. Furthermore, we investigated whether BTNs interact with CD45RO by generating tetramers expressing CD45RO and BTN3A1 extracellular domain. We demonstrated that the presence of BTN3A1 did not alter αβ T cell activation, proliferation, cytokine production or K562 cell cytotoxicity. CD45RO tetramer did not bind to HEK293T cells overexpressing BTN3A1. Likewise, the BTN3A1 extracellular domain tetramer did not bind human $CD45RO^+$ PBMCs. Hence, BTN3A1 does not appear to modulate $CD4^+$ and $CD8^+$ αβ T cell function, nor do they appear to interact with CD45RO which contradict published findings. Therefore, future studies are required to confirm if these BTN molecules indeed exert immunoregulatory activity on αβ T cells, and if targeting BTNs with agonist or antagonist antibodies could be beneficial for anti-cancer and viral therapeutics.

1. Payne, K. K. et al. BTN3A1 governs antitumor responses by coordinating alphabeta and gammadelta T cells. Science 369, 942-949, doi:10.1126/science.aay2767 (2020).

196

Role of socs3b in zebrafish innate immunity

Mohamed Luban Sobah[1], Clifford Liongue[1], Alister Ward[1]

1. Deakin University, Highton, VIC, Australia

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

197

Characterisation of CD1c-restricted T cells recognising mycobacterial lipids

Caroline Soliman[1], Katrina J Smith[1], Samuel J Redmond[1], D Branch Moody[2], Dale I Godfrey[1], Nicholas A Gherardin[1]

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

2. Division of Rheumatology, Inflammation and Immunity, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

The CD1 family are MHC I-like molecules that present self and foreign lipid antigens to T cells. CD1a, CD1b and CD1c are known for their presentation of lipids derived from the *Mycobacterium tuberculosis* (Mtb) cell wall, and CD1-restricted T cells are thought to play an important role in the immune response toward Mtb as well as the Bacillus Calmette–Guérin (BCG) vaccine. CD1c in particular has been shown to present mycoketide lipids including phosphomycoketide (PM) and mannosyl phosphomycoketide (MPM). However, little is known about the T cells that respond to these lipids. This is partly due to the fact that CD1c-restricted T cells are not present in mice, making them harder to study. In addition, until recently, the use of CD1c-lipid tetramers has been obfuscated by their reactivity toward receptors from the broadly expressed CD36 family, limiting their use for detecting T cell receptor (TCR)-specific staining in blood samples. Here, through the use of CD1c tetramers in conjunction with CD36 blocking antibodies, we isolate CD1c-restricted T cells ex vivo from human blood, and characterise them in the context of their lipid reactivity profile. Intriguingly, CD1c-PM restricted T cells are particularly abundant relative to CD1c-MPM and CD1c-autoreactive cells. These cells display a fine-specificity for PM, with minimal cross-reactivity to other lipids. The surface phenotype of these cells is generally diverse with CD1c-reactivity extending to both αβ and γδ T cells, and spread across CD4, CD8 and double negative αβ T cell populations. Their TCR repertoire is also diverse, with a TRBV gene bias. Furthermore, analysis of memory subsets suggests these cells may follow adaptive-like dynamics. By understanding the specificity and functionality of these cells, we aim to explore the unique role that CD1c-restricted T cells play in the immune system, and begin elucidating their uses as potential immunotherapeutic agents against foreign targets.

198

Investigating the mechanism and function of IFNb-induced 3'-UTR transcript isoform switching in macrophages

Sarah Straub[1], Linden J Gearing[1], Traude Beilharz[2], Ralf Schittenhelm[2], Joseph Rosenbluh[2], Eicke Latz[3], Paul J Hertzog[1]

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

2. Monash Biomedicine Discovery Institute, Monash University, Clayton

3. Institute of Innate immunity, Bonn, Germany

Type 1 interferons (IFNs) are cytokines with critical roles in innate immunity that can act on a large number of cell types. They induce changes on different cellular levels, such as transcriptional, translational and metabolic. This study began with our discovery of a novel aspect of global post-transcriptional changes, shortening of 3' untranslated regions (UTRs) of mature mRNAs.

An increase of transcripts with shorter 3'-UTRs was detected in IFNb-treated human and murine macrophages using Poly-A-Tail-sequencing. Genes expressed with shorter 3'-UTRs are not typical IFN-regulated genes (IRGs) and are not differentially expressed on gene level. They make up a new class of IRGs regulated through alternative polyadenylation rather than changes to overall expression.Initial investigations using a NanoString array have shown that 3'-UTR shortening is mediated by IRGs. A flow cytometry-based RNA detection method was adapted to measure 3'-UTR isoforms and used as a read-out for a genome-wide CRISPR/Cas9 knock out screen. This allowed further identification of IRGs and other components, such as RNA processing factors, as putative mediators of IFNb-induced 3'-UTR shortening.

A change in 3'-UTR length generally doesn't alter the protein coding sequence of a transcript or affect the abundance of the corresponding protein. Instead, recent studies have described a scaffold-like role for 3'-UTRs. They can facilitate the formation of different protein complexes depending on 3'-UTR length, which can affect the encoded proteins localization and function. Here, sets of overexpression constructs for selected candidates were used for a co-IP followed by mass spectrometry to identify 3'-UTR length-dependent protein-protein interactors. Network and ontology analyses of the identified interactors suggest differences in function and localization of candidate RNA and protein derived from different 3'-UTR isoforms, independent of protein coding sequence. This discovery expands our current understanding of IRGs and their functions and highlights the importance of including non-coding sequences in future research.

This study shows that global shortening of 3'-UTRs is a novel aspect of macrophage activation by IFNb. Genes regulated through alternative polyadenylation make up a new class of IRG that may have diverse, 3'-UTR length-dependent functions. Further investigations will show how the expression of distinct isoforms shapes innate immune responses.

199

Understanding the innate immune response to Group A Streptococcus pili

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

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

Despite the global disease burden imposed 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 on the cell surface key for the initiation of infection. Pili based vaccines have been demonstrated to stimulate robust production of protective antibodies, but the innate immune responses involved remain undefined. Thus the interaction between GAS pili and components of innate immunity was investigated to characterise the implication of pili-based-vaccines on the immune system.

Pilus recombinant proteins and recombinant *L. lactis* strains expressing GAS pili on the cell surface were utilised to investigate the immunomodulation capacity of this surface structure. Furthermore, interactions between pili and toll-like receptors (TLRs) was studied in HEK239 reporter cell lines expressing various human TLRs. Cytokine production in response to pili was analysed by a cell-based ELISA using human monocytic THP-1 cells.

Protein production downstream of TLRs in the HEK239 cell lines indicated the GAS pilus proteins are ligands of TLR2. Additionally, the interaction between TLR2 and pilus proteins was confirmed via binding assays as well as flow cytometry. Furthermore, the TLR2/6 heterodimer was pinpointed as the TLR2 heterodimer recognising pili. In the THP-1 cells, strong pili induced production of pro-inflammatory cytokines such as TNF was observed. This pili induced cytokine release decreased with the introduction of a TLR2 antagonist, consolidating the pili signalling pathway. Whilst both the tip and backbone subunit appeared to be involved in the innate immune response, the tip subunit was found to have higher affinity binding to receptor and induced higher levels of cell stimulation. Interestingly, differences in the levels of cellular response were seen between different GAS pilus types.

The GAS pilus was shown to be highly immunostimulatory ligands of TLR2, alluding to its ability to achieve a desirable immunisation outcome. This helps solidify the pili as a GAS vaccine candidate, and warrants further investigation into its use as an adjuvant.

200

LAT8881 and other naturally derived cytokine peptides limit influenza virus replication and the development of severe disease

Christopher M Harpur[1], Alison C West[1], Melanie Le Page[1], Christopher Hodges[1], Lauren Ely[2], Andrew J Gearing[2], Michelle D Tate[1]

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

2. Lateral Pharma Pty Ltd, Melbourne, Victoria, Australia

There is an urgent need to develop new host-targeted therapeutics that safely limit the severity of respiratory viral infections. Severe and fatal influenza virus (IAV) infections are associated with significant viral replication and damaging hyperinflammatory responses which can lead to morbidity, mortality and long-term multi-system organ damage. LAT8881 is synthetic form of the naturally occurring C-terminal fragment of human growth hormone (GH), acting independently of the GH receptor to reduce inflammation-induced damage and promote tissue repair in an animal model of osteoarthritis. Additionally, LAT8881 has been investigated in several clinical trials in healthy volunteers and for the treatment of obesity and has an established safety profile.

In light of its effects in improving inflammatory damage in animal models, we investigated the potential for LAT8881 and related compounds to treat severe HKx31 H3N2 IAV infection. Daily, intranasal delivery of LAT8881 or its metabolite LAT9991F from 1 day following infection significantly reduced disease susceptibility and resulted in a dose-dependent reduction in infectious viral loads and pro-inflammatory cytokines in the lungs, as well as an increased abundance of protective alveolar macrophages. LAT8881 had similar activity with that of the antiviral oseltamivir phosphate and when combined, therapeutic effects were improved. *In vitro* LAT8881 treatment enhanced the viability of multiple cell types, particularly in the presence of cytotoxic stress, which was countered by siRNA inhibition of host lanthionine synthetase C-like (LANCL) proteins.

These studies provide the first evidence identifying LAT8881 and LAT9991F as novel host-protective therapies that improve survival, limit viral replication, and reduce local inflammation during severe IAV infection.

201

Single-cell RNAseq analysis of HIV reservoir cells from viremic and virally-suppressed individuals living with HIV

Sushama Telwatte[1, 2, 3], Julie Frouard[4], Xiaoyu Luo[4], Holly A Martin[3], Nikhila G Kadiyala[3], Adam Wedrychowski[3], Pavitra Roychoudhury[6, 5], Rebecca Hoh[7], Steven G Deeks[7], Sulggi Lee[7], Nadia Roan[4], Steven A Yukl[2, 3]

1. The Peter Doherty Institute for Infection and Immunity, Melbourne, VICTORIA, Australia

2. Infectious Diseases, University of California, San Francisco, San Francisco, California, United States

3. San Francisco VA Medical Center, San Francisco, California, United States

4. Gladstone Institute of Virology and Immunology, University of California, San Francisco, San Francisco, California, United States

5. Laboratory Medicine and Pathology, University of Washington, Seattle, Washington, United States

6. Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, United States

7. University of California, San Francisco, San Francisco, California, United States

Latently-infected CD4+ T cells are considered the main barrier to a cure for HIV-1. While these cells do not produce HIV constitutively, they can be induced to produce infectious virus upon activation. During viral suppression under therapy, a proportion of infected cells remain transcriptionally-active and these are predictive of time to viral rebound after antiretroviral therapy (ART) cessation. Considerable technical challenges are posed by the low frequency of transcriptionally-active HIV-1 reservoir cells and the fact that many of those cells reside in lymphoid tissues such as the gut. Finally, there are currently no known biomarkers that reliably distinguish latently-infected cells from uninfected cell populations in vivo.

To address these limitations, we developed “HIV-Seq”, a new single-cell (sc)RNAseq approach that enables simultaneous characterization of the transcriptome and surface proteome of unstimulated HIV-infected cells from blood and gut tissue from people living with HIV (PWH). Using custom-designed HIV-specific capture sequences and DNA-barcoded antibodies directed to key cell surface proteins (CITE-seq) introduced into a single cell RNAseq workflow (10X Genomics), we describe an in-depth combined scRNAseq/CITE-seq analysis of HIV reservoir cells from blood in the context of both viremia and ART suppression.

This new approach was applied to longitudinal samples collected at Week 0 (prior to commencing ART) of acute infection, and Week 24 or 45 after ART suppression [n=5]. CD4+ T cells were enriched using bead-based isolation and stained with DNA-tagged antibodies. HIV capture sequences were incorporated during library preparation. We additionally characterised total immune cells (CD45+) and T cells (CD3+) from the blood and gut of one ART-suppressed individual. scRNAseq and CITE-seq analyses were performed and sequences were aligned to a constructed subtype B consensus reference sequence.

Based on viremic sample data, HIV-seq enables 32-72% increased capture of HIV RNA+ cells, relative to no capture. In total, we identified 1232 HIV RNA+ cells from viremic timepoints and 26 HIV RNA+ cells from the ART-suppressed timepoints representing the transcriptionally-active reservoir.

Our HIV-seq method enables efficient identification and characterization of HIV-infected cells including in the context of ART suppression, allowing for in-depth transcriptomic and surface phenotypic analysis of transcriptionally-active reservoir cells.

202

Investigating the antimalarial activity of novel heterospirocyclic compounds in malaria parasites

Liana Theodoridis[1], Carlo Giannangelo[2], Farrah El-Saafin[3, 4], MR Ranga Prabhath[5], Pallavi Sharma[5, 6], Delphine Merino[3, 4, 7, 8], Darren Creek[2], Teresa G Carvalho[1]

1. Department of Microbiology, Anatomy, Physiology and Pharmacology, La Trobe University, Bundoora, VIC, AUS

2. Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Science, Monash University, Parkville, VIC, AUS

3. Olivia Newton-John Cancer Research Institute, Heidelberg, VIC, AUS

4. School of Cancer Medicine, La Trobe University, Bundoora, VIC, AUS

5. School of Chemistry, University of Lincoln, Lincoln, LN6 7TS, UK

6. Cold Spring Harbor Laboratory, New York, NY, USA

7. Department of Medical Biology, The Faculty of Medicine, Dentistry and Health Science, The University of Melbourne, Melbourne, VIC, AUS

8. Molecular Medicine Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, AUS

Malaria remains a significant global health burden and a leading contributor to global mortality rates, particularly in Africa and South-East Asia. Although the first ever malaria vaccine has been approved by the World Health Organisation, its effectivity of 36% highlights the importance of antimalarial drugs as key tools for disease control. However, the recent rise in global malaria cases and the spread of antimalarial drug resistance creates an urgent need for novel treatments. Due to the lack of chemical diversity amongst existing antimalarial therapeutics, a focus on novel compounds with underrepresented chemical scaffolds is vital to combat malaria drug resistance. We have synthesised a new class of 3D-spiroheterocycle compounds with chemical connectivities never previously synthesised or explored as drug leads. The unique 3D architecture of these molecules allows them to interact with biological domains otherwise inaccessible to relatively flat structures, leading to increased efficiency. We have previously shown that two spirocyclic compounds, C25 and C26, induce death of the most lethal human malaria parasite *P. falciparum* within 48hrs and present IC_{50} values in the low micromolar range. Recently, we have shown both compounds to be non-toxic in kidney- (HEK293) and hepatic- (HepG2 and Huh-7) derived humans cell lines when compared to the known antimalarial chloroquine. Interestingly, both compounds have a killing effect on multiple drug-resistant *P. falciparum* strains, including artemisinin-sensitive Cam3.II^{rev}, artemisinin-resistant Cam3.II^{R539T} and Cam3.II^{C580Y}, and multi-drug resistant W2mef parasite lines. Untargeted metabolomics indicates that C25 perturbs pyrimidine biosynthesis, with further analysis ongoing to identify the specific target(s). Parasites resistant to C25 and C26 have been generated in both 3D7 and Dd2 genetic backgrounds, with whole genome sequencing to be performed on the resulting resistant clones. This biological information will allow the synthesis of refined derivatives with more potent antimalarial activity.

203

MLKL deficiency protects against sterile inflammation and immunosenescence in aged mice

Emma C. Tovey Crutchfield[1, 2, 3], Sarah E. Garnish[1, 2], Jessica Day[1, 2, 4], Holly Anderton[1, 2], Shene Chiou[1, 2], Anne Hempel[1], Cathrine Hall[1], Komal M. Patel[1], Pradnya Gangatirkar[1], Connie S.N. Li Wai Suen[1], Alexandra L. Garnham[1], Andrew J. Kueh[1, 2], Ueli Nachbur[1, 2], Andre L. Samson[1, 2], James M. Murphy[1, 2], Joanne M. Hildebrand[1, 2]

1. Inflammation Department, The Walter and Eliza Hall Institute, Melbourne, VIC, Australia

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

3. The Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Melbourne, VIC, Australia

4. Department of Rheumatology, Royal Melbourne Hospital, Melbourne, VIC, Australia

MLKL and RIPK3 are the core signaling proteins of the inflammatory cell death pathway, necroptosis, which is a known mediator and modifier of human disease. Recently, reports have suggested a novel role for necroptosis in aging, particularly in the male reproductive system. However, whether other age-related phenotypes emerge in necroptosis-deficient mice, remains unknown. Here we present the first comprehensive analysis of age-dependent phenotypes in a cohort of littermate-controlled, *Mlkl*$^{-/-}$ and *Ripk3*$^{-/-}$ mice on a congenic C57BL/6J genetic background. We show that genetic deletion of *Mlkl*, but not *Ripk3*, in female mice interrupts immune system aging, specifically delaying the age-related reduction of circulating lymphocytes. *Mlkl*$^{-/-}$ female mice were also protected against age-related, low-grade chronic sterile inflammation, with a reduced number of inflammatory infiltrates present in the connective and muscle tissue at 17 months relative to wild-type littermates. Our observations implicate MLKL in sterile inflammation and immunosenescence, the age-dependent decline in immune function, in mice. We anticipate our study to be a starting point for more in-depth analyses of necroptosis-driven aging in mice and humans. Furthermore, these results will inform ongoing studies into the potential human indications for necroptosis-targeted therapies.

204

Divergence in immune evasion capacity between different genotypes of Hendra and Nipah virus

Melanie Tripp[2, 1], Cassandra David[2], Jasmina Luczo[1], Kim Halpin[1], Gregory Moseley[2]

1. AAHL, CSIRO-ACDP, East Geelong, VIC, Australia

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

Hendra (HeV) and Nipah virus (NiV) are highly pathogenic paramyxoviruses that, with several related viruses, form the genus *Henipavirus*. The natural reservoir of HeV and NiV is fruit bats, and ongoing spillover events have resulted in severe disease with high mortality in humans, horses, and pigs (1). The ability of these viruses to evade the interferon (IFN)-mediated innate immune response is considered to be a critical pathogenesis factor, and is mediated principally by accessory proteins encoded by the P gene; P, V, W and C through a variety of mechanisms (2). HeV and NiV both comprise at least 2 genotypes (HeV-g1 and the recently defined HeV-g2, in addition to NiV Bangladesh [NiV B] and Malaysia [NiV M]) (3). Differences in outcomes of spillover of NiV B and NiV M suggest potential variation in molecular mechanisms of pathogenesis; however data relating to this is currently lacking (4). While most of the viral proteins have high amino acid homology, P, V and W show the greatest divergence.

Here, we examined potential differences between P, V and W of different genotypes in antagonism of the critical IFN induction and signaling pathways. Using approaches including fluorescence microscopy, reporter assays, and immunoprecipitation analysis, we found that the capacity to inhibit IFN pathways is broadly conserved. However, significant differences in the extent of antagonist function were identified between viral species and genotypes. Ongoing research will define the molecular basis of these differences and their significance in infection, using our established approaches and unique biosafety facilities (5, 6).

These data provide the first indications that substitutions between the P gene of henipavirus genotypes have impacted functions in IFN-antagonism. This provides insights into potential differences in disease outcomes and is important to understanding the risks associated with outbreaks of different viruses and genotypes.

1. Halpin K, Rota P. A Review of Hendra Virus and Nipah Virus Infections in Man and Other Animals. Zoonoses - Infections Affecting Humans and Animals. 2014:997–1012.
2. Audsley MD, Moseley GW. Paramyxovirus evasion of innate immunity: Diverse strategies for common targets. World J Virol. 2013;2(2):57-70
3. Wang J, Anderson DE, Halpin K, Hong X, Chen H, Walker S, et al. A new Hendra virus genotype found in Australian flying foxes. Virol J. 2021;18(1):197.
4. Mire CE, Satterfield BA, Geisbert JB, Agans KN, Borisevich V, Yan L, et al. Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. Sci Rep. 2016;6:30916
5. Brice AM, Rozario AM, Rawlinson SM, David CT, Wiltzer-Bach L, Jans DA, et al. Lyssavirus P Protein Isoforms Diverge Significantly in Subcellular Interactions Underlying Mechanisms of Interferon Antagonism. J Virol. 2022:e0139622.
6. Chen M, Tachedjian M, Marsh GA, Cui J, Wang LF. Distinct Cell Transcriptomic Landscapes Upon Henipavirus Infections. Front Microbiol. 2020;11:986.

205

Covalent probe reveals Ubc13 interactome in the malaria parasite

Anna Truong[2, 1], Baiyi Quan[1, 3], Ruitian Hu[1], Michael C Fitzgerald[1, 4], Emily R Derbyshire[1, 5]

1. Chemistry, Duke University, Durham, North Carolina, United States

2. Biochemistry & Pharmacology, University of Melbourne, Melbourne, Victoria, Australia

3. Proteome Exploration Laboratory, California Institute of Technology, Pasadena, California, United States

4. Biochemistry, Duke University, Durham, North Carolina, United States

5. Molecular Genetics & Microbiology, Duke University, Durham, North Carolina, United States

Malaria was responsible for over 600,000 deaths in 2020, of which more than 90% was caused by the protozoan parasite *Plasmodium falciparum*. Artemisinin-based combination therapies (ACTs) target the blood stage of the parasite life cycle and serve as the frontline antimalarial treatment. However, the emergence of drug resistance threatens the efficacy of ACTs. Thus, there is an urgent need to better understand the mechanism of action of ACTs and to identify novel malaria drug targets. Potential, underexplored drug targets in *P. falciparum* are the enzymes that mediate ubiquitination, an essential post-translational modification. The covalent attachment of ubiquitin to substrate proteins is mediated by an enzymatic cascade consisting of a ubiquitin-activating (E1), conjugating (E2), and ligase (E3) enzyme. Interestingly, previous studies have shown that deletion of the E2 PfUbc13 increases parasite sensitivity to dihydroartemisinin, suggesting that PfUbc13 has a role in ACT antimalarial activity. PfUbc13 is an essential gene and a central mediator of Lys63-linked polyubiquitin (K63-Ub) chains, also implicating this specific modification in ACT activity. Hence, targeting PfUbc13 and its interactome serves as a novel avenue for combating malaria.

The compound NSC697923, a known covalent inhibitor of HsUbc13, was identified as a covalent inhibitor of PfUbc13. Similar degrees of thermal destabilization were observed when recombinant HsUbc13 and PfUbc13 were treated with NSC697923, whereas no thermal shift was observed with the mutant protein PfUbc13-C86S. Using high-resolution mass spectrometry, the mass of the PfUbc13 covalently modified Cys86-containing tryptic peptide (929.4952 kDa) was detected. Furthermore, dose-dependent NSC697923 inhibition of PfUbc13~Ub conjugate formation was observed in Ub transfer assays monitored by anti-Ub western blots. The K_i was determined to be 0.36 μM using a fluorescence polarization assay. The NSC697923 EC_{50} values against the parasite blood, liver, and sexual stages were determined to be 6.7, 7.8, <10 μM, respectively. Additionally, 64 putative PfUbc13 substrate proteins modified with K63-Ub were identified through differential bottom-up proteomics. Overall, this work has identified the first-known chemical probe of PfUbc13 and provided insight into the PfUbc13 interactome, broadly informing future investigations into the role of PfUbc13 and K63-Ub in the mechanism of ACTs and furthering malaria drug discovery efforts.

206

Novel bacterial mediators of neutrophil chemotaxis to *Acinetobacter baumannii*

Muhammad Ikhtear MIU Uddin[1], Faye FM Morris[1], Xenia XK Kostoulias[1], Vahid VP Pazhakh[2], Graham GL Lieschke[2, 3], Anton AYP Peleg[1, 4]

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

2. Australian Regenerative Medicine Institute, Monash University, Melbourne, Victoria, Australia

3. Department of Clinical Haematology, Peter MacCallum Cancer Centre and The Royal Melbourne Hospital, Melbourne, Victoria, Australia

4. Department of Infectious Diseases, The Alfred Hospital and Central Clinical School, Monash University, Melbourne, Victoria, Australia

Acinetobacter baumannii is a Gram-negative, opportunistic pathogen that has become widespread in clinical settings. The emergence of strains that are multi-, extreme- and pan-drug resistant has created a significant healthcare burden due to the limitation of treatment options available, leading the WHO to declare the development of new drugs to treat this pathogen a critical priority. The study of key virulence factors and their impact on the immune response may help to identify new targets for novel therapeutics.

It is established that neutrophils play crucial role in the defence against *A. baumannii* infections. However, the bacterial factors that modulate the process of neutrophil chemotaxis toward the site of infection remain largely unknown. To identify novel bacterial-derived neutrophil chemoattractants, an *A. baumannii* transposon mutant library of a contemporary clinical isolate, AB5075-UW was screened using a high throughout human neutrophil chemotaxis assay. A total of 942 *A. baumannii* mutants were screened, and of these, 24 and 23 mutants induced an increase and reduction in chemotaxis, respectively. Further *ex vivo* and *in vivo* validation assays, identified two mutants (*ddc*, ABUW_2564 and *nfa*, ABUW_3555) that resulted in reduced neutrophil chemotaxis and the phenotype was restored to wild type level by supplying the genes *in trans*. The gene *ddc* (ABUW_2564) encodes a putative D-alanyl-D-alanine carboxypeptidase (DDCpase), which is thought to involve in peptidoglycan (PG) maturation and recycling. PG is a potent stimulator of host immune system that activates a protective inflammatory response. It was postulated that in the absence of DDCpase, there is a decrease in PG, which in turn decreases neutrophil chemotaxis. The gene *nfa*, (ABUW_3555) encodes for putative purine and pyrimidine nucleoside phosphorylase (PpnP), an enzyme of the nucleoside salvage pathway. It was hypothesised that the absence of PpnP may alter nucleotide metabolism, which in turn may impact biosynthetic pathways requiring nucleotide triphosphates, such as PG biosynthesis. This study confirmed the role of two novel *A. baumannii* genes as drivers of neutrophil chemotaxis, with further biochemical characterisation being a key future direction.

207

Functional IL-37 receptor cell surface expression on immune cell subsets in health and disease by flow cytometry

Holly K Ung[1, 2], Steven X Cho[1, 2], Devi Ngo[1, 2], Jason C Lao[1, 2], Ina Rudloff[1, 2], Marcel F Nold[1, 2, 3], Claudia A Nold-Petry[1, 2]

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

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

3. Monash Newborn, Monash Children's Hospital, Melbourne, Victoria, Australia

Background: Interleukin (IL)-37 is a potent anti-inflammatory cytokine of the IL-1 family that can broadly suppress inflammation and immunity. Extracellularly, IL-37 signals via formation of a functional complex with the receptor proteins IL-1R8 and IL-1R5 on the cell surface to initiate its anti-inflammatory signaling cascade. However, the expression and regulation of the IL-37 receptor (IL-37R) and its effect on the intracellular mechanism of action of IL-37 are not fully understood.

Methods: Here we characterised IL-37R expression in peripheral leukocyte subsets such as lymphocytes, monocytes, and dendritic cells in human whole blood from healthy donors and patients with inflammatory conditions including systemic lupus erythematosus, rheumatoid arthritis, and respiratory, neurological, and gastrointestinal disease. We also investigated the stimuli that induce IL-37R and the response of IL-37R+ cells to extracellular recombinant IL-37 stimulation.

Results: We show that T cells are the major contributor to the total IL-37R+ population in healthy adult whole blood and that monocytes and dendritic cells are highly inducible for IL-37R. We also observed differences in IL-37R expression between health and disease contexts which has implications for assessing donor-specific responses to IL-37.

Conclusion: IL-37 is a strategic target for drug development because of its ability to broadly suppress inflammation. Our study identifies the effector cells expressing IL-37R and their regulation and provides information for specific drug-target interaction studies.

208

Repeat immunisations to SARS-CoV-2 progressively increase recognition of Spike RBD-specific memory B cells to Omicron subvariants

Gemma E. Hartley[1], Holly Fryer[1], Emily S.J. Edwards[1], Nirupama Varese[2], Irene Boo[3], Scott Bornheimer[4], P. Mark Hogarth[2], Heidi E. Drummer[3], Robyn E. O'Hehir[1, 5], Menno C. van Zelm[1, 5]

1. Immunology and Pathology, Monash University, Melbourne, VIC, Australia

2. Immune Therapies Group, Burnet Institute, Melbourne, VIC, Australia

3. Viral Entry and Vaccines Group, Burnet Institute, Melbourne, Victoria, Australia

4. BD Biosciences, San Jose, California, USA

5. Allergy, Asthma and Clinical Immunology, Alfred Health, Melbourne, VIC, Australia

Background:

SARS-CoV-2 vaccination coverage is increasing worldwide; however, emerging Omicron subvariants challenge its protection. Booster vaccinations improve protection against severe disease from Omicron, but the underlying mechanism is currently unknown. We assessed the capacity of vaccine-induced antibodies and circulating memory B cells (Bmem) to recognize SARS-CoV-2 Omicron subvariants.

Methods:

Recombinant Nucleocapsid protein (NCP) and Spike receptor binding domain (RBD) from Wuhan and Omicron BA.2 an BA.5 subvariants were produced for ELISA-based serology, and biotinylated for fluorescent tetramer formation to identify RBD-specific Bmem by flow cytometry. Healthy adults vaccinated with the Pfizer mRNA (n=30) or AstraZeneca vector (n=35) vaccinations were sampled before and 1 month after 1st and 2nd dose immunisation, and 3rd dose mRNA booster.

Results:

None of the participants carried IgG to NCP or RBD before vaccination. IgG to RBD progressively increased after 1st, 2nd and 3rd dose of vaccination. AstraZeneca vaccination induced significantly lower levels of RBD IgG than Pfizer vaccination. Bmem numbers after double-dose vaccination were similar for AstraZeneca and Pfizer groups. First dose Pfizer vaccination elicited RBD-specific Bmem, which expressed predominantly IgM or IgG1; the 2nd and 3rd doses expanded the IgG1^{+} Bmem numbers. The proportions of RBD-specific Bmem recognizing Omicron BA.2 or BA.5 increased from ~20% to >40% after 3 vaccine doses.

Conclusions:

Double-dose Pfizer mRNA and AstraZeneca vector vaccinations generate RBD-specific antibodies with significant loss in recognition of Omicron subvariants. The 2nd vaccine dose as well as 3rd dose mRNA boosters progressively increase the capacity of RBD-specific Bmem to recognize Omicron subvariants. This suggests that the additional exposures elicit affinity maturation of RBD-specific Bmem thereby overcoming mismatches in variant RBD. In ongoing studies, we will evaluate the impact of the bivalent 4th dose booster vaccine (Wuhan and Omicron BA.1) on Bmem recognition of Omicron subvariants.

209

Structural and functional characterisation of a novel gasdermin protein

Joshua von Ameln[2, 3, 1], Shuhua Chen[4], Nicole Oelerich[4], Kay Hofmann[4]

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

2. University of Melbourne, Melbourne, VIC, Australia

3. University of Bonn, Bonn, NRW, Germany

4. Institute for Genetics, University of Cologne, Cologne, NRW, Germany

Pyroptosis is a form of regulated cell death (RCD) that is characterised by a necrotic phenotype and the potential to initiate or amplify inflammation. It is engaged in the immune system as a response to defend against pathogenic invasion.

Gasdermins (GSDMs) are a protein family that are considered to be the main executioners of pyroptosis by their ability to form pores in the cell membrane. Especially GSDMD has been thoroughly characterised. Its function is mediated by an N-terminal pore forming domain (PFD) that, in the inactive state, is inhibited by the C-terminal repressor domain. Upon cleavage by inflammatory Caspases, free PFDs translocate to the membrane and form pores leading to cytokine release, membrane rupture and finally to inflammation of the surrounding tissue.

In contrast to GSDMD, other members of this protein family have not been thoroughly investigated and a variety of questions, regarding expression profiles, molecular mechanism and involvement in RCD, among others, remain unanswered.

Here, we present insights into the structural and functional properties of GSDMX, a hitherto uncharacterised protein, that is a potential novel GSDM family member.

We demonstrate that GSDMX conserves main structures of the GSDM family which are critical for membrane binding and pore forming activity. Furthermore, it was shown that the cleavage of GSDMX is induced by intracellular Ca^{2+} overload but not by Etoposide treatment. Interestingly, the PFD of GSDMX seems to be able to induce cell death in murine L929 cells but not in human HEK293T cells.

Results presented here support the hypothesis of GSDMX being a hitherto unrecognised member of the GSDM family, but also indicate that it might be activated in a molecular mechanism different to GSDMDs activation. Our results underline the gap of knowledge and understanding of most members of the GSDM family.

210

A novel role for interferon epsilon in preventing Zika virus induced testicular damage

Rukmali Wijayarathna[1, 2], Eveline de Geus[2, 3], Rosemary Genovese[1], Michelle Tate[2, 3], Paul Hertzog[2, 3], Mark 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 HIV, mumps, Zika and SARS, infect the testis and disrupt fertility. This has been attributed to an inability of spermatogenic cells to produce anti-viral interferons (IFN) or IFN-induced proteins. Challenging this dogma, we discovered that interferon-epsilon (IFNe), a type-I IFN first identified in female reproductive epithelia, is constitutively expressed by meiotic and post-meiotic spermatogenic cells and testicular macrophages in mice and humans. We investigated the anti-viral role of IFNe in the testis, using an established mouse model of Zika virus infection and a human Sertoli cell-line (HSerc, ScienCell^{TM}). Adult wildtype mice (WT), *Ifne*$^{-/-}$ mice lacking IFNe, and *Ifnar1*$^{-/-}$ mice lacking the IFNAR1 receptor subunit required for IFN-signalling, received a single intraperitoneal injection of Zika virus (PRVABC59, $5x10^5$ pfu in saline). Controls received saline only. Reproductive organs were collected 7 days post-infection (peak illness). Infected WT mice lacked histological evidence of orchitis or epididymitis, but infected *Ifne*$^{-/-}$ and *Ifnar1*$^{-/-}$ mice displayed testicular hyperaemia, oedema and immune cell infiltrates. The epididymis of infected *Ifne*$^{-/-}$ mice displayed immune cell infiltrates, epithelial damage, luminal obstruction and fibrosis. Expression of critical Leydig cell (*Cyp11a1*, *Cyp17a1*) and spermatid genes (*Tnp1*) was also reduced in infected *Ifne*$^{-/-}$ and *Ifnar1*$^{-/-}$ mice. The human Sertoli cell-line was infected with 5 or 10 MOI Zika virus, and treated with 100IU recombinant human IFNe either 12h before or 1h after infection. qPCR for viral RNA and plaque assays for infectious virus performed 24h post-infection showed that IFNe pre-treatment reduced the viral load by ~98%. Post-infection IFNe treatment reduced

viral RNA by ~70% and infectious virus by 97%. These data indicate that IFNe plays a key role in protecting the testis against Zika virus, shifting the existing paradigm of testicular anti-viral defences, and identifying IFNe as a potential therapeutic and diagnostic target in male reproductive tract infection and infertility.

211

Pilus tip protein AP1 essential for Group A *Streptococcus* adhesion to the tonsil epithelium

Alisha M Wilson[1], Jana Haasbroek[1], Jua Iwasaki[1], Jessica Hillas[1], Jacelyn M Loh[2], Anthony Kicic[1], Tim Barnett[1]

1. Telethon Kids Institute, Subiaco, WA, Australia

2. University of Auckland, Auckland, New Zealand

Group A *Streptococcus* (GAS) is an obligate human pathogen that causes a range of superficial, invasive, and autoimmune diseases, resulting in a significant burden on the healthcare system. The human palatine tonsils are the only known reservoir for GAS infections, and infection of the tonsils likely precedes severe GAS diseases. Prevention of GAS adherence to the tonsils is a promising approach to develop a safe and effective vaccine to prevent GAS infection and associated antibiotic use, and the severe consequences of these infections. However, mechanistic understanding of GAS adhesion is limited due to the use of immortalised cell lines and other experimental models that don't accurately model the healthy tonsil. This project established an *in vitro* system to study GAS attachment to the primary tonsil epithelium. We screened a library of isogenic GAS virulence factor mutants for the ability to attach to tonsil epithelial cells. The pilus tip protein AP1 was found to be essential for GAS adhesion to terminally-differentiated primary tonsil cells that constitute the surface of the palatine tonsils, yet was dispensable for adhesion to tonsil cells grown under conditions that model epithelial repair. We conclude that AP1 is likely essential for GAS attachment to the healthy tonsil epithelium and is a promising vaccine candidate for the production of antibodies that prevent the initial stem in GAS infection - attachment to the surface epithelium of the palatine tonsils.

212

Differentiation of human induced pluripotent stem cells to tissue-resident-like macrophages using a serum-free protocol

Le (Christy) Ying[2, 1], Hani Hosseini Far[1], Jamie Gearing[1], Jodee Gould[1], Paul J. Hertzog[2, 1]

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

Macrophages play a central role in innate immunity and homeostasis. The most common human macrophage models used are mainly monocyte-derived macrophages or immortalized macrophage cell lines such as THP-1. These cells are either difficult to access or have been transformed or have a different origin than tissue-specific macrophages. Here, we developed a feeder-free and serum-free protocol that differentiates induced pluripotent stem cells (iPSCs) into macrophages that resemble tissue-resident macrophages. The protocol includes induction of the primitive streak, generation of meso-endothelial cells and hemogenic endothelium, production of hematopoietic progenitor cells, and macrophages.

We successfully applied the protocol to five different iPSC lines, including Kolf2 iPSCs, IFNAR1 null, and IFNAR2 null iPSCs and their corresponding isogenic control iPSC lines. The IFNAR1 null and IFNAR2 null iPSCs are reprogrammed from primary immunodeficiency patients. The fully differentiated Kolf2 iPSC-derived macrophages exhibited human macrophage markers such as CD45, CD14, CD16, CD11b, HLA-DR, and CD163, and they also showed tissue-resident markers such as CD169, CD71, and CD11c by flow cytometric analyses. A PCA plot from the Myeloid Atlas on the Stemformatics website indicated that the Kolf2 iPSC-derived macrophages are very similar to colon and lung macrophages. Moreover, the Kolf2 iPSC-derived macrophages show a potent phagocytic ability and cytokine production upon LPS stimulation. The responses of Kolf2 iPSC-derived macrophages to cytokines, pathogens, commensals, and synthetic ligands are currently being analysed by transcriptomics.

Establishing this feeder-free and serum-free protocol for iPSC macrophage differentiation provides a powerful tool to study macrophage signalling in innate immunity with the advantages of unlimited expansion *in vitro*, the potential to be genetically manipulated and polarised to organ-specific macrophages, which recapitulate the features of human primary macrophages. The successful application of this protocol in various iPSC lines that have been reprogrammed from primary immunodeficiency patients allows us to investigate the mechanisms of key components in innate immunity.

213

Kinetics of the innate antiviral response of equine peripheral mononuclear cells in response to Ross River virus

Ka Yuen[1], Lee McMichael[1], Mitchell Coyle[2], Helle Bielefeldt-Ohmann[3, 4]

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

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

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

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

Introduction

When bitten by an arbovirus-infected mosquito, peripheral blood mononuclear cells (PBMCs) are amongst the first cells to encounter the virus and may be vital for the development of antiviral activity. Ross River virus (RRV) infection in horses and humans is often subclinical, however, some individuals experience chronic debilitating arthritogenic signs. Some studies suggest that monocytes may contribute to the chronicity of the disease. However, little is known regarding the interactions between RRV and PBMCs in acute infection.

Methods

EDTA-stabilised blood samples were collected from nine RRV seronegative horses and PBMCs isolated using Ficoll-Histopaque, and the cells challenged with RRV (MOI = 1). Cells were harvested at 6 and 24 hours, and RNA was purified, cDNA synthesised, and qPCR performed for selected inflammatory biomarkers to quantify gene-expression. Results were expressed as means of fold changes (compared to control) ± SEM.

Results

Toll-like receptor 7 (TLR-7) was slightly upregulated from 0.9 ± 0.1 to 1.3 ± 0.2 over time. Interleukin (IL) 6 and 8 were both upregulated over time, from 1.4 ± 0.4 to 13.1 ± 11.7 and from 1.3 ± 0.1 to 2.5 ± 0.9, respectively. Interestingly, there was minimal change in interferon (IFN)-α gene expression, whereas, IFN-γ was markedly upregulated from 2.3 ± 0.6 to 12.9 ± 8.8. Monocyte chemoattractant protein (MCP) 1 was down-regulated from 1.4 ± 0.1 to 0.9 ± 0.2, while MCP-2 was unchanged.

Conclusion

Up-regulation of TLR-7 suggest that PBMCs are involved in early viral recognition and elicit an antiviral response via ILs and IFN-γ, when encountering RRV, to control viral replication and inflammation. The role of MCPs in the pathogenesis of RRV may be minimal. More studies are underway to elucidate the equine innate immune response to RRV.

INDEX OF ABSTRACT AUTHORS