LORNE INFECTION & IMMUNITY VIRTUAL CONFERENCE

17 - 19 February

2021

www.lorneinfectionimmunity.org

CONTENTS

WELCOME 2
ORGANISING COMMITTEE 2021 4
INVITED SPEAKERS 5
SPONSORS 2021 11
DELEGATE INFORMATION 12
- THE ORGANISERS – ASN EVENTS 12
- WHAT YOUR REGISTRATION INCLUDES 12
- VIRTUAL PLATFORM 12
- DISPLAYING YOUR POSTER 12
- STUDENT & EARLY CAREER RESEARCHER FUNCTION: Coffee with the Professor 12
- EXHIBITOR PRIZES 13
- SPECIAL ISSUE OF THE JOURNAL OF LEUKOCYTE BIOLOGY 13

SPONSOR AND EXHIBITOR LISTING 14
PROGRAM 16
- e-POSTER LISTING 27

ORAL ABSTRACTS 35
SCIENCE BITES ABSTRACTS 55
POSTER ABSTRACTS 63
INDEX OF ABSTRACT AUTHORS 108

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WELCOME

Dear Colleagues and Friends,

On behalf of the organising committee, we welcome you to our 11th Lorne Infection & Immunity conference and, through necessity, the first 'virtual' one!

Only a year ago, at the 2020 Lorne Infection and Immunity Conference, we discussed SARS-CoV-2 as an emerging global threat. Since that time, we have seen the world's first respiratory pandemic in over 100 years, extraordinary public health efforts to contain COVID-19 and a massive global research effort into disease pathogenesis, with new antiviral drugs and vaccines developed, approved and administered into millions of people in record-breaking time. We can be proud of our collective sacrifices to eliminate COVID-19 from our community, as well as the world-leading efforts by our colleagues to understand and combat SARS-CoV-2. Talk about infection & immunity in action!

In this year's program, we feature a cross-section of the exemplary, responsive research that arose in Australia and around the world to combat this novel virus. As always, there are outstanding presentations on the many other infectious and inflammatory diseases, immunological problems and microbiological questions that our scientific community endeavours to address. In face of the almost overwhelming challenges posed by the current pandemic, remember that the innovative discovery research you are doing today, will form the foundation for therapies, diagnostics and preventative measures necessary to combatting other disease challenges in the future.

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 acknowledging emerging trends in our field and by providing focus on particular topics from year to year. 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. Our program and activities will continue to evolve in new and exciting directions and we always welcome your feedback and suggestions.

We are delighted by the 11 years of support we've received from delegates, presenters of all kinds, our invited guests, conference committees, support teams, sponsors and exhibitors. This has been crucial in enabling us to establish this conference as a fixture in the scientific calendar, joining other Lorne Conferences, some of which have been running over 40+ years.

This year, we extend a special welcome to our international speakers, joining us from disparate time zones, and thank them for contributing to the high calibre of this conference. We hope you get the opportunity to visit Lorne in person in the future. We encourage all attendees to engage with them by asking questions through the chat function in each session and for students and ECRs to meet our invited speakers at Thursday's virtual coffee-break to '*Meet the Professor*'.

We are 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 virtual exhibition booths, attending the morning symposia on Thursday and Friday and by buying their quality products and services for your research. We especially thank our major sponsor CSL Ltd, a local-turned international pharma company, who have loyally supported this conference for all 11 years and whose

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capabilities and leadership in Australia have enabled us to produce the University of Oxford / AstraZeneca COVID-19 vaccine.

It is important to acknowledge the significant contributions made by the organising committee to eleven years of successful Lorne Infection and Immunity conferences. It has been a pleasure to work with knowledgeable, engaged and generous committee members. This year, the organising committee welcomes new members Antje Blumenthal from The University of Queensland Diamantina Institute, Michelle Boyle from QIMR Berghofer Medical Research Institute, Andrew Currie from Murdoch University, Phil Hansbro from Centenary Institute and associated organisations noted below, and Nikki Moreland from The University of Auckland. The committee extends special thanks to our Program Manager, Rebecca Smith, and Gemma-Ann Taylor, Jim Fawcett, Nitesh Patel and colleagues at ASN, without whom these conferences would not run so efficiently.

We have always been proudly and strongly committed to gender equity at the Lorne Infection and Immunity Conference and our track record on this can be viewed on our website. Please join us on Friday of the program for a special session by Prof Akiko Iwasaki on this topic. The organising committee will continue to work to find ways to make this meeting as diverse, accessible, relevant and enjoyable as possible, for all participants.

We encourage you to acquaint yourself with and make full use of the interactive features of our virtual platform, to engage with speakers, sponsors and each other to make this a rewarding event.
Until 2022, when we hope to 'see' you again at the beach in Lorne.

With best wishes,

Paul Hertzog
Co-Convenor

Heidi Drummer
Co-Convenor

On behalf of the Organising Committee

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Justine Mintern, University of Melbourne
Nicole Moreland, The University of Auckland
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Rebecca Smith, Victorian Infection and Immunity Network
Cameron Stewart, CSIRO Australian Animal Health Laboratory
Ana Traven, Monash University
Linfa Wang, Duke-NUS Medical School, Singapore

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INTERNATIONAL INVITED SPEAKERS

Prof Leah Cowen, *University of Toronto, Canada*

Leah E. Cowen, Ph.D., is Professor and Chair of the Department of Molecular Genetics at the University of Toronto, and co-Founder and Chief Scientific Officer of Bright Angel Therapeutics, a company that leverages state-of-the-art technologies for development of novel antifungal therapeutics. She has been appointed the University of Toronto's first Associate Vice-President, Research, a position that she will begin on March 1st, 2021. She received her undergraduate degree from the University of British Columbia, a PhD from the University of Toronto, and pursued postdoctoral studies at the Whitehead Institute, Massachusetts Institute of Technology. Her laboratory takes an interdisciplinary approach to understand what allows some microbes to exploit the host and cause disease, and to develop new strategies to treat life-threatening infectious disease. Dr. Cowen has an outstanding track record of excellence in research, scholarship, and education. She has published over 100 high impact research articles. She has been recognized with a myriad of awards including a Burroughs Wellcome Fund Career Award, Grand Challenges Canada Star in Global Health Award, Merck Irving S. Sigal Memorial Award, E.W.R. Steacie Award, and Canada Research Chair in Microbial Genomics & Infectious Disease (Tier I and Tier II). She has been elected as Fellow of the American Academy of Microbiology and Fellow of the American Association for the Advancement of Science. Dr. Cowen has cultivated an international network of excellence as co-Director of the CIFAR Fungal Kingdom: Threats & Opportunities program and is advancing knowledge translation as Chief Scientific Officer of Bright Angel Therapeutics.

Dr Kate Fitzgerald, *University of Massachusetts Medical School, United States of America*

Dr. Fitzgerald is Professor of Medicinem Vice Chair of the Department of. Medicine and. Director of the Program in Innate Immunity at the University of Massachusetts Medical School. She is also and President of the International Cytokine and Interferon Society.

Dr. Fitzgerald directs an internationally recognized laboratory focused on understanding the molecular mechanisms controlling the inflammatory response in both health and disease. Her group is interested in determining how the immune system distinguishes friend from foe to protect the host from infection and avoid damaging inflammatory diseases. She has been continuously funded by the NIH since 2004 and is also funded by several foundations and pharmaceutical companies.

Dr. Fitzgerald is a member of the Royal Irish Academy, a fellow of the American Academy of Microbiology and has received numerous awards. She also serves as a scientific advisor for several biotech companies.

Prof Tracy Hussell, *Lydia Becker Institute of Immunology and Inflammation, Manchester University, United Kingdom*

Tracy Hussell completed her PhD at University College London where she identified Helicobacter pylori as an aetiological agent in human gut lymphomas. After her PhD Professor Hussell moved to Respiratory Medicine at St Mary's Hospital to study immunity and pathology to respiratory syncytial virus. In 1998 she accepted a lectureship in the Centre for Molecular Microbiology and Infection (CMMI) at Imperial College led by Professors Gordon Dougan and Douglas Young. She was subsequently awarded a career development fellowship by the Medical Research Council. Professor Hussell was awarded a Personal Chair in inflammatory disease at Imperial College London in 2006 and developed a

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vibrant research group studying immune health and its deregulation in the lung. In 2012 Professor Hussell moved to The University of Manchester to be the Director of the Manchester Collaborative Centre for Inflammation Research (MCCIR). Professor Hussell also currently leads the Lydia Becker Institute of Immunology and Inflammation while continuing her research on immunological homeostasis on the respiratory tract and how this alters in acute and chronic inflammatory conditions.

Dr Aaron Irving, *Zhejiang University School of Medicine, China*

Dr Aaron Irving trained at Monash Institute of Medical Research, Melbourne and Emerging Infectious Diseases, Duke-NUS Medial School, Singapore. He recently opened his lab at ZJE, China with a focus on emerging zoonotic viral infections and the host-pathogen interactions triggered by these viruses. There is a special interest in comparative biology using bats as a model organism and identifying key factors from bats that may be utilized to improve the human immune responses to pathogen infections. Other projects in the laboratory include novel diagnostic techniques to identify current or historical infection of wildlife by zoonotic viruses, including SARS-CoV-2, and establishing/evaluating factors that may contribute to zoonotic potential.

Prof Akiko Iwasaki, *Yale University School of Medicine; Howard Hughes Medical Institute,*

United States of America

Professor Akiko Iwasaki has made major discoveries in innate anti-viral and mucosal immunity that have resulted in paradigm shifts in the understanding of the immune response to pathogens as well as in vaccine design. Her research focuses on the mechanisms of immune defense against viruses at mucosal surfaces, which are a major site of entry for infectious agents. The knowledge gained in her lab can be used to design more effective vaccines or microbicides to prevent transmission of viral and bacterial pathogens.

Professor Iwasaki's research group developed a new vaccine strategy, termed "Prime and Pull", that can be used to treat those infected with virus, unlike many vaccines that are given preventatively. This method is currently under phase 2 clinical trials for the treatment of high-grade cervical lesions caused by infection human papillomavirus (HPV).

Professor Iwasaki received her Ph.D. in Immunology from the University of Toronto and completed her postdoctoral training with the National Institutes of Health before joining Yale's faculty in 2000. She has received numerous awards and honors, including the Burroughs Wellcome Fund Career Award in Biomedical Sciences, the Wyeth Lederle Young Investigator Award, the BD Biosciences Investigator Award, and the Seymour & Vivian Milstein Award for Excellence in Interferon and Cytokine Research. Professor Iwasaki has been a Howard Hughes Medical Institute Investigator since 2014, a prestigious honor that provides the researcher long-term, flexible funding that gives them to freedom to explore new avenues of research. She was elected to the National Academy of Sciences in 2018, and to the National Academy of Medicine in 2019. Dr. Iwasaki is also well known for her Twitter advocacy on women and underrepresented minority in the science and medicine fields.

Currently, Professor Iwasaki is directing translational immunology team to investigate the role of immune response in COVID-19 disease outcome. She also co-directs the IMPACT (Implementing medical and public health actions against coronavirus in Connecticut) team to generate an extensive biorepository for specimens collected from patients and health care workers, as well as implementing viral testing in both groups.

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Dr Elizabeth Mann, *University of Manchester, United Kingdom*

Prof Dana Philpott, *University of Toronto, Canada*
Dana Philpott is a Professor in the Department of Immunology at the University of Toronto and co-director of the Host-Microbiome Research Network, where she has established the first gnotobiotic mouse facility in Toronto. Dr. Philpott's research employs animal models of inflammatory bowel disease (IBD) and considers how innate immunity and the microbiome shape immune homeostasis within the intestine. Specifically, her group studies three genes implicated in the pathogenesis of the IBD, Crohn's disease. This disease can affect the entire gastrointestinal tract and the chronic inflammation that ensues can put individuals at risk for developing colon cancer. Current treatment strategies, which include steroids, immunomodulatory drugs, and anti-TNFa biologics, calm the inflammatory response but do not cure CD. Continued basic research is needed to define disease mechanisms to uncover new targets for therapy and find a cure for this increasingly prevalent chronic disorder.

Dr Nassos Typas, *European Molecular Biology Laboratory, Germany*
Athanasios (Nassos) Typas is a trained biochemist, geneticist, and systems biologist. He leads a group at the Genome Biology Unit at EMBL, Heidelberg, Germany since 2011. His group combines systems microbiology with molecular mechanism to study bacterial cellular networks, and how bacteria interact with each other, the environment and the host. A key focal area of the group is on drug-microbe interactions: identifying new therapeutic strategies, understanding and predicting the drug mode of action and cellular resistance potential, and dissecting the interplay of medication with the gut microbiome. Nassos has received a number of awards (NIH K99/R00, Sofja Kovalevskaja Award- Humboldt Foundation, ERC consolidator grant) and is a member of the European Academy of Microbiology.

Dr Marit Vangils, *Amsterdam Umc, University of Amsterdam, Netherlands*
Marit van Gils (1982) studied medical pharmaceutical sciences at the University of Groningen, the Netherlands. She has performed her Ph.D. research (2007-2011) at the University of Amsterdam in the lab of Dr. Schuitemaker on the humoral immune response after HIV-1 infection. She continued her career as a postdoctoral fellow in the lab of Dr. Sanders working on the development of an HIV protein vaccine at the Academic Medical Center in Amsterdam, the Netherlands. Marit has also performed part of her postdoctoral research at the Scripps Research Institute in the lab of Dr. Burton (2013-2016) and in 2017 started her own research group at the Amsterdam UMC, the Netherlands, studying the antibody and B cell responses after infection and vaccination, including HIV-1, Influenza and SARS-CoV-2.

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NATIONAL INVITED SPEAKERS

Prof Gabrielle Belz, *University of Queensland, QLD*
Professor Gabrielle Belz trained in veterinary medicine and surgery from the University of Queensland in 1993 and has made major contributions to the field of immunology for which she received a DVSc. Her prime research interests are in the areas of infectious disease, particularly lung and gut diseases, where she works to unravel how protective innate and adaptive immune cells are wired to generate long-live protective memory. She has published over 200 peer-reviewed original papers in leading scientific journals, an H-index of 75, and has been cited > 21,000 times. She is currently the Chair of Immunology, University of Queensland Diamantina Institute.

Prof Warwick Britton, *Centenary Institute and Nhmrc Centre, NSW*
Warwick Britton is head of the Tuberculosis Research Program at the Centenary Institute and Emeritus Professor at the University of Sydney. He has longstanding interests in the immunology of mycobacterial infections, including the balance of protective immunity and inflammatory damage in the lung, and the development novel vaccines and drugs against TB. He contributes to ongoing research to improve the control of tuberculosis and leprosy globally. He is principal investigator on the NHMRC-funded Centre for Research Excellence in Tuberculosis Control on both sides of the border that promotes TB research, collaboration, training and translation within Australia and the Asia-Pacific, including Vietnam, Indonesia and Papua New Guinea. He is an investigator on the new MRFF-funded PEARL project, Pathway to Elimination of Anti-microbial Resistant and Latent Tuberculosis in the Pacific.

A/Prof Keith Chappell, *University of Queensland, QLD*
A/Prof Keith Chappell is a co-leader, together with Prof Paul Young, of the University of Queensland program to produce a vaccine for COVID-19. Keith is a Molecular Virologist whose research has focused primarily on vaccine development and is one of the inventors of a UQ's molecular clamp platform. Keith has played a leading role in designing and implementing an epidemic response vaccine pipeline which enabled the progression of UQ's COVID-19 vaccine candidate from sequence information to clinical trial dosing within 6months.

Prof Tania De Koning-Ward, *Deakin University, VIC*
Tania de Koning-Ward is a NHMRC Senior Research Fellow and Professor of Molecular Microbiology in the Institute of Mental and Physical Health and Clinical Translation at Deakin University. Here she heads a vibrant research team, with the main focus of her research being the dissection of key interactions that occur between malaria parasites and their host cells. Her laboratory uses the most cutting-edge approaches to genetically engineer human and rodent malaria parasites. Phenotype characterisation of these transgenic parasites, including in in vivo malaria infection models, at a molecular, cellular and biochemical level enable a deeper understanding of how malaria parasites secure their survival inside their host and cause disease. The overarching aim of Tania's research is to use this knowledge to rationally identify new targets for malaria vaccine and drug development.

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Dr Jennifer Juno, *University of Melbourne, VIC*
Dr. Jennifer Juno is an early career researcher at the Peter Doherty Institute for Infection and Immunity with a strong interest in T cell immunology in the context of infectious disease. She completed her PhD in HIV immunology in 2014 at the University of Manitoba in Canada, where she defined the impact of HIV disease progression on the depletion and exhaustion of unconventional T cells. She expanded this work into studies of HIV/TB co-infection during a post-doctoral fellowship with the Public Health Agency of Canada. In 2016, she moved to Melbourne, Australia where she has developed an interest in understanding how T follicular helper cells can influence serological responses to vaccination and infection. Many of her studies focus on influenza vaccines, and more recently, SARS-CoV-2. She has received numerous fellowships including a Canadian Institutes of Health Research post-doctoral fellowship and a NHMRC Early Career Fellowship.

A/Prof Maria Liaskos, *La Trobe University, VIC*
Maria Kaparakis-Liaskos is an Associate Professor in the Department of Physiology, Anatomy and Microbiology at La Trobe University, and Deputy Director of the Research Centre for Extracellular Vesicles. Her primary research interests aim to understand the cellular and molecular mechanisms whereby bacteria and their products modulate host immunity, with particular focus on Helicobacter pylori and bacterial membrane vesicles.
Maria obtained her PhD in Microbiology and Immunology from The University of Melbourne, undertook postdoctoral studies at Monash University and then established a research group at the Hudson Institute of Medical Research. In 2017, Maria moved to La Trobe University where she currently leads the Bacterial Membrane Vesicles and Host-Pathogen Interactions laboratory.

Dr Mike Mcdonald, *School of Biological Sciences, Monash University, ViIC*
Mike completed his Ph.D. in Evolutionary Genetics at Massy University in New Zealand in 2009 and then took up a Distinguished Postdoctoral fellowship at the Institute of Molecular Biology at Academia Sinica in Taiwan. In 2012 Mike moved to the USA to take up a position as a postdoc, in OEB at Harvard University. Mike started a research group in the School of Biological Sciences at Monash University in 2016. Mike and his group grow populations of bacteriophage, bacteria, and fungi for 1000s of generations in a variety of environments and use the tools of whole-genome sequencing, statistics, and genetic engineering to observe evolution as it happens. In particular, they are interested in how horizontal gene transfer and coevolution changes the rules of evolution in prokaryotes and contributes to the evolution of antibiotic resistance.

A/Prof Zoe Mcquilten, *Monash University, VIC*
A/Prof McQuilten is a consultant haematologist at Monash Health and a NHMRC Emerging Leadership Fellow. She is the Deputy-Director of the Transfusion Research Unit at Monash University and a senior research fellow with the Australian and New Zealand Intensive Care Research Centre (ANZIC-RC). Her other appointments include Chair of the Supportive Care Group for the Australasian Leukaemia and Lymphoma Group and she is an Associate Editor for Transfusion. She leads a research program focused on interventions to improve supportive care and transfusion in areas of major blood use, including haematological malignancies, critical care and trauma.

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Dr Lindi Masson, *Burnet Institute, VIC*
Lindi joined the Burnet Institute in 2020 as a Senior Research Fellow and head of the HIV, Inflammation and Microbiome Working Group. She also continues to lead a research group in the Department of Pathology at the University of Cape Town (UCT), is an Associate Member of the Institute of Infectious Disease and Molecular Medicine at UCT, Honorary Scientist at the Centre for the AIDS Programme of Research in South Africa (CAPRISA) and Adjunct Senior Lecturer at Monash University. Lindi has been involved in HIV and genital microbiome and immunology research for over 10 years. She completed her Ph.D. in Medical Virology at UCT in 2012, followed by a two-year postdoctoral fellowship, also at UCT. She is currently leading or co-leading two clinical cohort studies and a translational research study in South Africa. The primary focus of this research is to understand the socio-behavioural and biological factors associated with HIV acquisition risk in women and to develop tools to reduce this risk.

Prof Matt Sweet, *University of Queensland, QLD*
Matt Sweet is a Group Leader at the Institute for Molecular Bioscience (IMB). He completed his PhD under the supervision of David Hume in 1996 at The University of Queensland, Australia. He then undertook an NHMRC CJ Martin post-doctoral training fellowship at the University of Glasgow (Scotland, UK) in the laboratory of Eddy Liew. After returning to Australia, he had a number of key roles within the Cooperative Research Centre for Chronic Inflammatory Diseases, before establishing his own group at the IMB in 2007. His laboratory studies the innate immune system, with an emphasis on the roles of pattern recognition receptors, their signaling components and their downstream target genes in regulating both infectious and inflammatory disease processes. His recent work has focused on immune cell metabolism in the context of inflammation and host defence.

Prof Jamie Triccas, *University of Sydney, NSW*
Jamie Triccas is Director of the Infection, Immunity and Inflammation Program in the School of Medical Sciences, University of Sydney. He is a bacteriologist who uses a multidisciplinary approach to define immunity to chronic bacterial pathogens and develop new treatments to control infection. His group has progressed tuberculosis vaccines from the initial discovery phase (e.g. antigen discovery) to the development of products that are being prepared for clinical trials. He has also established platforms for the detailed assessment of vaccine-induced immunity, which have been used as part of an international TB vaccine consortium (TBVAC2020), to aid clinical progression of live TB vaccine candidates. He has also established vaccine programs for important Cystic Fibrosis pathogens, in particular Pseudomonas aeruginosa and Mycobacterium abscesses. In recent years he has diversified his research program to include drug discovery and development for important human pathogens, involving a large network of national and international collaborators, with a focus on TB.

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WHAT YOUR REGISTRATION INCLUDES

The Delegate and Student registrations include:

- Access to the sessions of your choice
- Access to presentations and conference content for 12 months post conference
- Live chat function enabling interaction with speakers, delegates and exhibitors

VIRTUAL PLATFORM

Log in information for the Virtual Platform, Pheedloop will be sent to the email address registered with. If it is not in your inbox, please check the junk box.

The Virtual Platform will give you opportunity to view all sessions live, visit with exhibitors, view e-Posters and network with other delegates, speakers and sponsors.

To learn more about how to maximise your virtual platform, go to https://www.lorneinfectionimmunity.org/virtual-platform-information

DISPLAYING YOUR POSTER

You will be able to converse with delegates and answer any questions through the chat functions within the virtual platform. Delegates will post questions in the Public Chat on the right-hand side of your e-Poster Presentation page on the virtual platform. They also have the option to start a private chat with you during the conference. Please make sure that you refer back to your e-Poster page to answer any questions that are asked.

All presentations (unless permission is withheld) for the conference will displayed on the online platform for up to 12 months post meeting.

STUDENT & EARLY CAREER RESEARCHER FUNCTION: Coffee with the Professor

Thursday 18th February, 11:15am – 12:15pm

An opportunity for ECRs to join Invited Speakers with a virtual coffee. There will be a Zoom link provided on the Virtual Platform for you to join.

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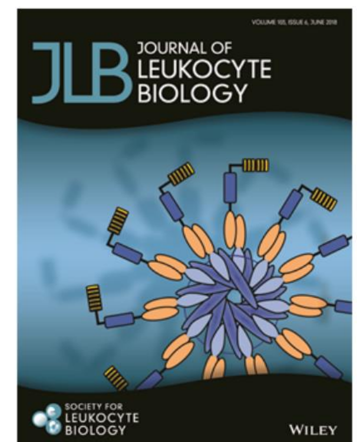
EXHIBITOR PRIZES

Interested in winning a prize? At the conclusion of the conference, lucky delegates who maximise their engagement with our virtual exhibitors will win one of many prizes! To enter, check out the virtual booths and interact with all of our exhibitors. Prizes include:

- Abcam - One free antibody or kit of your choice, to the value of $650 AUD
- BMG Labtech - $100 Providoor gift voucher
- Genesearch - $50 ColesMyer voucher
- Mimotopes - $100 voucher for their online store
- MP Bio – 30% discount voucher + $150 JB-HiFi gift card
- Sapphire Bioscience - $50 Westfield gift card
- Scientifix – Portable Carry Case with PPE Essentials
- Solve Scientific - $100 Dan Murphy's voucher
- Transnetyx - $75 Amazon gift card

Please note that by visiting the virtual booths, contact details from your online profile will be available to the sponsor.

SPECIAL ISSUE OF THE JOURNAL OF LEUKOCYTE BIOLOGY



JLB Dedicated Issue

Be part of a special issue of JLB focused on the meeting! Get a flyer at the information desk or go online to https://slb.memberclicks.net/lorne-infection-and-immunity-2021---jlb-special-issue to learn more. Submit by May 17, 2021!

Breaking news! EXTENDED TO MAY 31, 2021!

Contact jlbstaff@leukocytebiology.org with questions.

Be sure to indicate your submission is from Lorne 2021 when submitting!

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CSL is headquartered in Melbourne, Australia. Our global R&D activities support our existing licensed products and development of new therapies that align with our technical and commercial capabilities in Immunology and Neurology, Haematology and Thrombosis, Transplant, Respiratory, Cardiovascular and Metabolic. Over the last five years CSL has invested more than USD$2.6 billion in research and development and employs more than 1,400 people in R&D.

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Find out more at www.wehi.edu.au

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Website: www.abcam.com

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Genesearch is home of the e•Freezer and Australian distributor of Cell Signaling Technology antibodies; PerkinElmer/Cisbio HTRF assays; Thompson filter vials and flasks; GENEWIZ synthesis and NGS services; Preomics sample prep kits; Promise Proteomics; Hello Bio small molecules; Geneware plasticware, and New England Biolabs molecular biology reagents.

Mimotopes Pty Ltd **Virtual Exhibitor**

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Website: www.scientifix.com.au

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Website: www.solvescientific.com.au

Solve Scientific represents FortéBio's (Sartorius) Octet and Pioneer instrument platforms provide label-free kinetics for characterising disease targets and drug interactions based on BLI and SPR.

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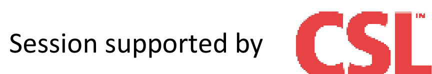
PROGRAM

Wednesday 17th February 2021

Session 1 - Welcome Address and Plenary

11:00AM - 12:45PM
Chair: Paul Hertzog, *Hudson Institute of Medical Research*



11:00 AM Welcome Address by **Paul Hertzog**, *Hudson Institute of Medical Research*

11:10 AM **Akiko Iwasaki,** *Yale University School of Medicine, Howard Hughes Medical Institute*
Immune response to SARS-COV-2

abs# 1

12:00 PM **Niall D Geoghegan**, *The Walter & Eliza Hall Institute*
Quantitative 4D microscopy of *P.falciparum* invasion of erythrocytes

abs# 2

12:15 PM **Marios Koutsakos**, *Peter Doherty Institute, University of Melbourne*
Integrated immune dynamics define correlates of COVID-19 severity and antibody responses

abs# 3

12:30 PM **Melanie Neeland**, *Murdoch Children's Research Institute*
Innate cell profiles during the acute and convalescent phase of SARS-CoV-2 infection in children

abs# 4

Virtual Break

12:45PM - 1:15PM

Session 2 - Viruses and their hosts 1

1:15PM - 2:30PM
Chair: Gregory Moseley, *Monash University*

1:15 PM **Aaron Irving,** *Zhejiang University - University of Edinburgh Institute*
Bats as reservoirs of zoonotic viruses and SARS-Like viruses

abs# 5

1:45 PM **Joshua A Hayward,** *Burnet Institute*
Infectious KoRV-related retroviruses circulating in Australian bats

abs# 6

2:00 PM **Ebony A Monson,** *La Trobe University*
Analysis of the dynamics and composition of lipid droplets during an antiviral response

abs# 7

2:15 PM **Hannah Sloane,** *Monash University*
CD4+ T cell recognition of haemagglutinin epitopes across different influenza strains

abs# 8

The conference acknowledges CSL

Virtual Break

2:30PM - 3:00PM

Session 3 - COVID-19 research and development in Australia

3:00PM - 4:50PM
Chair: Cameron Stewart, *CSIRO*

3:00 PM **Jennifer Juno,** *University of Melbourne*
CD4 T cell responses to SARS-CoV-2 infection and vaccination
abs# 9

3:20 PM **Zoe McQuilten,** *Monash University*
Convalescent plasma for COVID-19
abs# 10

4:00 PM **Jamie Triccas,** *The University of Sydney*
The rebirth of BCG in the era of COVID-19
abs# 12

4:20 PM **Stephanie Gras,** *La Trobe Univeristy*
Characterization of SARS-CoV-2 peptides presented by Human Leukocyte Antigen molecules
abs# 13

4:35 PM **Huy Van,** *Burnet Institute*
Dimeric IgA as a biomarker of incident SARS-CoV-2 infection
abs# 14

Virtual Break

4:50PM - 5:40PM

Session 4 - Microbiota and the immune system

5:40PM - 7:00PM
Chair: Philip Hansbro, *Centenary Institute*

5:40 PM **Annabell Bachem,** *University of Melbourne*
Microbiota-derived butyrate promotes metabolism and memory potential of effector $CD8^+$ T cells
abs# 15

5:55 PM **Lucille C Rankin,** *Walter and Eliza Hall Institute of Medical Research*
Dietary Trp deficiency promotes gut $Rorgt^+$ T_{regs} cells at the expense of $Gata3^+$ T_{regs} cells and alters commensal microbiota metabolism.
abs# 16

6:10 PM **Lindi Masson,** *Burnet Institute*
Microbial function and genital inflammation in young South African women at high risk of HIV infection
abs# 17

6:30 PM **Elizabeth Mann,** *University of Manchester*
Modulation of mucosal macrophage function by the gut microbiota
abs# 18

The conference acknowledges CSL

Thursday 18th February 2021

Session 5 - Perkin Elmer Morning Symposium

7:30AM - 8:30AM

High-throughput screening for drug and biomarker discovery: the present and the future

Speaker: Dr Amee George & Shima Hamidi

Dr Amee George is a Research Fellow in the ACRF Department of Cancer Biology and Therapeutics and heads the ANU Centre for Therapeutic Discovery (ACTD), at the John Curtin School of Medical Research at The Australian National University in Canberra, Australia. She has a keen interest in high-throughput screening and the use of functional genomics-based approaches to investigate the molecular basis of disease. She leads screening-based research investigating diseases of the ribosome (ribosomopathies), in particular, the congenital bone marrow failure disorder Diamond Blackfan Anaemia (DBA), receiving funding from the Australian National Health and Medical Research Council (NHMRC), the Captain Courageous Foundation and Maddie Riewoldt's Vision. Dr George also collaborates with national and international researchers from diverse medical research areas who wish to undertake drug and biomarker discovery projects.

This presentation will provide a broad overview of the technology and approaches for high-throughput screening, as well as provide some short vignettes into screening-related research. New screening approaches and examples for how this technology could assist in the infection and immunity research field will also be discussed.

This conference acknowledges the support of

Virtual Break

8:30AM - 9:00AM

Session 6 - Host pathogen interactions 1

9:00AM - 9:40AM
Chairs: Darren Creek, *Monash University* & Michelle Boyle, *Menzies School of Health Research*

9:00 AM **Warwick Britton,** *Tuberculosis Research Program, Centenary Institute and NHMRC Centre of Research Excellence in Tuberculosis Control*
Pulmonary vaccines against Tuberculosis - effective and feasible

abs# 19

9:20 AM **Tania de Koning-Ward ,** *Deakin University*
How malaria parasites gain access to nutrients to facilitate their survival

abs# 20

Session 7 - Host pathogen interactions 2 - Concurrent session

9:40AM - 10:45AM
Chairs: Darren Creek, *Monash University* & Amy Baxter*, La Trobe Institute for Molecular Science*

9:40 AM **Mark A. T. Blaskovich,** *University of Queensland*
OPX: A new class of antibiotics for drug-resistant Gram-negative infections

abs# 21

The conference acknowledges CSL

9:55 AM **David MP De Oliveira,** *University of Queensland*
Repurposing a neurodegenerative drug to treat Gram-negative antibiotic resistant bacterial infection

abs# 22

10:10 AM **Cristina Giogha,** *Hudson Institute of Medical Research*
A unique glycosyltransferase effector from enteropathogenic *Escherichia coli* that targets innate immune signalling proteins

abs# 23

10:25 AM **Marina Zupan,** *University of Melbourne*
The molecular basis for zinc uptake via *Streptococcus pneumoniae* AdcAII

abs# 24

Session 8 - Host pathogen interactions 3 - Concurrent session

9:40AM - 10:45AM
Chair: Michelle Boyle, *Menzies School of Health Research*

9:40 AM **Rhea Longley,** *Walter and Eliza Hall Institute of Medical Research*
Humoral Immune Responses To *Plasmodium vivax* Malaria: Application To Sero-Surveillance For Accelerated Malaria Elimination

abs# 25

9:55 AM **Merryn Roe,** *Burnet Institute & Monash University*
Evaluation of serosurveillance to understand the micro-heterogeneity of sub-microscopic malaria and other malaria risk factors in Western Cambodia.

abs# 26

10:10 AM **Megan Soon,** *QIMR Berghofer Medical Research Institute*
Defining transcriptome dynamics underlying memory CD4+ T cell development during malaria

abs# 27

10:25 AM **Lisa J Ioannidis,** *Walter and Eliza Hall Institute of Medical Research*
High dimensional mass cytometry analysis dissects T cell and B cell heterogeneity in the immune response to *Plasmodium vivax* malaria and identifies signatures predicting immunity to infection

abs# 28

Virtual Break

10:45AM - 11:15AM

Session 9 - ECR Networking Function: coffee with the professor

11:15AM - 12:15PM

An opportunity for ECRs to join Invited Speakers with a virtual coffee

Session supported by QIMR Berghofer Medical Research Institute

Virtual Break

12:15PM - 1:00PM

The conference acknowledges CSL

Session 10 - Host pathogen interactions 4

1:00PM - 3:05PM
Chair: Antje Blumenthal, *The University of Queensland*

1:00 PM **Dana Philpott,** *University of Toronto*
Regulation of NOD-like receptor signaling: role in colorectal cancer

abs# 29

1:30 PM **Maria Kaparakis Liaskos,** *La Trobe University*
Immune responses mediated by bacterial membrane vesicles and their intracellular fate.

abs# 30

1:50 PM **Jennifer A E Payne,** *Monash University*
New drugs for superbugs: immunotherapeutics that enhance neutrophil clearance of *Staphylococcus aureus*

abs# 31

2:05 PM **Jaclyn S Pearson,** *Hudson Institute of Medical Research*
RIPK1: a master regulator of cellular immunity in protection against mucosal infection

abs# 32

2:20 PM **Reuben McGregor,** *University of Auckland*
Mapping Autoantibody Responses in Children with Acute Rheumatic Fever

abs# 33

2:35 PM **Stefan H Oehlers,** *Centenary Institute*
Rough and smooth variant *Mycobacterium abscessus* infections are differentially controlled by host immunity during chronic infection

abs# 34

2:50 PM **Caroline M Weight,** *University College London*
Mutations in *Streptococcus pneumoniae* biosynthesis genes influence pneumococcal interactions with human respiratory epithelial cells and alter the epithelial-innate immune response

abs# 35

Virtual Break

3:05PM - 3:30PM

Session 11 - Designated Poster Viewing Session

3:30PM - 4:30PM

Poster listing for this session can be found on page 27. Be sure to view the e-Poster Hall throughout the conference. All e-Poster and Science Bite presenters will be online to answer any questions during both Poster Sessions.

Session 12 - Science Bites 1 - Concurrent session

4:30PM - 5:00PM

4:30 PM **Ariane Lee,** *The University of Melbourne/The Peter Doherty Institute*
The role of the short chain fatty acid butyrate in CD4+ T cell immunity

abs# 101

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4:33 PM **Felicia Schlotthauer**, *Burnet Institute*
Characterization of a monoclonal antibody towards the N-terminal hypervariable region 1 (HVR1) and epitope I of Hepatitis C Virus Glycoprotein E2

abs# 102

4:36 PM **Lucy Cooper**, *Monash University*
Regulation of immune memory formation and function during viral infection

abs# 103

4:39 PM **Matthias H Enders**, *University of Melbourne*
Liver resident CD4 T cell in malaria infection

abs# 104

4:42 PM **Meghanashree M Shreenivas,** *The Peter Doherty Institute for Infection and Immunity*
B cells are required for optimal $CD4^{+}$ T cell memory response against *Salmonella* infections

abs# 105

4:45 PM **Yi Wang,** *St Vincent's Institute*
Characterising interacting thymocytes and thymic stromal cells for mapping physical thymocyte-stroma interactions during T cell development

abs# 106

4:48 PM **Isabella A. Joubert,** *Murdoch University*
Optimizing a dual RNA-sequencing protocol for the analysis of host-pathogen interactions during neonatal sepsis

abs# 107

4:51 PM **Md Jahangir Alam,** *Monash University*
Therapeutic blockade of CXCR2 rapidly clears inflammation in Arthritis and Atopic Dermatitis models: Demonstration with surrogate and humanized antibodies

abs# 108

*Note that all Science Bite presentations can also be found in the e-Poster Hall

Session 13 - Science Bites 2 - Concurrent session

4:30PM - 5:00PM

4:30 PM **Madel V Tutor,** *University of Melbourne*
The role of Kelch 13 protein in the malaria parasite *Plasmodium falciparum*

abs# 109

4:33 PM **Nicholas L Dooley,** *QIMR Berghofer / Griffith University*
Identifying novel Vgamma9Vddelta2 T cell phenotypes during human *Plasmodium falciparum* infection

abs# 110

4:36 PM **Hui-Chi Lai,** *UNSW/Ingham Institute*
The role of SMG1 in regulating innate immunity

abs# 111

4:39 PM **Richard M Lucas,** *University of Queensland*
The transmembrane TLR adaptor SCIMP scaffolds Erk1/2 to drive macrophage pro-inflammatory responses

abs# 112

4:42 PM **Stephanie Huang,** *Hudson Institute of Medical Research*
Characterisation of a novel type I interferon pathway and its implications in inflammatory disease

The conference acknowledges CSL

abs# 113

4:45 PM **Sven Engel,** *The University of Melbourne*
The flexible usage of diverse cell death pathways ensures host protection against *Salmonella* Typhimurium infection

abs# 114

4:48 PM **Wang Cao,** *The Walter and Eliza Hall Institute*
Intestinal microfold cells orchestrate the interactions between microbiota and immunity

abs# 115

4:51 PM **Jilong Qin,** *Queensland University of Technology*
Structural and functional heterogeneity in pathoadaptive FimH adhesin variants in *Escherichia coli*

abs# 116

4:54 PM **Nadia Rajab,** *The University of Melbourne*
An integrated analysis of myeloid cells identifies gaps in *in vitro* models of *in vivo* biology

abs# 117

4:57 PM **Sarah Straub,** *Hudson Institute*
Global 3'-UTR length changes mediated by interferon beta in murine and human macrophages

abs# 118

*Note that all Science Bite presentations can also be found in the e-Poster Hall

Virtual Break

5:00PM - 5:55PM

Session 14 - Reversing the evolution of AMR with systems microbiology

5:55PM - 7:00PM
Chair: Begoña Heras, *La Trobe University*

Session supported by

5:55 PM **Ka Pui Sharon Yau,** *Monash University*
A role of *C. albicans* Rpn4 in regulating antifungal drug susceptibility

abs# 36

6:10 PM **Michael McDonald,** *Monash University*
The experimental evolution of antibiotic resistance reveals chinks in the armor of multidrug-resistant pathogens

abs# 37

6:30 PM **Nassos Typas,** *European Molecular Biology Laboratory*
Solving bottlenecks and providing new strategies for AMR crisis

abs# 38

The conference acknowledges CSL

Friday 19th February 2021

Session 15 - Scientifix Morning Symposium

8:00AM - 9:00AM

Latest advancements in immune-receptor profiling and single cell mrna sequencing

Speaker: Bryan Bell, PhD

The immune system is composed of a complex hierarchy of cell types that protect the organism against disease and maintain homeostasis. Understanding the heterogeneity of the full array of cells involved, such as T cell and B cell lymphocytes, is the key to understanding the immune system. Takara Bio's next-generation sequencing (NGS) technologies have revolutionized our understanding of immunological phenomena and human disease by enabling analyses of gene expression, gene regulation, and immune receptor information with unprecedented sensitivity. With the results of our best-in-class bulk profiling kits and our innovative single-cell chemistry, scientists can gain new insights into the immune repertoire. Here, we will present our latest advancements in immune-receptor profiling and single cell mRNA sequencing.

This conference acknowledges the support of scientifix

Session 16 - Designated Poster Viewing Session

9:00AM - 9:40AM

Poster listing for this session can be found on page 27. Be sure to view the e-Poster Hall throughout the conference. All e-Poster and Science Bite presenters will be online to answer any questions during both Poster Sessions.

Session 17 - Innate immunity

9:40AM - 11:15AM
Chairs: Ashley Mansell, *Morningside Biopharma Advisory* & Larisa Labzin, *The University of Queensland*

9:40 AM **Matthew J Sweet,** *University of Queensland*
Organelle wars and the enemy within

abs# 39

10:00 AM **Jennifer K Dowling,** *Royal College of Surgeons in Ireland*
Arginase-2 is essential for IL-10 metabolic reprogramming of inflammatory macrophages at the mitochondria

abs# 40

10:15 AM **Snehlata Kumari,** *The University of Queensland Diamantina Institute, Translational Research Institute*
Z-DNA-binding protein-1 regulates necroptosis-mediated skin inflammation

abs# 41

 The conference acknowledges CSL

10:30 AM **Kaustav Das Gupta,** *Institute for Molecular Bioscience, The University of Queensland*
Histone deacetylase 7 coordinates innate immune defence by promoting phagocytosis, NADPH oxidase-dependent reactive oxygen species production and bacterial clearance

abs# 42

10:45 AM **Kate Fitzgerald,** *University of Massachusetts Medical School*
Succination inactivates gasdermin D and blocks pyroptosis

abs# 43

Virtual Break

11:15AM - 11:45AM

Session 18 - Equity and diversity

11:45AM - 12:15PM
Chair: Nicole Moreland, *University of Auckland*

Session supported by

11:45 AM **Akiko Iwasaki,** *Yale University School of Medicine; Howard Hughes Medical Institute*
My career path and advocacy

abs# 44

Virtual Break

12:15PM - 12:45PM

Session 19 - Eukaryotic pathogens and their hosts 1

12:45PM - 1:45PM
Chair: Ana Traven, *Monash University*

12:45 PM **Wieland Meyer,** *Sydney Medical School - Westmead Hospital, University of Sydney*
Bringing metagenomics to the patient bedside to combat the emerging global health threat: the rise of invasive fungal infections

abs# 58

1:00 PM **Hamsa Puthalakath,** *La Trobe University*
TREML4 receptor regulates inflammation and innate immune cell death during polymicrobial sepsis

abs# 46

1:15 PM **Leah Cowan,** *University of Toronto*
Genomic Analysis of Fungal Morphogenesis and Interaction with Host Immune Cells

abs# 47

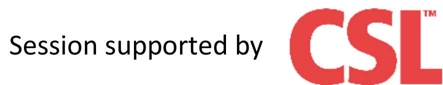
Virtual Break

1:45PM - 2:15PM

The conference acknowledges CSL

Session 20 - Vaccines, adaptive and mucosal immunology

2:15PM - 2:55PM
Chairs: Andrew Currie, *Murdoch University* & Justine Mintern, *University of Melbourne*

Session supported by 

2:15 PM **Keith Chappell,** *University of Queensland*
Creating Vaccines at Pandemic Speed: Molecular clamp stabilized subunit vaccine
abs# 48

2:35 PM **Gabrielle Belz,** *University of Queensland, Diamantina Institute*
Pre-empting danger to protect against gut infections
abs# 49

Session 21 - Vaccines and vaccination - Concurrent session

2:55PM - 4:00PM
Chair: Andrew Currie, *Murdoch University*

2:55 PM **Nicole L Messina,** *Murdoch Children's Research Institute*
Immunomodulatory effects of neonatal Bacillus Calmette–Guérin vaccination on responses to unrelated pathogens
abs# 50

3:10 PM **Danika L Hill,** *Monash University*
Immune system development varies according to age, location, and anemia in African children
abs# 51

3:25 PM **Liriye Kurtovic,** *Burnet Institute*
Novel virus-like particle encoding the circumsporozoite protein is an immunogenic malaria vaccine in mice
abs# 52

Session 22 - Adaptive and mucosal immunology - Concurrent session

2:55PM - 4:00PM
Chair: Justine Mintern, *University Of Melbourne*

2:55 PM **Mitra Ashayeripanah,** *The University of Melbourne*
Dendritic cell paralysis contributes to immunosuppression and secondary infections long after severe infection or trauma; investigation on the mechanisms, diagnostic markers and restorative therapies
abs# 53

3:10 PM **Seungyoul Oh,** *St. Vincents Institute of Medical Research*
Single cell analysis of αβ versus γδ T cell development
abs# 54

3:25 PM **Eveline D de Geus,** *Hudson Institute of Medical Research*
Interferon ε as a novel regulator of intestinal homeostasis
abs# 55

Virtual Break

4:00PM - 4:45PM

The conference acknowledges CSL

Session 23 - Viruses and their hosts 2 and Closing Remarks

4:45PM - 6:00PM
Chair: Heidi Drummer, *Burnet Institute*

4:45 PM **Tracy Hussell,** *University of Manchester*
Immune features associated with COVID-19
abs# 56

5:10 PM **Marit van Gils,** *Amsterdam UMC, University of Amsterdam*
HIV-1 has paved the way in the battle against SARS-CoV-2
abs# 57

Q&A Panel with **Tracy Hussell** and **Marit can Gils**

Closing Remarks by **Heidi Drummer**, Burnet Institute

The conference acknowledges CSL

Designated e-Poster Sessions

Abstracts #201 to #312 are found on page 63.

All e-Poster presenters are encouraged to be online to answer questions during both Poster Sessions. Be sure to view the e-Poster Hall throughout the conference. e-Posters will be available on the Virtual Platform for 12 months.

Alana L Whitcombe
Developing a high-throughput multiplex immunoassay that characterises antibody responses to StrepA vaccine antigens abs# 201

Andrea Nguyen
CD8+ T cell cross-reactivity across conserved Influenza A and B epitopes abs# 202

Daniel Thiele
Age related epigenetic changes associated with and causative of intrinsic CD8+ T cell dysfunction abs# 203

Sophia Hawas
Humoral immune responses during acute bacterial urinary tract infection in mice abs# 204

Theinmozhi Arulraj
Modeling the immune complex cycling in follicular dendritic cells abs# 205

Adi Idris
Targeting SARS-CoV-2 using stealth nanoparticles loaded with siRNAs abs# 206

Cecy R Xi
A novel purification procedure for active recombinant human DPP4 and the inability of DPP4 to bind SARS-CoV-2 abs# 207

Larisa Labzin
SARS-CoV-2 infects but does not replicate in human macrophages and triggers pro-inflammatory and anti-viral cytokine responses. abs# 208

Manisha Pandey
Antibodies to neutralizing epitopes synergistically block the interaction of the receptor binding domain of SARS-CoV-2 to ACE 2 abs# 210

Naveen Vankadari
Structure of SARS-CoV-2 Nsp1/5'-UTR Complex and Implications for Potential Therapeutics, Vaccine & Virulence abs# 211

Aidan Fletcher
The alternative sigma factor RpoE2 is involved in the stress response to hypochlorite and in vivo survival of *Haemophilus influenzae* abs# 212

Akila Pilapitiya
Molecular insights into secreted toxins from *Escherichia coli* pathogens reveal approaches to combat diarrheal infections abs# 213

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Alice Trenerry
Hijacking of lipid synthesis and storage during flavivirus infection abs# 214

Amy Pham
Inferior outcomes in lung transplant recipients with serum *Pseudomonas aeruginosa* specific cloaking antibody abs# 215

Daniel Ellis
Genetic diversity and antibiotic resistance rates amongst recent Australian NTHi clinical isolates abs# 216

Elizabeth Peterson
Shape-shifting bacteria are key to infection abs# 217

George Ashdown
Spatiotemporal quantification of host membrane lipid order during *Mycobacterium tuberculosis* infection abs# 218

Jennifer Hosmer
H. influenzae nutritional virulence determines outcomes of interactions with human host cells during intra- and extracellular growth abs# 219

Jessica R Loughland
Age-dependent innate and adaptive cellular immune responses in malaria-naive children and adults. abs# 220

Jessica L Rooke
Elucidating genes that are required for chronic infections of *Salmonella enterica* abs# 221

Jiyao Gan
The *Salmonella* Effector SseK3 Targets Small Rab GTPases abs# 222

Johannes Hoehensteiger
Novel serum-resistance mechanism in patients with *P. aeruginosa* bacteraemia abs# 223

John M Atack
Defining the glycointeractome of the multidrug resistant pathogen *Acinetobacter baumannii:* towards novel treatments and therapeutics abs# 224

Laura Cook
Induction of stable human FOXP3+ Tregs by a parasite-derived TGF-β mimic abs# 225

Lauren Zavan
Antimicrobial activity of outer membrane vesicles against Gram-negative and Gram-positive bacteria is altered by their mechanism of biogenesis abs# 226

Lilian Hor
Characterisation and inhibition of bacterial virulence factors abs# 227

Madeleine Wemyss
Salmonella Typhimurium induces cIAP1 degradation to promote death in macrophages abs# 228

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Michelle P Clark
'Unleishing' host cell death pathways to promote clearance of *Leishmania donovani* abs# 229

Mohini A Shibu
Maurer's cleft tethers – is tethering important for malarial adhesion? abs# 230

Praveena Thirunavukkarasu
Understanding the molecular recognition of Bacteroides fragilis glycosphingolipids by Natural Killer T-cell receptor abs# 231

Sachintha Wijegunasekara
Host and pathogen genetic determinants that contribute to Buruli ulcer severity abs# 232

Sandra Chishimba
Complement and Fcγ-receptor mediated antibody effector functions target merozoites and are associated with protection from severe malaria in children abs# 233

Sathya N Kulappu Arachchige
Pathological and immune responses to chronic *Mycoplasma gallisepticum* infection and protective immunity of the ts-304 vaccine in chickens abs# 234

Sonja Chua
Characterization of Sec13 as a host-interactor in influenza using a Yeast-based system and Mammalian cells abs# 235

Sonja Frolich
Super-resolved view of PfCERLI1, a rhoptry associated protein essential for *Plasmodium falciparum* merozoite invasion of erythrocytes abs# 236

Tayla Williamson
Drug addiction in the human malaria parasite *Plasmodium falciparum* abs# 237

Thorey Jonsdottir
Investigating the role of *Plasmodium falciparum* exported proteins that bind the new permeability pathway complex protein RhopH2 abs# 238

Adriana Pliego
The gastrointestinal tract is a central site of dengue virus pathology and possible key to severe disease abs# 239

Aidil Zaini
T follicular helper (Tfh)-germinal centre (GC) B cell response is required for sterile immunity during enteric helminth infection abs# 240

Garrett Z Ng
Depletion of alveolar macrophages by cigarette smoke causes delayed clearance of *Legionella pneumophila* infection abs# 241

Giang Hoang Bao Le
Stabilization of tristetraprolin modulates inflammation during *Mycobacterium tuberculosis* infection abs# 242

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Hui Xu
Functional cure of chronic hepatitis B is associated with co-occurrence of HBsAg/anti-HBs immune complex peaks with ALT flares, and seroconversion to potently neutralising anti-HBs abs# 243

Jaehyeon Kim
TLR4 signalling pathway in the gut attributes to the intestinal inflammation during dengue infection abs# 244

Joanne M Hildebrand
Missense mutations in the gene encoding the cell death effector MLKL lead to lethal neonatal inflammation in mice and are present in high frequency in humans. abs# 245

Julie Hibbert
Neonatal sepsis: exploring the plasma proteome abs# 246

Madeeha Afzal
Antimicrobial susceptibility of *Staphylococcus aureus* from different ocular conditions abs# 247

Manjulatha Mrs Sara
Synergistic activity of ciprofloxacin and azithromycin against staphylococci and streptococci abs# 248

Maxwell Stevens
Essential protective role for miR-652 during intracellular bacterial infection abs# 249

Rosa Coldbeck-Shackley
The unique type-I Interferon-epsilon (IFNε) constitutively protects the female reproductive tract from Zika Virus infection. abs# 250

Neeraj Tuladhar
PI3K gamma suppresses macrophage mediated inflammation in cystic fibrosis cells abs# 251

Rebecca Ambrose
Monophasic *Salmonella* Typhimurium strains exhibit increased replication capacity in human macrophages by modulating intracellular host responses. abs# 252

Rukshan AM Rafeek
Simultaneous Induction of Cardiac and Neurobehavioral Autoimmune Pathology Following Exposure to Streptococcal Antigens abs# 253

Shipra Pant
Screening for *Chlamydia trachomatis* infection in tubal ectopic pregnancy patients in India: Is serology the answer abs# 254

Tabinda Hussain
Investigating the impact of helminth infection on young and aged TVM cells abs# 255

Yizhuo Wang
Histone deacetylase 7 promotes innate immune defence against bacterial infection, as well as Pkm2-dependent production of the monocyte recruiting chemokine Ccl2 abs# 256

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Alexis Bonfim-Melo
Epithelial lytic cell death promotes monolayer permeability, apical elimination and lamellipodia-driven mechanism of epithelia resealing abs# 257

Anita A Pinar
Therapeutic targeting of the myofibroblast NLRP3 inflammasome as a novel approach to treating fibrosis abs# 258

Bangyan Xu
Application of activity-based probes to interrogate the contribution of cathepsin X to dendritic cell function abs# 259

Calvin Xu
MAIT cells expand in the absence of NKT and γδT cells abs# 260

Caroline L Holley
Live imaging of pyroptotic macrophages reveals novel GSDMD-dependent cytoskeletal remodelling abs# 261

Ee Shan Pang
Activation of stimulator of interferon genes in dendritic cells induces interferon-lambda and subset- and species-specific dendritic cell death abs# 262

Gregory Moseley
Viral Immune Evasion: There are more than two STATs abs# 263

Ina Rudloff
IL-37 employs multiple strategies to suppress inflammasome-mediated IL-1β bioactivity and inflammation abs# 264

Isanka U Ratnasekera
Bactericidal capacity of ascitic fluid from patients with decompensated cirrhosis abs# 265

James Harris
Exploring the MIF family of cytokines: Different roles for MIF and D-DT in inflammation abs# 266

Jessica B von Pein
Tracking variability in the antimicrobial zinc toxicity response of human macrophages against *Escherichia coli* abs# 267

Johanna K Ljungberg
WNT signatures are an integral part of macrophage responses to infection abs# 268

Joshua G Dubowsky
Dengue Infection Causes Membrane Recruitment of FH Through Ligands other than Glycosaminoglycans abs# 269

Louise M Randall
Ficolin-1 is an innate pattern recognition receptor that binds *Plasmodium falciparum*-infected red blood cells and may promote parasite clearance abs# 270

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Maialen Sebastian-delaCruz
Enteric viral infections prior to gluten intake exacerbate type I interferon response through an RNA methylation dependent mechanism abs# 271

Mariam Bafit
Interferon-lambda: a key cytokine for dendritic cell development? abs# 272

Nazneen Jahan
The role of intracellular pattern recognition receptors in recognition and immunomodulation of dendritic cells in response to daptomycin resistant *Staphylococcus aureus.* abs# 273

Nicole K. Campbell
Interferon epsilon alters peritoneal myeloid cell populations in a murine model of ovarian cancer abs# 274

Sean W Cutter
Development of a 3D cell culture model to study macrophage biology and infection with *Leishmania mexicana* abs# 275

Syeda Farhana Afroz
Unravelling mechanisms of TLR-inducible mitochondrial fission in macrophages abs# 276

Tomalika Ms Ullah
Characterizing a novel anti-inflammatory activity of Genistein abs# 277

William J Gilmore
Differential modulation of innate immunity by *Bacteroides fragilis* bacteria and their secreted outer membrane vesicles abs# 278

Brianna Jesaveluk
Lactic acid produced by an optimal vaginal microbiota promotes cervicovaginal epithelial barrier integrity: implications for HIV transmission abs# 279

Emily L Gulliver
The gut microbiome: A source of antimicrobial resistance for pathogens abs# 280

Gemma L DAdamo
Identification of novel bacterial species associated with paediatric inflammatory bowel disease through direct mucosal sampling abs# 281

Michelle Chonwerawong
Characterisation of host-microbiome interactions using microfluidic organ-on-a-chip models abs# 282

Remy Young
Functional Characterisation of Gastrointestinal Microbes abs# 283

Tapoka T Mkandawire
Identification of suitable host conditions for the hatching of *T. muris, T. suis* and *T. trichiura* in the mucosal microbiota of pigs, humans and a humanised mice model to understand the molecular basis of hatching in *Trichuris* species abs# 284

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Ankita Ray
MicroRNA-16: Potential role in *Chlamydia trachomatis*-induced recurrent spontaneous abortion abs# 285

Astha Dimri
Is there potential clinical significance of miR-323-3p in *Chlamydia trachomatis*-associated tubal ectopic pregnancy? abs# 286

Carlos Fernando Santos Martin
Disulphide bond dependent bacterial pathogenesis abs# 287

Dulcie Lautu
Investigating molecular markers of *Plasmodium falciparum* artemisinin resistance in Papua New Guinea abs# 288

Ella L Johnston
Bacterial membrane vesicles: a potential reservoir for antimicrobial resistance genes within bacterial communities abs# 290

Jason Paxman
Molecular mechanisms of autotransporters abs# 291

Mahjabeen Khan
Genomic characteristics of exoU and exoS ocular *Pseudomonas aeruginosa* isolates abs# 292

Brandon E.K. Tan
Identification of dengue virus (DENV) NS1 protein residues that are critical to its secretion abs# 293

Tanu Bhati
Galectin-1 Plays a Significant Role in Preterm Birth in Women Infected with *Ureaplasma urealyticum* abs# 294

Natalia Rosas Bastidas
Investigating the molecular evolution of carbapenem-resistance in *Klebsiella quasipneumoniae* abs# 295

Abarna V. M. Murugan
Evolutionary Glycomics: A Comprehensive Study of Vertebrate Host Serum/Plasma Glycome Using Orthogonal Glycomics Techniques abs# 296

Michael Bentley
Bioinformatics analysis of chemotaxis related genes in gram negative bacterial colonisers of the gastrointestinal tract abs# 297

Paolo Bareng
Understanding *Plasmodium vivax* antigen diversity and identifying targets of immune selection abs# 298

Tinashe G Chabikwa
Single cell transcriptional profiles of immune cell subsets from children and adults with malaria. abs# 299

Amlan Chakraborty
Nanoparticle-mediated pulmonary delivery of the peptide hormone relaxin abrogates chronic allergic airways disease abs# 300

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Bing Zhang
Rapid profiling of polymyxin B susceptibility in gram-negative bacteria with a novel polymyxin-derived fluorescent probe abs# 301

Brodie L Bailey
Development of a Novel Antimalarial Class with a Slow Onset of Parasite Activity abs# 302

Celeste M Donato
Genetic characterisation of zoonotic rotavirus strains identified in children and adults with acute rotavirus gastroenteritis in Australia abs# 303

Jessica L Horton
Keep holding on: the role of antibody avidity in immunity to malaria abs# 304

Joey McGregor
Investigation of the optimal strategy to deliver a B cell immunogen for a HCV vaccine. abs# 305

Kia C Ferrell
Intrapulmonary vaccination with delta-inulin adjuvant stimulates non-polarised chemotactic signalling and diverse cellular interaction abs# 306

Mohammad AK Azad
Synchrotron-based X-ray fluorescence microscopy reveals accumulation of polymyxins in single human alveolar epithelial cells abs# 307

Nicholas S Eyre
Identification of estrogen receptor modulators as inhibitors of flavivirus infection abs# 308

Ranja Salvamoser
Fortrexo: New approach to prevent and treat viral infectious diseases abs# 309

Geqing Wang
Taming the beast: disarming pathogenic bacteria with novel anti-virulence agents abs# 310

Zuleima Pava
Molecular surveillance over 14 years confirms reduction of *Plasmodium vivax* and *falciparum* transmission after implementation of Artemisinin-based combination therapy in Papua, Indonesia abs# 311

Ahmed Al Mushafi
Human Amniotic Epithelial Stem Cells attenuate Crescentic Glomerulonephritis abs# 312

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1

Immune response to SARS-COV-2

Akiko Iwasaki[1]

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2

Quantitative 4D microscopy of *P.falciparum* invasion of erythrocytes

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Invasion of erythrocytes during the asexual blood stage of malaria is an area of intense research owing to its promise a target for anti-malarial treatments. Difficulties in the development of efficient therapies lie, in part, due to a fundamental lack of insight into the molecular processes and biophysical mechanisms which govern host-pathogen interactions at this stage. Invasion is a highly dynamic process involving the micron sized merozoite, numerous ligand receptor interactions and critical biomechanical forces [1, 2, 3, 4]. An important part of the invasion process is the formation of the parasitophorous vacuole membrane (PVM) at the point of entry to the host red blood cell. This membrane provides a physical barrier and an exchange surface between the parasite and the host cell. The formation of the PVM and subsequent remodelling of the host membrane during invasion are incredibly dynamic events and are very challenging to study in real time [5]. Studies using advanced microscopy techniques are often limited to fixed points in time or rely heavily on qualitative analysis. Until now a high-resolution 4D view of this complex invasion process has remained an insurmountable technical challenge.

We built a custom high-speed multi-dimensional lattice light sheet microscope to assess the molecular and biophysical formation of the PVM during invasion in 4-dimensions. Using various functional fluorescence imaging methods, we show, for the first time, temporal changes in the physical and molecular properties of the forming PVM. In addition, a computational framework to measure geometric features, such as membrane curvature, membrane surface area and volume has been developed. The combined spatial and temporal resolution of the lattice light sheet microscope offers unprecedented insights into the dynamic processes underpinning host-pathogen interactions.

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3

Integrated immune dynamics define correlates of COVID-19 severity and antibody responses

Marios Koutsakos[1], Louise C Rowntree[1], Luca Hensen[1], Brendon Y Chua[1, 2], Carolien E van de Sandt[1, 3], Jennifer A Habel[1], Wuji Zhang[1], Xiaoxiao Jia[1], Lukasz Kedzierski[1], Thomas M Ashhurst[4], Givanna H Putri[4], Felix Marsh-Wakefield[4], Mark V Read[4], Davis N Edwards[4], Bridie E Clemens[1], Chinn Yi Wong[1], Francesca L Mordant[1], Jennifer A Juno[1], Fatima Amanat[5], Jennifer Audsley[6], Natasha E Holmes[7, 8], Carly M Hughes[9], Mike Catton[10], Justin Denholm[11], Steven YC Tong[11, 12], Denise L Doolan[13], Tom C Kotsimbos[14, 15], David C Jackson[1, 2], Florian Krammer[5], Dale I Godfrey[1], Amy W Chung[1], Nicholas JC King[4], Sharon R Lewin[14, 15, 6], Adam K Wheatley[1], Stephen J Kent[1], Kanta Subbarao[1, 16], James McMahon[9, 14, 15], Irani Thevarajan[11, 6], Thi HO Nguyen[1], Allen C Cheng[14, 15], Katherine Kedzierska[1, 2]

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SARS-CoV-2 causes a spectrum of disease outcomes, ranging from asymptomatic to critical COVID-19. As immunological basis remains ill-defined, we analyzed 77 SARS-CoV-2-infected individuals at acute and/or convalescent timepoints, up to 103 days post-symptom onset, quantifying 154 innate and adaptive immunological parameters. Acute COVID-19 was associated with high levels of IL-6, IL-18 and IL-10, elevated neutrophil-to-lymphocyte and neutrophil-to-T cell ratios, frequencies of activated CD38^{+} neutrophils, CD38^{+} eosinophils, CD38^{+}/HLA-DR^{lo} monocytes, CD38^{+}CD56^{dim} NK cells, CD38^{+} γδ T-cells, antibody-secreting cells, CD38^{+}ICOS^{+} circulating T follicular helper cells, CD38^{+}/HLA-DR^{+}CD4^{+} T-cells, and effector CD27^{-}CD45RA^{+} and CD38^{+}CD8^{+} T-cells. During convalescence, elevated seroconversion and neutralisation activity were prominent and were correlated with acute cT$_{FH}$ cell activation. Strikingly, severe COVID-19 in ICU patients displayed elevated soluble IL-6R levels, higher IL-18 concentration, and hyperactivation of innate, adaptive and myeloid compartments. Our analyses provide a comprehensive map of longitudinal immunological responses in COVID-19 patients at acute and convalescent phases of SARS-CoV-2 infection, and integrate key cellular pathways of complex perturbed immune networks that underpin severe COVID-19, providing important insights into potential biomarkers and immunotherapies.

4

Innate cell profiles during the acute and convalescent phase of SARS-CoV-2 infection in children

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Children have mild COVID-19 disease compared to adults, and up to one-third are asymptomatic. The immunological basis for this difference is unclear and data on the cellular immune response in children with SARS-CoV-2 exposure and infection are limited. In a previous case study of two parents with PCR-confirmed symptomatic SARS-CoV-2 infection and their three SARS-CoV-2 PCR-negative children, we showed that all family members had a cellular immune response characterised by striking changes in the frequency of innate immune cells over time, particularly among the children. Here, we report acute and convalescent innate immune responses of 48 children and 70 adults infected with, or exposed to, SARS-CoV-2. We found that clinically mild SARS-CoV-2 infection in children was characterised by low proportions of all three circulating subsets of monocytes (classical, intermediate, non-classical), dendritic cells and natural killer cells during the acute phase, whereas SARS-CoV-2-infected adults showed reductions in the non-classical monocyte fraction only. Increased proportions of CD63^{+} activated neutrophils during the acute phase were an additional feature unique to SARS-CoV-2 infected children. Both children and adults exposed to SARS-CoV-2 but negative on PCR testing had increased proportions of low-density immature neutrophils that were observed out to 7 weeks post exposure. This study describes a role for the innate immune response during SARS-CoV-2 infection in children and suggests that exposure to SARS-CoV-2 induces a change in the immune response irrespective of evidence of active viral infection. Our study provides insights to explain differences in disease severity between children and adults.

5

Bats as reservoirs of zoonotic viruses and SARS-Like viruses

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6

Infectious KoRV-related retroviruses circulating in Australian bats

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Bats are reservoirs of emerging viruses that are highly pathogenic to other mammals including humans. Despite the diversity and abundance of bat viruses, they had previously not been shown to harbor exogenous retroviruses. Here we report the discovery and characterisation of a group of Koala retrovirus-related (KoRV-related) gammaretroviruses in Australian and Asian bats (1). These include the Hervey pteropid gammaretrovirus (HPG), the first reproduction-competent retrovirus found in bats, a close relative of KoRV and the Gibbon ape leukemia virus (GALV). The host animals of KoRV (koalas) and GALV (gibbons) respectively exist in non-overlapping habitats in Australia and Asia, separated by an oceanic boundary.

In this study we extracted and assembled the complete HPG genome from the scat of a single black flying fox, captured in Hervey Bay (QLD). We then chemically synthesized a proviral molecular clone that is capable of generating reproduction-competent virions. Electron microscopy and virion-associated reverse transcriptase (RT) assays reveal that HPG has the virion morphology and Mn^{2+}-dependent RT activity typical of a gammaretrovirus, and that *in vitro*, HPG is capable of infecting bat and human cells, but not mouse cells. Through an envelope protein pseudotyping assay, HPG displays a similar pattern of cell tropism as KoRV-A and GALV. Population studies reveal the presence of HPG and KoRV-related sequences in several locations across north-east Australia, as well as serological evidence for HPG in multiple pteropid bat species.

Combined, these results reveal bats to be important reservoirs of KoRV-related gammaretroviruses that can potentially be transmitted to other mammalian species, and positions bats as the most likely transmitters of KoRV-related retroviruses between Asia and Australia.

1. Hayward, J. A., et al. (2020). "Infectious KoRV-related retroviruses circulating in Australian bats." Proceedings of the National Academy of Sciences 117(17): 9529-9536.

7

Analysis of the dynamics and composition of lipid droplets during an antiviral response

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We have previously demonstrated that lipid droplets (LDs) are upregulated as a host repose to viral infections of IAV, ZIKV, DENV and HSV-1 both *in vitro* and *in vivo,* and this upregulation of LDs helps drive an effective interferon response. However, the mechanism by which they do this, and their role during a host antiviral repose has not been examined.

Microscopic examination of LDs very early following activation of antiviral signaling pathways showed a significant upregulation in both LD velocity and distance traveled, as well as displayed directional movement of LDs. As LDs are known to interact with other organelles, and transfer both lipid species and protein cargo, we developed techniques to determine the changing lipidome and protein landscape of LDs at early time points following activation of both dsRNA and dsDNA signalling pathways. Lipidome analysis revealed limited alterations in the cellular lysates at early time points, in direct contrast to the significant changes observed in multiple major lipid species within LDs themselves. Additionally, an upregulation of long chain fatty acids was also observed in virally driven LDs. Proteomics analysis demonstrated a significant upregulation of 83 proteins, including multiple antiviral proteins, and members of the early innate antiviral signaling pathways, demonstrating for the first time that the lipid droplet may act as a signaling platform during an effective antiviral response.

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

8

CD4+ T cell recognition of haemagglutinin epitopes across different influenza strains

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The 2019/20 SARS-Cov2 pandemic is a modern-day reminder of the impact that novel viral strains can have on a population without pre-existing immunity. The influenza A virus (IAV) has caused five pandemics in the last 150 years and is particularly prone to mutations in the surface glycoprotein haemagglutinin (HA) which is the primary target of the $CD4^+$ T cell and antibody responses.

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

We expanded $CD4^+$ T-cells from healthy donors expressing HLA-DRB1*07:01 or HLA-DRB1*11:01, against a haemagglutinin-peptide and its variants from different IAV strains. Using intracellular cytokine staining and HLA-II tetramer staining combined with multiparameter flow cytometry, we compared the responses of $CD4^+$ T cells in regard to their polyfunctionality, cross-reactivity and memory phenotype. We then used fluorescence polarisation to identify the relative affinity of peptide binding for each of the HA peptide variants to HLA-DRB1*07:01 and HLA-DRB1*11:01. The molecular basis of peptide presentation was then determined using X-ray crystallography. HLA-DRB1*11:01$^+$ individuals were found to have more cross-reactive responses towards HA peptides derived from different IAV strains than HLA-DRB1*07:01$^+$ individuals, and the cross-reacting $CD4^+$ T cells generally had lower polyfunctionality than specific $CD4^+$ T cells. Each of the HA peptides were found to have different relative affinities for HLA-DRB1*07:01 and HLA-DRB1*11:01. Moreover, we provide the first insight into the molecular and functional basis of IAV epitope presentation by different HLA-II molecules.

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9

CD4 T cell responses to SARS-CoV-2 infection and vaccination

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CD4+ T follicular helper (TFH) cells are critical to the induction and maintenance of germinal centre reactions and B cell maturation. To better understand how neutralising antibody responses are generated in response to SARS-CoV-2 infection or vaccination, we studied circulating TFH responses to SARS-CoV-2 and 'common cold' human coronaviruses (hCoV). By comparing the frequency and phenotypic profile of these cells in uninfected individuals, people recovered from COVID-19 infection, or animal trials of SARS-CoV-2 vaccines, we gain insight into biomarkers of potent neutralising antibody responses and better understand how coronaviruses induce serological responses.

10

Convalescent plasma for COVID-19

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Convalescent plasma from blood donors with anti-SARS-CoV-2 antibodies is a potential therapy for prevention and/or treatment of COVID-19. Since the beginning of the pandemic, there have been more than 100 clinical trials registered globally evaluating convalescent plasma or hyperimmune globulin for COVID-19. Considerations for evaluating convalescent plasma as a therapy include selection of patient populations most likely to benefit, blood donor eligibility and characteristics, optimal product characteristics and study design. Clinical trial results, including preliminary results from large, randomised controlled trials, have recently started to be reported. More data is required on the efficacy and safety of convalescent plasma early in the course of disease and in higher risk patient populations and on the optimal product characteristics.

12

The rebirth of BCG in the era of COVID-19

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The BCG vaccine has been used for control of tuberculosis (TB) since 1921 but also has remarkable 'off target' effects that affords protection against other pathogens, particularly respiratory infections in children and the elderly. For this reason BCG vaccination is currently being trialed as a strategy to reduce the incidence and severity of COVID-19. Modified forms of BCG are also being used as 'new generation' vaccines for TB.

Our research program aims to develop new vaccines for important lung pathogens, with an emphasis on TB and COVID-19. We hypothesised that BCG may interact sub-optimally with dendritic cells (DCs), the cell type pivotal in shaping the adaptive immune response to infectious agents, and thus strengthening this interaction could improve BCG protective efficacy against TB. BCG engineered to express the single-chain variable fragment recognizing the endocytic C-type lectin receptor DEC205 (BCG:DEC), expressed predominately on DCs, resulted an increased functional ability of BCG:DEC to interact with DEC205-expressing cells compared to BCG alone. In mice, BCG:DEC vaccination conferred greater protection than BCG after aerosol challenge with virulent *M. tuberculosis*; efficacy lasted up to 20 weeks post infection, even when BCG protection had waned. We are further assessing the suitability of BCG:DEC as a strategy to improve protection against TB in humans. In parallel studies, we are also defining the suitability of BCG as part of a vaccine strategy for COVID-19. Combination of BCG with a stabilized, trimeric form of the SARS-CoV-2 spike antigen promoted rapid development of virus-specific IgG antibodies in the sera of vaccinated mice, which could be further augmented by the addition of alum. This vaccine formulation, termed BCG:CoVac, induced a Th1-biased response both in terms of IgG antibody subclass and cytokine release by vaccine-specific $CD4^+$ and $CD8^+$ T cells. A single dose of BCG:CoVac was sufficient to induce high-titre SARS-CoV-2 neutralizing antibodies (NAbs) that were detectable as early as 2 weeks post-vaccination; NAb levels were greater than that seen in the sera of SARS-CoV-2-infected individuals. BCG:CoVac would be broadly applicable for all populations susceptible to SARS-CoV-2 infection and in particular could be readily incorporated into current vaccine schedules in countries where BCG is currently used. The vaccine is undergoing late stage pre-clinical testing and being prepared for clinical assessment.

13

Characterization of SARS-CoV-2 peptides presented by Human Leukocyte Antigen molecules

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To date, the COVID-19 pandemic has claimed over a million lives and afflicted more than 40 million individuals. Although a global effort has been enacted for vaccine and drug discovery, our rudimentary understanding of SARS-CoV-2 infection and our own immune defence against this infection remains unclear. Our immune system can naturally overcome viral infection through the presentation of viral protein fragments or peptides (p) via human leukocyte antigen (HLA) molecules.

These peptide-HLA complexes (pHLAs) are recognized by cytotoxic T cells that can activate, proliferate, and kill infected cells. T cells also retain memory of their encounter with the virus, and will respond faster during re-infection. How peptides from coronavirus are presented on the cell surface by HLA molecules can impact T cell recognition and influence the outcome of viral clearance, and therefore, outcome of the disease.

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Although SARS and SARS-CoV-2 cause severe acute respiratory syndrome, seasonal coronaviruses such as 229E, OCE43, HKU1, and NL63 only cause the common cold. These coronaviruses share protein homology that can also be presented by HLAs, meaning that prior exposure to a less severe strain of coronavirus may infer immunity via memory T cells if similar peptides are presented in the same structural fashion.

We used protein crystallography to further understand the presentation of SARS-CoV-2 peptides by HLA molecules, determine their stability which impact on T cell activation. In addition, we have determine the T cell response to those peptides, providing information that help map the epitopes from the virus that could influence vaccine strategies and provide a basis for research in T cell based vaccine.

14

Dimeric IgA as a biomarker of incident SARS-CoV-2 infection

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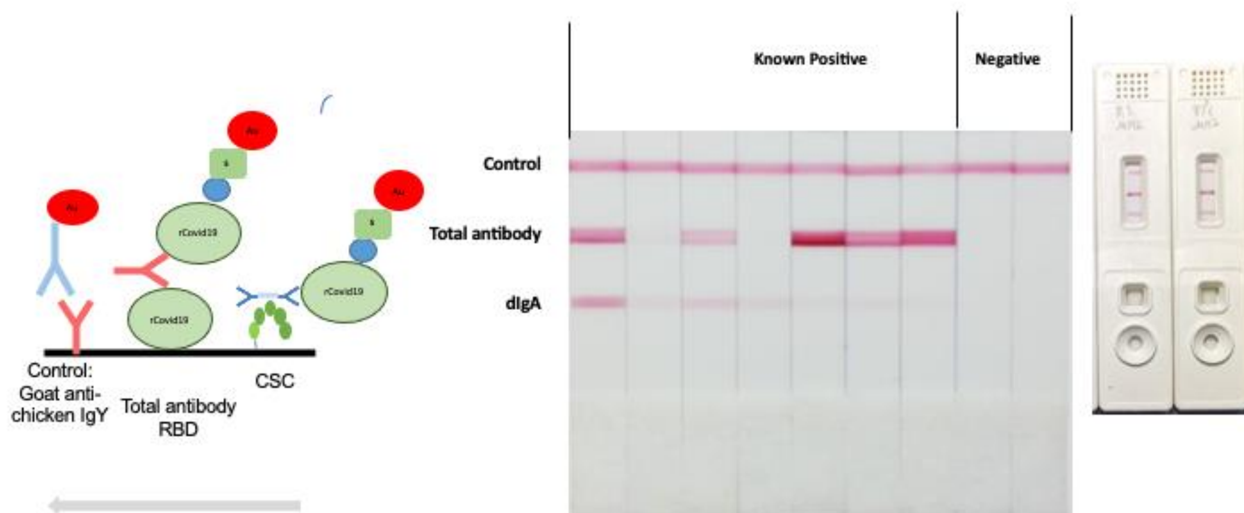

Serological testing for acute viral infections usually relies on detection of pathogen-specific IgM, but IgM typically has significant levels of cross-reactivity that hamper its utility, especially for infections such as SARS-CoV-2 (COVID-19) in low-prevalence settings. Indeed the proliferation of commercial ELISA and point of care (POC) tests for SARS-CoV-2 resulted in many inaccurate reports of incidence and prevalence, and IgM has not proven useful during the pandemic. We have developed a novel method for detection of the dimeric form of IgA (dIgA) using a chimera of rabbit- and human polymeric Ig receptor, termed CSC (chimeric secretory component), which binds only dIgA and not IgM. We have previously shown that dIgA is an acute phase biomarker in hepatitis A, hepatitis C and hepatitis E (Mohd-Hanafiah et al, 2018) and HIV (Seaton et al, 2017). UsingSARS-CoV-2 RBD antigen and recombinant CSC for capture (lateral flow POC) or detection (ELISA) of dIgA, we demonstrate that RBD-specific dIgA detects early seroconversion, reaching 100% sensitivity by 11 days after symptom onset. Importantly, the dIgA response was transient, declining rapidly after around 35-40 days after symptom onset and becoming negative on average around 80 days after symptom onset. Specificity of the dIgA tests appears to be very high, with no cross-reactivity observed in a limited number of acute seasonal coronavirus infected patients, and we will soon be testing a large panel of pre-COVID plasma samples. The SARS-CoV-2 dIgA assays should prove useful as an adjunct to detection of RNA/antigen in diagnosis, screening, and contact tracing, and for estimating the incidence of infection in serological surveys. In particular, the lateral flow POC dIgA tests will allow community-based testing and screening that will be important in responding to future waves of COVID-19, and we are pursuing commercial development of the tests.

1. Mohd Hanafiah K, et al, 2018. BMC Res Notes 11(1): 688
2. Seaton K, et al, 2017. JCI Insight 2(24)

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15

Microbiota-derived butyrate promotes metabolism and memory potential of effector CD8+ T cells

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Interactions with the microbiota influence many aspects of immunity, including immune cell development, differentiation and function. Here we examined the impact of microbiota on one of the key functions of CD8+ T cells, the transition to long-lived and protective memory. Antigen-activated CD8+ T cells transferred into germ-free mice failed to transition into long-lived memory cells with enhanced recall capacity and had transcriptional impairments in oxidative metabolism. To the contrary, the microbiota-derived short-chain fatty acid (SCFA) butyrate promoted cellular metabolism, enhanced memory potential of activated CD8+ T cells and was required for optimal recall responses upon antigen re-encounter. Mechanistic experiments revealed that the SCFA butyrate increased turnover of glycolysis and oxidative phosphorylation (OXPHOS) of effector CD8+ T cells but led to a partial uncoupling of the tricarboxylic acid cycle from glycolytic input. This allowed preferential fueling of oxidative phosphorylation through short-chain fatty acids. Our findings reveal a role for the microbiota in promoting CD8+ T cell long-term survival as memory cells and suggest that microbial metabolites potentially guide the metabolic rewiring of activated CD8+ T cells that enables this transition.

16

Dietary Trp deficiency promotes gut Rorgt+ T_{regs} cells at the expense of Gata3+ T_{regs} cells and alters commensal microbiota metabolism.

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Metabolic pathways encoded within the gut microbiome utilize dietary nutrients to produce bio-active molecules that interact with host gene products. For example, bacterial-derived secondary bile acids and short chain fatty acids, and dietary antigens support the expansion of gut resident Rorγt+ Treg cells. Rorγt+ Treg cells are crucial for maintaining host-microbiota homeostasis, however, the molecular network governing Treg cell maintenance in the gastro-intestinal tract still remains poorly understood. The essential amino acid Tryptophan (Trp) is metabolized by commensal microbiota to produce bioactive indoles and indole-based metabolites. These bacterially-derived Trp metabolites behave as Aryl-hydrocarbon receptor (AhR) ligands, which activate AhR mediated transcription in many cell types. AhR is an important regulator of barrier integrity, metabolism and immunity.

Here we have utilized a model of acute dietary Trp deficiency to investigate the effect of Trp metabolism in regulating immune-microbiota crosstalk., We first wanted to understand how acute dietary Trp deficiency impacted the microbiota, metabolite profile and transcriptome of the small intestine by using “multi-omics” approach. We placed mice on control or Trp-deficient diets and compared their microbial diversity by 16S, metabolomics profile and the transcriptional landscape of the intestinal ileum. As to be expected, Trp depletion significantly altered the fecal metabolite profile and the alpha and beta diversity of the microbiota. More specifically, a significant reduction in *L.reuteri* was observed, an important Trp metabolizing probiotic. Trp deficiency further resulted in robust transcriptional changes of key immune processes in a microbiota dependent manor.

As intestinal Treg cells are acutely sensitive to the intestinal microbiota and its metabolites, we next investigated whether microbial and metabolic changes resulting from a Trp deficient diet were impacting Treg homeostasis. Interestingly, acute dietary Trp depletion resulted in the expansion and proliferation of Rorγt+ Treg cells in a microbiota dependent manor. Furthermore, introduction of the AhR ligand Indole-3-Carbinol to our Trp deficient diet was sufficient to revert the effects of Trp-deficiency on Rorγt+ Treg cells. Therefore, our findings bring to light the importance of Trp metabolism in the maintenance of Rorγt+ Treg and suggests that AhR ligands negatively regulate the expansion of Rorγt+ Treg cells.

17

Microbial function and genital inflammation in young South African women at high risk of HIV infection

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Background: Female genital tract (FGT) inflammation is an important risk factor for HIV acquisition. The FGT microbiome is closely associated with inflammatory profile, however, the relative importance of microbial activities has not been established. Since proteins are key elements representing actual microbial functions, this study utilized metaproteomics to evaluate the relationship between FGT microbial function and inflammation in 113 young and adolescent South African women at high risk of HIV infection. Women were grouped as having low, medium or high FGT inflammation by K-means clustering according to pro-inflammatory cytokine concentrations.

Results: A total of 3,186 microbial and human proteins were identified in vaginal swabs using liquid chromatography-tandem mass spectrometry, while 94 microbial taxa were included in the taxonomic analysis. Both metaproteomics and 16S rRNA gene sequence analyses showed increased non-optimal bacteria and decreased lactobacilli in women with FGT inflammatory profiles. However, differences in the predicted relative abundance of most bacteria were observed between 16S rRNA and metaproteomics analyses. Bacterial protein functional annotations (gene ontology) predicted inflammatory cytokine profiles more accurately than bacterial relative abundance determined by 16S rRNA gene sequence analysis, and functional predictions based on 16S rRNA data ($p<0.0001$). The majority of microbial biological processes were underrepresented in women with high inflammation compared to those with low inflammation, including a Lactobacillus-associated signature of reduced cell wall organization and peptidoglycan biosynthesis. This signature remained associated with FGT inflammation in a subset of 74 women nine weeks later, was upheld after adjusting for Lactobacillus relative abundance, and was associated with in vitro inflammatory cytokine responses to Lactobacillus isolates from the same women. Reduced cell wall organization and peptidoglycan biosynthesis were also associated with high FGT inflammation in an independent sample of ten women.

Conclusions: Both the presence of specific microbial taxa in the FGT and their properties and activities are critical determinants of FGT inflammation. Our findings support those of previous studies suggesting that peptidoglycan is directly immunosuppressive, and identify a possible avenue for biotherapeutic development to reduce inflammation in the FGT. To facilitate further investigations of microbial activities, we have developed the FGT-METAP application that is available at (http://fgtdb.org/).

18

Modulation of mucosal macrophage function by the gut microbiota

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19

Pulmonary vaccines against Tuberculosis - effective and feasible

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More effective vaccines are essential to control the continuing pandemic of Tuberculosis (TB) globally. Two new developments in pulmonary TB vaccines will be presented. First, we have synthesised a whole protein conjugate vaccine linking the adjuvants, Pam2Cys and Pam3Cys, to the *M. tuberculosis* secreted protein ESAT-6. Delivery of these vaccines to the lungs stimulated antigen-specific Th1 and Th17 T cell responses that were associated with protection against *M. tuberculosis* lung infection in mice. Second, we have defined the characteristics of lung tissue-resident memory CD4 T cells induced by two different TB vaccines, viral (rIAV-P25) and protein/adjuvant (CysVac2/Advax) vaccines, that are protective when delivered to the lungs. The transcriptional similarities and differences between the lung TRM stimulated by these vaccines will be discussed.

20

How malaria parasites gain access to nutrients to facilitate their survival

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21

OPX: A new class of antibiotics for drug-resistant Gram-negative infections

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Infections caused by highly drug resistant Gram-negative bacteria such as *K. pneumoniae* and *P. aeruginosa* cause high mortality infections. Treatment options are limited, relying on 'last resort' historical agents with known toxicity liabilities such as the polymyxins. The evolution of high levels of resistance is making the last-resort polymyxin lipopeptide antibiotics (colistin and Polymyxin B) obsolete, with alternate antibiotics urgently required. The octapeptins are naturally derived products, first reported in the 1970s, that are structurally similar to the polymyxins. They retain activity against polymyxin resistant isolates, but to date have been largely ignored.

This talk will discuss our research program focused on developing octapeptin analogs (OPX) as a new class of antibiotics. This program recently received substantial funding from global agency CARB-X (the Combating Antibiotic-Resistant Bacteria Biopharmaceutical Accelerator), the first to an Australian organisation, and the first to any university in the world [1].

OPX retain activity against polymyxin-resistant Gram-negative bacteria, despite their structural similarity [2]. Mode of action studies, employing surface plasmon resonance, membrane probe assays, and fluorescently-labelled analogues prepared for membrane localization and permeabilisation studies, highlighted subtle variations in membrane interactions and permeability between the classes. A structure-activity-relationship campaign examined substituent effects and identified compounds with improved properties. Analogs were profiled for MIC potency, cytotoxicity and nephrotoxicity using primary human kidney cells. Promising compounds were advanced into *in vivo* mouse studies, including infection efficacy models, pharmacokinetic studies, and nephrotoxicity models.

We have also begun to investigate the application of OPX as potentiators (OPX-P), 'adjuvants' that help restore the activity of existing antibiotics that have become obsolete due to the development of resistance.

The octapeptins show promising activity against polymyxin- resistant MDR Gram-negative bacteria. Importantly, they appear to be much less susceptible to development of resistance than the polymyxin. Given the paucity of Gram-negative candidates, the octapeptins are a rare beacon of light in the fight against antimicrobial resistance.

1. [1] https://carb-x.org/carb-x-news/carb-x-funds-university-of-queensland-to-accelerate-the-development-of-a-new-class-of-last-resort-antibiotics-to-treat-deadly-superbug-infections/
2. [2] "Biosynthesis, structure and function of octapeptin antibiotics active against extremely drug resistant Gram-negative bacteria" Velkov, Gallardo-Godoy, Swarbrick, Blaskovich, Elliott, Han, Thompson, Roberts, Huang, Becker, Butler, Lash, Henriques, Nation, Sivanesan, Marco Sani, Separovic, Mertens, Bulach, Seemann, Li, Cooper. Cell Chemical Biology, (2018), 25 (4) 380-391. DOI: 10.1016/j.chembiol.2018.01.005, PMID: 22183171

22

Repurposing a neurodegenerative drug to treat Gram-negative antibiotic resistant bacterial infection

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The continual emergence of multi- and extensively-drug resistant (MDR; XDR) bacteria is a critical threat to human health, and alternative treatment strategies are urgently required. PBT2 is an orally bioavailable hydroxyquinoline ionophore, which is able to mediate the transfer of metal ions such as zinc across biological membranes. PBT2 has progressed to phase 2 human clinical trials for the treatment of Huntington's and Alzheimer's diseases, with once-daily oral doses of 250 mg shown to be generally safe and well-tolerated when administered for periods of six to 24 months. Here, we investigated the ability of the ionophore PBT2 to restore antibiotic sensitivity in MDR and XDR, ESBL-producing, carbapenem-resistant Gram-negative human pathogens. PBT2 resensitised *Klebsiella pneumoniae*, *Escherichia coli, Acinetobacter baumannii* and *Pseudomonas aeruginosa* to last-resort polymyxin class antibiotics, including the less toxic next-generation polymyxin derivative FADDI-287. PBT2 also resensitised several *A. baumannii* clinical isolates to the tetracycline class antibiotics - tetracycline, tigecycline and minocycline. The mechanism of action responsible for these affects appear to be mediated through the dysregulation of bacterial metal homeostasis. Using a highly invasive *K. pneumoniae* strain engineered for polymyxin resistance through *mgrB* mutation, we successfully demonstrated the efficacy of PBT2 + polymyxin (colistin or FADDI-287) for the treatment of Gram-negative sepsis in immune-competent mice. Moreover, PBT2 mediated tetracycline resensitisation was also efficaciously demonstrated *in vivo* for the treatment of tetracycline-resistant *A. baumannii* pneumonia. In comparison to either polymyxin or tetracycline antibiotic alone, the combination of PBT2 with either polymyxin or tetracycline significantly improved survival and reduced the bacterial burden in the major organs of infected mice. These data present a treatment modality to break antibiotic resistance in high priority antimicrobial resistant Gram-negative pathogens.

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23

A unique glycosyltransferase effector from enteropathogenic *Escherichia coli* that targets innate immune signalling proteins

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Gut bacterial pathogens including enteropathogenic *Escherichia coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC) are significant causes of diarrhoeal disease worldwide. During infection, the bacteria directly manipulate various aspects of host cell function by utilising a type III secretion system (T3SS) to translocate effector proteins directly into host cells. These effector proteins are essential for the bacteria to survive, replicate and cause disease.

We have identified several T3SS effectors with highly novel enzymatic activities, including the glycosyltransferase NleB1 of EPEC (1). Unlike other glycosyltransferases which add sugars to serine, threonine or asparagine residues, NleB1 transfers a single N-acetylglucosamine (GlcNAc) sugar to arginine residues, mediating Arg-GlcNAc modifications. NleB1 specifically glycosylates the death-domain of the adapter protein FADD and blocks host cell death during infection by preventing formation of the death-inducing signaling complex (DISC) in response FasL stimulation.

Homologues of NleB1 with conserved glycosyltransferase motifs are found within EPEC (termed NleB2) and *Salmonella* Typhimurium. Those from *Salmonella* appear to have similar enzymatic activities to NleB1. However, using Arg-GlcNAc-specific antibodies we found that NleB2 of EPEC does not catalyse this type of glycosylation when expressed in mammalian cells or during infection. In vitro glycosylation assays combined with mass spectrometry identified that in contrast to NleB1, NleB2 can utilise different sugar donors including UDP-GlcNAc, UDP-glucose and UDP-galactose to glycosylate the death domain of human RIPK1. Sugar donor competition assays revealed that NleB2 prefers UDP-glucose, and peptide sequencing identified the modification site within RIPK1 as an arginine residue, indicating that NleB2 catalyses arginine-glucose modifications.

We identified the residue in NleB1 and NleB2 that dictates this unique catalytic activity, using site-directed mutants and in vitro glycosylation assays. Although these mutations switch sugar donor preference, we found they do not affect the ability of these enzymes to inhibit inflammatory or cell death signaling during transfection or EPEC infection. Thus, this is the first identification of a bacterial enzyme that can catalyse arginine-glucose modifications, which are rare and previously reported only in plants. The switch in sugar donor preference that has arisen in NleB2 may allow for adaption to changes in sugar donor availability within the host cell cytoplasm.

1. 1. Pearson JS, Giogha C, Ong SY, et al. (2013). A type III effector antagonizes death receptor signalling during bacterial gut infection. Nature 501:247- 251.

24

The molecular basis for zinc uptake via *Streptococcus pneumoniae* AdcAII

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Streptococcus pneumoniae is a globally significant human pathogen that scavenges essential zinc [Zn(II)] ions from the host during colonization and infection. This is achieved by the ATP-binding cassette transporter, AdcCB, and two solute-binding proteins (SBPs), AdcA and AdcAII. Although both SBPs are required for full virulence of *S. pneumoniae*, AdcAII plays a greater role during the early stages of infection and under zinc limitation. However, the molecular details of how AdcAII acquires Zn(II) ions remain poorly defined. This can be attributed to the inability of crystallographic approaches to determine a high-resolution structure of ligand-free AdcAII. Here, we overcame this issue by systematically mutating each of the four Zn(II)-coordinating residues and performing structural and biochemical analyses on the variant isoforms. Structural analyses of Zn(II)-bound AdcAII variant proteins revealed how specific regions within the SBP undergo conformational changes via their direct coupling to each of the metal-binding residues. Quantitative *in vitro* metal-binding assays, combined with affinity determination and phenotypic analyses, revealed the relative contribution of each coordinating residue to the Zn(II)-binding mechanism. These analyses also revealed that in contrast to AdcA, AdcAII is permissive for interacting with other first-row transition metal ions. Intriguingly, the impact of mutant *adcAII* alleles on the growth of *S. pneumoniae* did not generally correlate with SBP affinity, but was instead consistent with the degree of structural perturbation exhibited in mutant AdcAII proteins. Taken together, our data show, for the first time, that SBP conformation rather than affinity is the primary determinant of efficacious Zn(II) uptake in *S. pneumoniae*. Collectively, our data reveal a novel metal-binding mechanism for AdcAII and highlight how ligand affinity and protein conformational

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changes are coupled within ligand-receptor proteins. These mechanistic insights provide a foundation for novel antimicrobial design to disrupt this process in bacterial metal-receptor proteins.

25

Humoral Immune Responses To *Plasmodium vivax* Malaria: Application To Sero-Surveillance For Accelerated Malaria Elimination

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Malaria infections due to *Plasmodium vivax* are a major challenge for elimination in the Asia-Pacific region. Improved surveillance tools appropriate for use in low transmission settings are required. Improving our understanding of naturally acquired immune responses induced following *P. vivax* infections could enable novel strategies to be implemented. In this study we aimed to characterise the *P.* vivax-specific antibody response in individuals from endemic areas by assessing the acquisition and decay of total IgG, IgG subclass, IgM and functional antibodies.

Using a panel of 60 *P. vivax* proteins, we identified a bi-phasic pattern of IgG antibody decay over 9 months, following a peak 1-2 weeks after clinical *P. vivax* infection in Thai patients (n=34). In these individuals with limited past exposure, IgG1 was the dominant subclass and followed similar kinetics to total IgG. We observed that IgM responses are long-lived in these Thai patients. To assess functional antibody responses, we have developed and validated a novel multiplexed assay to measure the interaction between *P. vivax*-specific IgG and complement (C1q). We are currently measuring the presence and decay of C1q-fixing antibodies in the same cohort of Thai patients.

We tested the use of antibody responses to all 60 proteins for classifying individuals as recently exposed to *P. vivax* parasites or not. Using total IgG responses to a panel of 8 *P. vivax* proteins, we could accurately classify recent exposure with > 80% sensitivity and specificity using large yearlong observational cohort studies in Thailand (n=829), Brazil (n=928) and the Solomon Islands (n=754). These serological markers of recent exposure can enable efficient targeting of limited resources for malaria elimination, and highlight the value in increasing our understanding of naturally acquired immune responses to malaria.

26

Evaluation of serosurveillance to understand the micro-heterogeneity of sub-microscopic malaria and other malaria risk factors in Western Cambodia.

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In low transmission settings approaching malaria elimination, such as the Greater Mekong Subregion, a large proportion of *Plasmodium* spp. infections are sub-microscopic. Sub-microscopic infections are molecular-detectable low parasite density infections which go undetected, and therefore untreated, using routine field diagnostics. Antibody serosurveillance, has the potential to detect sub-microscopic infections, as both current and recent exposure events. In order understand the potential use of serosurveillance to identify sub-microscopic infections and high-risk populations in low transmission settings, we determined seroprevalence and levels of antibodies specific for *Plasmodium* spp. antigens by ELISA in 990 participants living in 20 Western Cambodian villages. Within this population sub-microscopic *Plasmodium* spp. prevalence was at 9.2% (91/990), with few (n=7) detectable by microscopy. Seroprevalence was high, with 77.7% (769/990) of the total cohort being seropositive to *Plasmodium falciparum* apical membrane antigen 1 (Pf-AMA1). Both Pf-AMA1 levels and seroprevalence varied across villages (p<0.001), with seroprevalence ranging from 65% (28/50) to 100% (50/50). Variation of seroprevalence was quantified using multivariate mixed effects regression which found marked heterogeneity between villages (adjusted ICC Rho [95%CI]) (0.280 [0.147, 0.467]). Risk factors for seropositivity identified from the multivariate models included the presence of sub-microscopic infections and frequency of self-reported malaria history whereby the odds of sero-positivity approximately doubled in

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those with current or more than 2 historical infections. The odds of sero-positivity also increased with age, but no association was found with sex, overnight forest stays, international travel, or bed net use. This study supports the utility of malaria antibodies as a serosurveillance tool to determine the micro-heterogeneity of malaria transmission in low transmission areas as well as current sub-microscopic infections and historical (clinical) infections. Its application will allow the micro-stratification of malaria risk in a population to enable spatially targeted interventions to advance progress towards the target of malaria elimination in the Greater Mekong Subregion by 2030.

27

Defining transcriptome dynamics underlying memory CD4+ T cell development during malaria

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Hallmark features of adaptive CD4⁺ T-cells include differentiation into multiple effector T helper (Th) fates and subsequent emergence of memory states. Fate mapping, adoptive transfer and single-cell tracking approaches in mouse models of acute viral or bacterial infection suggest that memory $CD4^+$ T cells develop directly from effector precursors. However, molecular pathways governing these developmental transitions remain undefined. Here we tested the hypothesis that $CD4^+$ Th cells directly convert into memory CD4+ T cells during malaria *in vivo*, and in doing so defined underlying transcriptomic processes. Tracking endogenous TCR sequences via TraCeR analysis firstly confirmed that sibling clones populated both Th1 and Tfh fates. Instead of transitioning through a memory precursor population, two memory cell lineages were detected to originate directly from either T helper 1 (Th1) or follicular T helper (Tfh) effector cells. Memory development in both lineages were characterized over three weeks by a progressive 50% reduction in the numbers of genes expressed, as well as partial transcriptomic convergence towards memory. Low-level persisting infection in this model diverted but did not block memory development, which was associated instead with onset of partial exhaustion. We inferred within the Th1-lineage a linear transition from Th1 via Type 1 regulatory T (Tr1) to effector memory T (T_{EM}) cells, with T_{EM} cells poised for Th1 re-call. In contrast, the Tfh-lineage exhibited a modest Th1-signature throughout, no evidence of Tr1 development, and lineage-specific co-expression of central memory T (T_{CM}) markers. A subset of memory-associated genes was also defined, including transcription factors *Id2* and *Maf*, whose expression increased progressively against a background of transcriptomic quiescence. Thus, by examining transcriptional dynamics using scRNA-seq, we describe the molecular pathways taken by effector CD4+ T cells towards memory.

28

High dimensional mass cytometry analysis dissects T cell and B cell heterogeneity in the immune response to *Plasmodium vivax* malaria and identifies signatures predicting immunity to infection

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T follicular helper (T_{FH}) cells and MBCs play a crucial role in the induction, maintenance and recall of antibody responses, which are a key component of clinical immunity to malaria. Previous studies have shown that shown that inflammatory pathways associated with the development of clinical symptoms during acute infection induce alterations in both the composition and function of the T_{FH} and MBC compartments, including up-regulation of the T_{H1}-transcritpion factor T-bet. However, there are conflicting reports on the association of these T-bet⁺ $CD4^+$ T cells and MBCs with human malaria.

Most of our current knowledge on immune responses to malaria are derived from bulk population data, which assumes that cells belonging to the same sub-type are homogeneous. These bulk-like analyses often oversimplify the immune response, leading to the conflicting view that the same $CD4^+$ T cell and MBC responses appear to associate with opposite infection outcomes. To address this issue, we used high-dimensional single cell mass cytometry to untangle the complexity of the $CD4^+$ T cell and MBC response induced in response to *Plasmodium vivax* infection. Our main results identified a specific subset of T_{H2}-like circulating T_{FH} cells strongly associated with protection from infection. Unlike previous reports in *P. falciparum* infection, class-switched but not IgM^+ MBCs appeared to be required for protection. Furthermore, our approach was able to identify distinct T-bet⁺ sub-populations within the $CD4^+$ T cell and MBC compartments that were associated with either increased or reduced risk of symptomatic and asymptomatic *P. vivax* infection, supporting the notion that inflammatory responses to malaria are not unanimously detrimental and might also support immune networks contributing to the control of infection.

29

Regulation of NOD-like receptor signaling: role in colorectal cancer

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30

Immune responses mediated by bacterial membrane vesicles and their intracellular fate.

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Bacterial membrane vesicles are released by all bacteria as part of their normal growth. Both Gram-negative and Gram-positive bacteria produce membrane vesicles, referred to as outer membrane vesicles (OMVs) and membrane vesicles (MVs), respectively. Although initially considered to be artifacts of bacterial growth, it is now well established that bacterial membrane vesicles are a *bone fide* secretion mechanism used by all bacterial to facilitate intracellular communication, bacterial survival and to promote disease. To date, most of our knowledge regarding the composition and functions of bacterial membrane vesicles is about OMVs produced by Gram-negative bacteria. In contrast, less is understood about MVs produced by Gram-positive bacteria, and in particular we have very limited knowledge regarding their cargo composition, immunogenicity and intracellular fate once within host cells.

Here we discuss factors that regulate the size, composition and immunogenicity of OMVs produced by Gram-negative bacteria. In addition, we discuss our recent findings identifying the cargo composition of MVs, the mechanisms whereby they mediate innate immune responses and their intracellular fate. Collectively, the findings advance our limited knowledge regarding the regulation of bacterial membrane vesicle size, composition and immunogenicity, and identify key factors that need to be considered for the refinement of their use in a range of medical and therapeutic applications.

31

New drugs for superbugs: immunotherapeutics that enhance neutrophil clearance of *Staphylococcus aureus*

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S. aureus has two superpowers: invisibility and invincibility. *S. aureus* has evolved a diversity of attack and counter-attack measures to our immune response, allowing it to hide and evade the innate immune defences and establish infections - almost as if it were invisible. Additionally, *S. aureus* readily develops resistance to antibiotics, becoming almost invincible to our treatment strategies. Methicillin-resistant *S. aureus* (MRSA) strains are all too common in hospital settings and cause 10,000 death a year in the USA alone. Antibiotic resistance in *S. aureus* is often associated with a decrease in neutrophil recruitment - a vital step in ensuring a successful immune response to the infection. In this work, we have developed improved antibiotic agents that act as dual-function agents, enhancing neutrophil recruitment and directly killing of *S. aureus.* Our new antibiotics exploit formylated peptides as chemoattractants for neutrophil recruitment, which is combined with the targeted binding of vancomycin to the bacterial cell wall. This attachment of a chemoattractant directly to the bacteria by vancomycin, generates a chemotactic gradient around S. aureus, guiding neutrophils to the invading *S. aureus.* Using the combination of *in vitro* assays, infection-on-a-chip and *in vivo* mouse models, we have determined that these antibiotic-chemoattractants improve the recruitment, engulfment and killing of *S. aureus* by neutrophils. This offers a new paradigm in combating antibiotic-resistant bacteria by countering the superpowers of superbugs.

32

RIPK1: a master regulator of cellular immunity in protection against mucosal infection

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RIPK1 (receptor-interacting serine/threonine-protein kinase 1) is a master regulator of cellular fate: its activation can define whether cells live or die when exposed to cellular stress. RIPK1-mediated cell death leads to disruption of epithelial barriers and release of damage-associated molecular patterns, cytokines, and chemokines, propagating inflammatory and degenerative diseases, such as Alzheimer's disease, multiple sclerosis and amyotrophic lateral sclerosis. Thus, RIPK1 has emerged as a compelling drug target for the treatment of human neurodegenerative, autoimmune, and inflammatory diseases. The intriguing converse of RIPK1-driven disease pathogenesis and inhibitory therapeutic development, is the recent discovery of humans with complete *RIPK1* deletion. Here, patients suffered severe, recurrent mucosal infections, intestinal inflammation, and a 40% mortality rate. The past two decades of research has shed much light on the mechanistic roles of RIPK1, however its role *in vivo* in pathogen infection remains largely unresolved. Here we explored the role of RIPK1 in controlling infection by the global human and animal pathogen *Salmonella* Typhimurium, responsible for ~153 million cases of gastroenteritis and 57,000 deaths annually. There is currently no effective vaccine or therapeutics for *S.* Typhimurium in humans and

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antibiotics are only indicated if disease is invasive, as treatment can promote convalescent carriage. We showed that in the absence of RIPK1, infection with *S.* Typhimurium induces highly dysregulated cytokine responses (IFN gamma, TNF, and IL-6), uncontrolled bacterial burden, and significant mortality in mice. Surprisingly, we found that just one allele of RIPK1 is sufficient to rescue mice from this severe disease phenotype, restoring cytokine homeostasis, controlling bacterial load and enabling 100% survival of animals. Importantly, we have demonstrated for the first time that the kinase activity of RIPK1 is dispensable for controlling *S.* Typhimurium infection, suggesting that the role of RIPK1 in protecting against infection is unrelated to its role in cell death signaling, but rather highly related to its role in inflammatory signaling. This data, along with the recent characterisation of RIPK1-deficient patients, highlights the critical role of RIPK1 in tissue homeostasis, and raises the important question of how the inevitable introduction of RIPK1 inhibitors as therapeutics may impact host susceptibility to microbial infections.

33

Mapping Autoantibody Responses in Children with Acute Rheumatic Fever

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Acute rheumatic fever (ARF) and associated rheumatic heart disease (RHD) are serious sequelae of Group A *Streptococcus* (GAS) infection. Rates of ARF/RHD remain unacceptably high in Indigenous Aboriginal children in Australia and Māori and Pacific children in New Zealand. Pathogenesis remains poorly understood, with the current prevailing hypothesis based on molecular mimicry and the notion that antibodies generated in response to GAS infection cross-react with cardiac proteins such as myosin. Specific laboratory tests for ARF are lacking, with diagnosis relying on presentation of a set of clinical symptoms. This presents a major hurdle in disease control efforts, with an accurate diagnosis requiring a series of assessments over a period of days. The aim of this study is to map autoantigens targeted by serological antibodies in ARF patients using a multi-platform approach to both inform pathogenesis models and identify new biomarkers for the disease.

Sera from patients with ARF, matched healthy controls and patients with uncomplicated GAS pharyngitis was analysed for autoreactivity using high content protein arrays (Protoarray, 9000 autoantigens). Autoantigens were validated using a second high content protein array (HuProt Array, 16,000 autoantigens) and 2-D gel electrophoresis using human heart lysate with LC-MS/MS mass spectrometry. Individual autoantigens were further validated using conventional immunoassays using sera from a nationwide case-control study of ARF conducted in New Zealand (RF RISK Study). Disease pathway analysis revealed several auto-antigens within pathways linked to arthritic and myocardial disease. Notably autoantigen profiling revealed known disease-associated autoantibodies (anti-Myosin and anti-collagen antibodies) as well as novel candidates. These novel candidates are potential ARF biomarkers and provide new insight into disease pathogenesis.

34

Rough and smooth variant *Mycobacterium abscessus* infections are differentially controlled by host immunity during chronic infection

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Infections caused by *Mycobacterium abscessus* are increasing in prevalence within patient groups with respiratory comorbidities. Initial colonisation by the smooth colony *M. abscessus* (S) can be followed by an irreversible genetic switch into a highly inflammatory rough colony *M. abscessus* (R), often associated with a decline in pulmonary function. Our understanding of the role of adaptive immunity in *M. abscessus* pathogenesis is largely unknown. Here, we have used intraperitoneal infection of adult zebrafish to model *M. abscessus* pathogenesis in the context of fully functioning host immunity. Infection with either variant resulted in sustained burdens of infection for over one month, a significant improvement on existing mouse models of *M. abscessus* infection. We find infection with the R variant penetrates host organs causing an inflammatory immune response leading to necrotic granuloma formation within 2 weeks. The R bacilli are targeted by T cell-mediated immunity and burden is constrained. Strikingly, the S variant colonises host internal surfaces at high loads and is met with a robust innate immune response but little T cell-mediated immunity. Invasive granuloma formation is delayed in S variant infection compared to R variant infection upon which T cell-mediated immunity is required to control infection. In mixed infections, the S variant outcompetes the R variant. We also find the R variant activates host immunity to the detriment of S variant *M. abscessus* in mixed infections. These findings demonstrate the applicability of the adult zebrafish to model persistent *M. abscessus* infection and provide insight into the immunopathogenesis of chronic *M. abscessus* infection.

35

Mutations in *Streptococcus pneumoniae* biosynthesis genes influence pneumococcal interactions with human respiratory epithelial cells and alter the epithelial-innate immune response

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Human colonization by *Streptococcus pneumoniae* is a prerequisite for transmission and pneumococcal disease, causing 800,000 deaths/year. It is not fully understood how the bacteria transitions to cause invasive disease. The first line of defense occurs during interactions with the nasopharyngeal epithelium where carriage induces protective immunity. We have previously demonstrated using an experimental human pneumococcal challenge model (EHPC) and *in vitro* culture models, that pneumococci form surface micro-colonies

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and micro-invade nasal epithelial cells. Epithelial cell transcriptomic activation responses coincide with bacterial clearance in the EHPC, suggesting that the epithelium is central to the control of pneumococcal colonization.

In this study, we characterized the impact of a serotype 6B strain with deletions in pneumococcal biosynthesis genes (ΔX/PiaA and ΔY/PiaA) on epithelial-innate immune responses *in vitro*. We explored the hypothesis that these mutations influence pneumococcal interactions and the epithelial cell transcriptomic profile during micro-invasion events.

Using Detroit 562 cells, we observed a ten-fold reduction in both mutants' association to the epithelium and a five-fold reduction in transmigration, compared to wild type colony forming unit counts. However, both mutants exhibited a higher micro-invasive index, and led to elevated epithelial secretion of IL-6 and IL-8. In contrast to the similarity between the mutant strains in the functional assays, the epithelial transcriptional responses following infection revealed a strong similarity between wild type and ΔX/PiaA strains, including induction expression of cytokine and chemokine genes and NF-κB transcriptional regulators. Although ΔY/PiaA induced these pathways to a lesser extent, we also discovered enhanced intracellular signaling, sorting and processing responses, which associated with elevated IL-33 protein secretion.

These data reveal a surprising uncoupling between the host cellular transcriptional response and the ability of pneumococcal strains to cross the epithelial barrier. Using the EHPC model, we will test the mutants' ability to establish carriage and micro-invasion and, determine the epithelial transcriptomic signatures.

36

A role of *C. albicans* Rpn4 in regulating antifungal drug susceptibility

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Publish consent withheld

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37

The experimental evolution of antibiotic resistance reveals chinks in the armor of multidrug-resistant pathogens.

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Experimental evolution is a powerful method for testing fundamental questions in evolution and ecology. Horizontal gene transfer has been shown by comparative genomics to be important for microbial evolution. However, it has been difficult to carry out evolution experiments that track the flow of genetic elements into evolving populations of microbes. This means that many of the lessons learned from the field of experimental evolution may not apply in natural populations. Here we use a new model for experimental evolution – *Helicobacter pylori* – which is able to take up DNA that is added to its growth media. We use whole population metagenomics sequencing to confirm that these genes are taken up by the evolving population, and then track them over 200 generations of evolution. We find that gene flow from outside the population, and between individuals, allows for the introgression and maintenance of neutral or mildly deleterious alleles. We measure the fitness effects of thousands of genetic variants in media with and without antibiotic and find that abrogation of many gene targets is strongly deleterious in multidrug environments but neutral in antibiotic-free conditions. This study shows how horizontal gene transfer can potentiate antibiotic resistance and suggests a new set of genetic targets for antimicrobial strategies.

38

Solving bottlenecks and providing new strategies for AMR crisis

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Antimicrobial resistance (AMR) is emerging as one of most serious public health threats of our times. Here I will be presenting our recent efforts on applying systems-based approaches to different angles of the problem – from identifying the mode-of-action of new compounds to understanding new AMR risks, and from exploring the potential of drug combinations for new therapies to their use to reduce the collateral damage of antibiotics to the gut microbiota.

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39

Organelle wars and the enemy within

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Mitochondria are derived from endosymbiotic bacteria, with remnants of their proteobacterial past still evident today. These organelles replicate within cells through the process of mitochondrial fission. We reasoned that fission may mimic intracellular bacterial replication, thus serving to alert immune cells of a bacterial threat. Here we show that Toll-like receptor (TLR) signalling triggers activating post-translational modifications of the fission-promoting GTPase, Drp1, as well as Drp1-dependent mitochondrial fission in murine macrophages. Genetic and pharmacological approaches revealed that TLR-inducible fission promotes bacterial killing by macrophages, with both the mitochondrial unfolded protein response and lipid droplets playing a role in antimicrobial defence. The lysine deacetylase histone deacetylase 6 (HDAC6) constrains TLR-inducible fission in macrophages, via its actions on the mitochondrial fusion-promoting GTPase, Mfn1. Consequently, genetic targeting or inhibition of HDAC6 amplifies fission and enhances clearance of the intramacrophage pathogen Salmonella enterica serovar Typhimurium *in vitro* and *in vivo*. Fission-mediated bacterial killing is also apparent in the nematode *Caenorhabditis elegans* (*C. elegans*), highlighting the broad conservation of this host defence pathway across the animal kingdom. Agents that amplify fission may have applications as host-directed therapies to combat antibiotic-resistant bacterial infections.

40

Arginase-2 is essential for IL-10 metabolic reprogramming of inflammatory macrophages at the mitochondria

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Recent evidence highlights the importance of mitochondrial bioenergetics and dynamics in regulating macrophage polarisation. We demonstrate that Arginase-2 (Arg2), one of two arginase isoforms, is a miR-155 and interleukin-10 (IL-10) regulated gene localized at mitochondria in inflammatory macrophages, and is critical for IL-10 induced restoration of oxidative respiration. We show that IL-10 radically affects mitochondrial dynamics by promoting a state of enhanced 'fusion', which likely facilitates the higher oxidative bioenergetics we observe. Mechanistically, we show that both the catalytic activity and presence of Arg2 at the mitochondria is crucial for boosting oxidative phosphorylation (OxPhos). We further show Arg2 mediates this process by influencing activity of complex II (also known as succinate dehydrogenase (SDH)), a bi-functional enzyme that links the mitochondrial electron transport chain (ETC) and the TCA cycle. We subsequently show Arg2 is crucial for IL-10 mediated downregulation of inflammatory mediators including succinate, hypoxia inducible factor 1α (HIF-1α) and interleukin 1β (IL-1β) *in vitro*. Additionally, we show Arg2 expression was significantly downregulated in IL-10 deficient mice in an LPS-induced acute model of inflammation. Furthermore, we observed elevated levels of HIF-1α and IL-1β in this model using Arg2 deficient mice. These findings shed light on a new arm of IL-10 mediated metabolic regulation via Arg2, where it works to resolve the inflammatory status of the cell.

41

Z-DNA-binding protein-1 regulates necroptosis-mediated skin inflammation

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Z-DNA Binding Protein-1 (ZBP1) is a cytoplasmic innate immune sensor, which contains nucleic acid binding domains (Za) and RIP Homotypic Interaction Motif (RHIM). ZBP1 mediates host defense against some viruses by sensing viral nucleic acids. Here we have identified a pro-inflammatory role of ZBP1 in inducing necroptosis-mediated skin inflammation in mice.

RIPK1 controls inflammation and cell death and is important to maintain tissue homeostasis. Keratinocyte-specific deletion of RIPK1 ($RIPK1^{E\text{-}KO}$) leads to progressive inflammatory skin disease in mice, which is characterized by epidermal hyper-proliferation, infiltration of immune cells and up-regulation of pro-inflammatory cytokines. Genetic deletion of RIPK3/MLKL, which are essential for necroptosis, completely protected the inflammatory skin lesions in $RIPK1^{E\text{-}KO}$ mice *(Nature, 2014)*, showing necroptosis as a major trigger of skin inflammation. However, the mechanism by which RIPK1 counteracts RIPK3-MLKL-dependent inflammation remains unknown. We observed up-regulation of ZBP1 protein in the epidermis and reasoned that ZBP1 may regulate inflammation in $RIPK1^{E\text{-}KO}$ mice. To address the specific role of ZBP1 and its Za and RHIM domains, we generated $RIPK1^{E\text{-}KO}$ mice lacking ZBP1 ($RIPK1^{E\text{-}KO}$*Zbp1*$^{-/-}$) or having mutation in the Za domain ($RIPK1^{E\text{-}KO}$*Zbp1*$^{mZa2/mZa2}$) or RHIM domain ($RIPK1^{E\text{-}KO}$*Zbp1*$^{mR1/mR1}$). Remarkably, inhibition of ZBP1 or its Za- or RHIM- mediated function prevented the development of inflammatory skin lesions, and inhibited the up-regulation of pro-inflammatory factors in the skin of $RIPK1^{E\text{-}KO}$ mice, demonstrating that endogenous nucleic acid sensing and RHIM-domain of ZBP1 trigger skin inflammation in $RIPK1^{E\text{-}KO}$ mice. Additionally, we detected endogenous retroelements-derived complementary reads in the epidermal RNA, and treatment with a combination of reverse transcriptase inhibitors ameliorated the skin inflammation in $RIPK1^{E\text{-}KO}$ mice, suggesting that double-stranded RNA derived from retroelements may trigger Za-domain-dependent activation of ZBP1 *(Nature, 2016; Nature, 2020).*

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Collectively, we identified that epidermal RIPK1 counteracts RIPK3/MLKL-dependent necroptosis by inhibiting Za-dependent sensing of endogenous nucleic acid and RHIM-dependent function to prevent skin inflammation.

42

Histone deacetylase 7 coordinates innate immune defence by promoting phagocytosis, NADPH oxidase-dependent reactive oxygen species production and bacterial clearance

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Histone deacetylases (HDACs) remove acetyl groups from lysine residues of histone and non-histone proteins. Class IIa HDACs (HDAC4, 5, 7, 9) drive macrophage inflammatory responses, but their contributions to host defence have not been explored. Here, we show that pharmacological or genetic targeting of Hdac7 led to greatly increased bacterial loads and dissemination of a representative strain (EC958) of the globally-disseminated uropathogenic *Escherichia coli* clone ST131, in an intraperitoneal challenge model in mice. Challenge of primary mouse macrophages with *E. coli* also resulted in a rapid increase in class IIa HDAC enzyme activity. Pharmacological or genetic targeting of Hdac7 impaired phagocytosis of *E. coli* and bacterial clearance by primary murine macrophages. Conversely, primary macrophages from Mac-Hdac7 mice that selectively over-express Hdac7 in myeloid cells displayed increased phagocytosis and intracellular killing in comparison to control cells. The antimicrobial effects of Hdac7 were dependent on its enzymatic activity, since an enzyme dead mutant was unable to reconstitute antimicrobial functions of Hdac7. Interestingly, the antimicrobial effects of Hdac7 were independent of its proinflammatory functions, which are mediated by the glycolytic enzyme pyruvate kinase isoform M2. Instead, we provide evidence that Hdac7 drives antimicrobial responses via the pentose phosphate pathway enzyme, 6-phosphogluconate dehydrogenase (6-PGD). Hdac7 promotes phagocyte oxidase-dependent reactive oxygen species production, with inhibition of either glucose uptake or phagocyte oxidase blocking the antimicrobial phenotype of Mac-Hdac7 macrophages. Mechanistically, genetic targeting or pharmacological inhibition of Hdac7 significantly reduces the activity of 6-PGD, an enzyme generating NADPH for phagocyte oxidase function. Finally, in contrast to its role in promoting lipopolysaccharide (LPS)-driven IL-1b production, Hdac7 limits *E. coli*-induced IL-1b, suggesting that Hdac7 discriminates between different types of danger. We propose that HDAC7 responds to local threats by coordinating effective innate defence and limiting leukocyte recruitment, whereas it drives a systemic inflammatory response upon sensing distal danger.

43

Succination inactivates gasdermin D and blocks pyroptosis

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Activated macrophages undergo a metabolic switch to aerobic glycolysis accumulating Krebs cycle intermediates that alter transcription of immune response genes. Here we extend these observations by defining fumarate as an inhibitor of pyroptotic cell death. We found that dimethyl fumarate (DMF) delivered to cells or endogenous fumarate reacts with gasdermin D (GSDMD) at critical cysteine residues to form S-(2-succinyl)-cysteine. This succination of GSDMD prevents its interaction with caspases, limiting its processing, oligomerization, and capacity to induce cell death. Administration of fumarate protects in mice against LPS shock, alleviates familial Mediterranean fever and experimental autoimmune encephalitis (EAE) by targeting GSDMD. Collectively, these findings identify GSDMD as a target of fumarate and reveal a novel mechanism of action for fumarate-based therapeutics including dimethyl fumarate used to treat multiple sclerosis.

44

My career path and advocacy

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45

Bringing metagenomics to the patient bedside to combat the emerging global health threat: the rise of invasive fungal infections

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Invasive fungal diseases (IFDs) cause 1.6 million deaths/year globally and account for 10% of all hospital-acquired infections. Identification of the causative agents is essential in the management of IFDs, with the ideal diagnostic method being rapid, accurate and informative whilst remaining cost-effective. If diagnosed in a timely manner, IFDs are treatable, but despite effective antifungal therapies, the death rate from IFDs can still reach ~50%, which is largely due to the fact that fungal infections are under-recognized, and their diagnosis using conventional diagnostic techniques is typically delayed by days to weeks and lacks specificity and sensitivity. The evolution of next generation sequencing has revolutionised genomic studies to generate more data at a cheaper cost. Long-read/third generation sequencing specifically offers a unique advantage over other diagnostic methods, namely the simultaneous detection of disease agents

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(bacteria, viruses, fungi, etc.), their interaction, their resistance profiles and their genetic relatedness directly from clinical samples in real-time. The application of long-read sequencing to clinical diagnosis has begun to be explored through both metagenomic and metabarcoding approaches. These preliminary studies highlighted the need for further improvement and optimization, especially to increase the pathogen:host DNA ratio, high-quality DNA preparations from clinical specimens and the improvement of reference datasets and bioinformatic tool. This talk will discuss the cons and pros as well as give samples for its use in the clinical diagnosis of IFDs. The implementation of long-read based metagenomics as diagnostic tool for IFDs will result in a drastic reduction in turnaround times, from days/weeks (now) to <24h, and a timely initiation of tailored antifungal treatment, which will, in turn, improve patient outcomes, reduce morbidity and mortality, and greatly reduce health care costs.

46

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

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Sepsis is a biphasic disease characterized by an acute inflammatory response, followed by a prolonged immunosuppressive phase. Therapies aimed at controlling inflammation help to reduce the time sepsis patients spend in intensive care units, but they do not lead to a reduction in overall mortality. Recent focus has been on addressing the immunosuppressive phase, often caused by apoptosis of immune cells. However, molecular triggers of these events are not yet known. Using a whole genome CRISPR screen in mice we identified Trigger Receptor Expressed in Myeloid-Like 4 (TREML4) as a key receptor that regulates inflammation and immune cell death in sepsis. Genetic ablation of *Treml4* in mice demonstrated that TREML4 regulates calcium homeostasis, the inflammatory cytokine response, myeloperoxidase activation, the ER stress response and apoptotic cell death in innate immune cells, leading to an overall increase in survival rate, both during the acute and the chronic phases of polymicrobial sepsis including Pseudomonas aeruginosa pneumonia and invasive candidiasis.

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47

Genomic Analysis of Fungal Morphogenesis and Interaction with Host Immune Cells

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Fungi pose a serious threat to human health, with *Candida albicans* as both a leading fungal pathogen of humans and a member of the human mucosal microbiome. A major virulence trait of *C. albicans* is its ability to transition between yeast and filamentous morphologies. The yeast form of *C. albicans* is crucial for colonization and dissemination via the bloodstream, while the filamentous form enables tissue invasion and deep-seated infection. Here, I discuss recent work exploring genomic analyses that implicate core regulators of proteostasis in governing temperature-dependent morphogenesis. I also discuss recent work that identifies determinants of *C. albicans* filamentation in host immune cells, given that internalization of *C. albicans* by macrophages induces a transition from yeast to filamentous growth that promotes macrophage cell death and fungal escape. The filamentation response within macrophages has been attributed to conditions within the phagosome such as nutrient deprivation, alkaline pH, and oxidative stress, however, the impact of other host-derived factors has remained unknown. Here, I show that lysates prepared from macrophage-like cell lines robustly induce *C. albicans* filamentation and implicate a phosphorylated, immunomodulatory peptide. I discuss the relationship between filamentation and cell wall remodelling in the induction of pyroptosis, a macrophage inflammatory cell death program. Together, this work explores mechanisms governing a key virulence trait that is central to a complex host-pathogen interaction.

48

Creating Vaccines at Pandemic Speed: Molecular clamp stabilized subunit vaccine

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49

Pre-empting danger to protect against gut infections

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The maintenance of immune homeostasis in the gastrointestinal tract guarantees our health and prevents the development of serious infection. How this occurs in the gut in the face of the massive load of food material ingested throughout a day, has been unclear. Here, we examine the tempo-spatial mechanism describing how innate lymphoid cells orchestrate this protection and physiologically integrate the multitude of antigenic inputs composed of food and microbes to restrict the development of inflammation and prevent invasion of harmful bacteria.

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50

Immunomodulatory effects of neonatal Bacillus Calmette–Guérin vaccination on responses to unrelated pathogens

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The live attenuated vaccine Bacillus Calmette–Guérin (BCG) is given to the majority of infants worldwide to protect against tuberculosis (TB). In addition to protecting against TB and other mycobacterial infections, BCG vaccination has beneficial off-target effects associated with an up to 45% reduction in all-cause infant mortality in high-mortality settings. This is proposed to result from BCG-mediated protection against non-mycobacterial infections. Neonatal BCG vaccination may also reduce infant allergy and eczema.

We have established a randomised controlled trial, the Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR)[1], in which 1272 neonates were randomised to receive BCG vaccination or not, to determine if BCG protects against allergic sensitisation, eczema and infections in infancy. Using samples from MIS BAIR participants, we investigated the immunomodulatory effects of neonatal BCG vaccination.

Our studies revealed that neonatal BCG vaccination reduces *in vitro* cytokine and chemokine responses to non-mycobacterial pathogens[2]. In addition to altering the level of cytokine secretion, we found that in BCG vaccination induced an interferon (IFN)-γ 'non-responder' state in infants resulting in failure to produce IFN-γ following *in vitro* stimulation with a range of pathogens[3]. Consistent with BCG's heterogeneous actions in protecting against TB, we observed heterogeneity in the effect of BCG vaccination on immune responses to mycobacterial and non-mycobacterial pathogens. We identified several modifiable and non-modifiable factors that contributed to this heterogeneity and thus may impact the beneficial off-target effects of BCG.

Like BCG, measles containing vaccines (MCV) reduce all-cause infant mortality in high-mortality settings. Using samples taken from MIS BAIR participants, we also investigated the effects of MCV on infant immune responses to unrelated pathogens to determine whether the beneficial off-target effects of BCG and MCV are mediated by similar mechanisms.

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51

Immune system development varies according to age, location, and anemia in African children

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Children from low- and middle-income countries, where there is a high incidence of infectious disease, have the greatest need for the protection afforded by vaccination, but vaccines often show reduced efficacy in these pop- ulations. An improved understanding of how age, infection, nutrition, and genetics influence immune ontogeny and function is key to informing vaccine design for this at-risk population. We sought to identify factors that shape immune development in children under 5 years of age from Tanzania and Mozambique by detailed immuno-phenotyping of longitudinal blood samples collected during the RTS,S malaria vaccine phase 3 trial. In these cohorts, the composition of the immune system is dynamically transformed during the first years of life, and this was fur- ther influenced by geographical location, with some immune cell types showing an altered rate of development in Tanzanian children compared to Dutch children enrolled in the Generation R population–based cohort study. High-titer antibody responses to the RTS,S/AS01E vaccine were associated with an activated immune profile at the time of vaccination, including an increased frequency of antibody-secreting plasmablasts and follicular helper T cells. Anemic children had lower frequencies of recent thymic emigrant T cells, isotype-switched memory B cells, and plasmablasts; modulating iron bioavailability in vitro could recapitulate the B cell defects observed in anemic children. Our findings demonstrate that the composition of the immune system in children varies according to age, geographical location, and anemia status.

52

Novel virus-like particle encoding the circumsporozoite protein is an immunogenic malaria vaccine in mice

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Plasmodium falciparum malaria is a major cause of global morbidity and mortality, and the development of an efficacious vaccine would greatly aid control and elimination efforts. RTS,S is the leading malaria vaccine candidate, but has demonstrated only moderate protective efficacy in clinical trials. RTS,S is a virus-like particle (VLP) that uses human hepatitis B virus as a scaffold to display the malaria antigen, circumsporozoite protein (CSP). Particle formation requires fourfold excess scaffolding antigen compared to the target CSP antigen, which may limit vaccine immunogenicity and efficacy.

Here, we describe a novel VLP that uses the small surface antigen (dS) of duck hepatitis B virus to display the CSP antigen. The CSP-dS fusion protein successfully formed particles without the need for excess scaffold antigen, and therefore CSP represented a larger portion of the vaccine construct compared to conventional VLP-based vaccines, such as RTS,S. CSP-dS VLPs formed large particles up to 70 nm in size that correctly displayed CSP on the surface.

The novel CSP-dS VLP formulated with an alum adjuvant was highly immunogenic in mice and induced antibodies to multiple regions of the CSP, even when administered at a lower vaccine dose. We recently established that functional antibody responses were associated with protective efficacy in human clinical trials of the RTS,S malaria vaccine. These included antibody-mediated fixation and activation of the complement system, and antibody interactions with Fcγ-receptors to promote opsonic phagocytosis. Importantly, our novel CSP-dS VLPs also induced functional antibodies in mice that fixed complement and interacted with Fcγ-receptors.

The platform we describe to produce VLPs without the need for excess scaffolding antigen is highly novel and warrants further evaluation in pre-clinical efficacy studies and as a platform to produce other candidate malaria antigens as VLP-based vaccines.

53

Dendritic cell paralysis contributes to immunosuppression and secondary infections long after severe infection or trauma; investigation on the mechanisms, diagnostic markers and restorative therapies

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Dendritic cells (DC) are the most potent antigen presenting cells which link the adaptive and innate arms of immune response. This normal functioning of DC is severely impaired following recovery from acute inflammation caused by sepsis or severe trauma, leading to protracted immunosuppression. This so-called “DC paralysis” results in greater risk of secondary infections and higher rates of mortality and morbidity in patients. We modelled systemic inflammation, DC paralysis and immunosuppression in mice after injecting Toll-like receptor (TLR) ligands or malaria infection. Functional characterization of paralyzed DC following recovery from systemic inflammation and/or infection showed impairments in uptake of antigen, defects in antigen processing and presentation by MHC molecules, diminished cytokine production, and elevated production of inhibitory molecules, altogether leading to impaired priming of antigen-specific T cells by paralyzed DC. We were able to improve paralyzed DC function by targeting antigen to a surface receptor or by blocking interferon type I signaling. We are currently addressing the role of intrinsic and extrinsic factors that induce DC paralysis in our mouse models. In parallel, we are translating our findings to the clinic, characterizing paralyzed DC in peripheral blood of COVID-19 or trauma patients admitted to Intensive Care Units. We ultimately aim to develop diagnostic tests of DC paralysis to identify patients at-risk of secondary infections, and potential therapies to prevent, ameliorate or shorten DC paralysis in critically-ill patients.

54

Single cell analysis of αβ versus γδ T cell development

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T cells develop in the thymus, where they acquire a range of distinct functional identities. Studies over the years have defined, at a population level, many of the specific genes that have to be activated or silenced as T cell progenitors differentiate toward either the αβ (alphabeta) or γδ (gammadelta) lineages. However, it is still unknown whether this combination of activation/silencing actually occurs in each and every cell as they differentiate. To investigate this, single cell RNA sequencing (scRNAseq) technology has been employed to re-assemble *de novo* a model of the early stage in T cell development based on the transcriptional profiles of individual cells. Two sets of cells were analyzed using Chromium 10x Genomics scRNAseq. A pure pool of CD4-CD8- double negative (DN) and γδ thymocytes were sorted for the first run, which yielded 5,527 high quality transcriptomes. For the second run, only DN1 and DN2 thymocytes were analysed, which generated 8,837 high quality transcriptomes. Hierarchical clustering identified 19 and 26 sub-populations within the first and second run respectively. This included 8 different populations that fall within the previous DN1 definition indicating much more complex than standard view of early T cell development. Assembly of these novel thymocyte populations into developmental trajectories based on gene expression revealed key findings. It indicated that the decision to differentiate into αβ or γδ T cells occurs at a much earlier stage than the current model and that there are distinct DN2 sub-populations and potentially DN1 that are specific to only one of the two developmental pathways. Analysis in the OP9-DL1 cultures confirmed that specific sub-populations of DN1s and DN2s differentiated into only αβ or γδ thymocytes. This is consistent with the αβ or γδ lineage decision being determined from the earliest stages of T cell development.

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55

Interferon ε as a novel regulator of intestinal homeostasis

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Disruption of intestinal immune balance can lead to inflammatory bowel diseases (IBD). Type I interferon (T1IFN) production has been implicated in maintaining intestinal homeostasis and T1IFN receptor (IFNAR) signalling has protective effects in experimental colitis. T1IFN, namely IFNα and IFNβ have been trialled in IBD patients, with conflicting results. This suggests another T1IFN ligand is important for maintaining intestinal homeostasis. We previously showed IFNε is highly expressed by epithelial cells of the female reproductive tract, where it is involved in protection against pathogens. IFNε expression has recently also been shown in epithelial cells of jejunum and rectum in rhesus macaques.

Here we show IFNε is expressed in human and mouse intestinal epithelium and expression is lost in inflamed conditions. Furthermore, our results show IFNε limits intestinal inflammation in the DSS colitis model, as IFNε-/- mice had more severe disease when compared to wildtype (WT) mice. Regulatory T cells (Treg) are crucial for maintaining intestinal homeostasis, and we observed FoxP3+ Treg frequencies were decreased in DSS-treated IFNε-/- mice, suggesting a role for IFNε in maintaining the intestinal Treg compartment. To rule out the involvement of IFNβ in experimental inflammation, we compared susceptibility to DSS colitis in WT and IFNβ-/- mice and found no significant differences in disease severity, nor in intestinal Treg frequencies. Finally, our data indicates that, as shown previously for IFNβ, IFNε can bind to IFNAR1 in the absence of IFNAR2 resulting in a distinct non-canonical gene signature. This non-canonical IFNAR signalling is relevant in experimental colitis, as IFNAR2-/- mice showed more severe clinical symptoms than both WT and IFNAR1-/- mice after DSS treatment. The importance of T1IFN signalling in maintaining intestinal homeostasis was confirmed in a cohort of paediatric IBD samples (n=150), where we found a dysregulated T1IFN response when compared to non-IBD control samples. Furthermore, this T1IFN response correlated with specific bacterial strains present in these samples.

These findings show IFNε is a new factor involved in the pathogenesis of IBD, and non-canonical IFNAR signalling may be a mechanism for its protective effect. This makes the IFNε-IFNAR pathway a promising therapeutic target for the treatment of IBD.

56

Immune features associated with COVID-19

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57

HIV-1 has paved the way in the battle against SARS-CoV-2

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Antibody responses are vital in the protection against infectious diseases and inducing potent antibodies is the major goal in vaccine research. Two major advances over the last few years have further invigorated research towards an HIV vaccine that induces broad and potent neutralizing antibodies. First, the discovery of many different potent antibodies has highlighted new targets on the Env trimer. The second major advancement is the development of soluble stable native-like trimer mimics. These developments have paved the way for active and passive immunizations against SARS-CoV-2 developing stabilized SARS-CoV-2 spike protein constructs as well as isolating extremely potent antibodies. Within a year several vaccines as well as monoclonal antibody treatment have been approved and will most likely have a major impact on the pandemic. With the emergence of new virus variants, vaccine immunogens and monoclonal antibodies need to evolve and detailed information on antibody – spike protein interactions are crucial to win the battle with SARS-CoV-2.

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101

The role of the short chain fatty acid butyrate in CD4+ T cell immunity

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$CD4^+$ T cells can differentiate into distinct effector subsets upon activation. Changes in immunometabolism impact this important fate decision due to different bioenergetic requirements of $CD4^+$ T cell subsets. The short chain fatty acid butyrate is a metabolite generated by the intestinal microbiota as a by-product of dietary fibre fermentation. It has been shown to be metabolised by many cell types. Although the effects of butyrate under T_{reg} and Th17-polarising conditions have been intensively studied, its impact on Th0 cell differentiation and function in the absence of polarising conditions is poorly understood. We aimed to investigate how butyrate impacts a key function of $CD4^+$ T cells: their capacity to differentiate into distinct subsets. Therefore, we treated $CD4^+$ T cells with butyrate *in vitro* to characterise its effects on $CD4^+$ T cell differentiation, function and metabolism. Additionally, we adoptively transferred butyrate-treated cells and challenged recipient mice with HSV-1.

We demonstrated that butyrate promotes activated $CD4^+$ T cell mitochondrial respiration, but does not influence glycolysis. This metabolic change was accompanied by a significantly elevated T-bet expression, indicating greater polarisation into Th1 cells even under non-polarising conditions. Moreover, Eomes co-expression suggested differentiation into $CD4^+$ cytotoxic lymphocytes – an underappreciated but important subset that directly eliminates target cells. Cytotoxicity was hence tested using *in vitro* killing assays, in which butyrate induced a 75% increase in $CD4^+$ T cell-mediated direct killing of B16 melanoma cells. This correlated with significantly upregulated expressions of IFN-g, granzyme B, perforin, and Fas ligand. Greater polarisation of butyrate-treated $CD4^+$ T cells into Th1 cells was maintained long-term after adoptive transfer and HSV-1 infection *in vivo*. Butyrate may therefore prime $CD4^+$ T cells to become more effective killers. These findings highlight the importance of metabolite availability, such as butyrate, in the microenvironment for pro-inflammatory responses towards infection and cancer.

102

Characterization of a monoclonal antibody towards the N-terminal hypervariable region 1 (HVR1) and epitope I of Hepatitis C Virus Glycoprotein E2

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A Hepatitis C virus vaccine is urgently needed to achieve global elimination. Hepatitis C is one of the most antigenically variable human pathogens and an effective vaccine must generate immunity in the majority of the human population. Glycoprotein E2 is present on the virion surface and is a major target of neutralizing antibodies which can prevent infection. All neutralizing domains identified to date work by blocking interaction between E2 and cell surface receptor CD81. The N-terminal hypervariable region 1, HVR1 (384-408) is an immunodominant region within E2 and elicits neutralizing antibodies that are usually type specific. HVR1 is known to play an essential role in binding of infectious serum derived HCV particles to scavenger receptor class B type 1 and glycosaminoglycans on the cell surface, essential for HCV entry. Cross neutralizing antibodies that include amino acids within HVR1 have not been characterized.

We have identified a novel rodent monoclonal antibody, MAb33, that binds to an unusual epitope bridging HVR1 and the adjacent target of broadly neutralizing antibodies referred to as epitope I (408-423). MAb33 potently neutralizes genotype 1a viruses, and also has the ability to cross-neutralize 3 different HCV genotypes, however, it only weakly blocks the interaction between E2 andCD81 suggesting its mechanism of neutralization is distinct from previously defined bNAbs.

We have defined the epitope of MAb33 and for the first time resolved its structure in complex with its epitope at 2Å resolution. The structure of the epitope is a helix and is in a different conformation to what is observed in unliganded structures that include this region of E2. The results suggest that this region could be flexible, raising the question whether there is a preferred conformation detected by neutralizing antibodies.

Studies using MAb33 and full length E2 will provide novel insight into the structure of HCV E2 and properties of antibodies directed towards HVR1. We will further perform a sero-survey to establish whether MAb33-like antibodies occur in infected individuals, which will help inform antigen development for HCV vaccines.

103

Regulation of immune memory formation and function during viral infection

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Chronic viral infections such as HIV and Hepatitis C result in poor humoral immune responses, characterised by the delayed generation of neutralising antibodies and dysregulation of memory B cell formation. Little is known regarding the mechanisms dictating the fundamental changes that result in dysfunctional B cell memory during chronic viral infection. Understanding these factors will be key for developing effective vaccines. We used an *in vivo* mouse model utilising acute and chronic strains of lymphocytic choriomeningitis virus (LCMV) in conjunction with a B cell tetramer specific for the LCMV nucleoprotein to examine antigen-specific memory B cell subset formation following infection. We performed phenotypic analysis of genetically modified mouse strains, BrdU-labelling studies and single cell RNA-sequencing to examine memory B cell formation, phenotype and function in response to acute vs. chronic LCMV infection. We found that memory B cells generated during chronic viral infection are highly proliferative, unlike classical memory B cells. Single-cell RNA-seq identified key genes downregulated in memory B cells generated in chronic infection including CD21, which promotes enhanced B cell responses to antigen, CD23, which binds C3 (complement)-coated immune complexes to mediate selection of B cells, CD55, which protects host cells from complement-mediated damage, and GILZ, a lack of which is associated with immune complex glomerulonephritis. To examine the role of excess immune complexes in the formation of effective B cell memory, we utilised mice in which B cell-intrinsic T-bet, which regulates antibody production to viral infection, was ablated. T-bet deletion resulted in increased expression of classical memory B cell markers, decreased circulating LCMV-specific immune complexes and reduced viremia. Understanding the factors driving altered humoral immune memory formation and the roles of memory B cells formed in the context of chronic viral infection is integral for improving therapeutics.

104

Liver resident CD4 T cell in malaria infection

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Malaria is caused by different *Plasmodium* species that can infect a variety of animals including humans and rodents. The life cycle of these parasites is complex, including a liver-stage followed by a blood-stage in their vertebrate hosts. While the host's immune response against each of these stages is incompletely understood, CD4 T cells are known to play an important role in immunity to *Plasmodium* infection during both stages. This project aims to examine the specific CD4 T cell response to a novel MHC II-restricted epitope in *Plasmodium in C57BL/6* mice and to characterize the protective capacity of these T cells. Given the importance of tissue-resident memory T cells (Trm cells) in peripheral immunity, we focused our study on the formation of CD4 Trm cells in the liver and protection against liver-stage parasites. To do this, we made use of a recently generated TCR transgenic mouse line, termed PbT-II, that responds to an epitope, abbreviated as YYI, expressed by both rodent and human *Plasmodium* parasites. Different priming methods were used to induce PbT-II priming; these included injection of anti-Clec9A antibody attached to the YYI epitope (Clec9A-YYI) or infection with radiation attenuated Plasmodium sporozoites (RAS), which infect the liver but do not progress to the blood.

To test whether PbT-II T cells formed tissue-resident memory T cells, GFP-expressing PbT-II transgenic T cells were injected into naïve B6 mice that were then primed with Clec9A-YYI or RAS. Flow cytometric analyses 35 days later revealed the existence of a memory PbT-II cell population in the liver expressing surface markers associated with tissue-residency. Parabiosis surgery on RAS vaccinated mice confirmed the residency of this PbT-II cell population in the liver. Gene expression analysis of these CD4 T cells further revealed a similar expression profile to that of CD8 liver resident T cells. Our results indicate that CD4 Trm cells form in the liver during malaria infection and share gene expression profiles with CD8 liver Trm cells. Our ongoing studies will determine whether CD4 T cell lineage-specific differences affect formation of liver Trm cells and their role in protection from infection.

105

B cells are required for optimal CD4$^+$ T cell memory response against *Salmonella* infections

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Mitchell *et al.* showed more than 50 years ago that B cells and T cells co-operate to generate an effective immune response. In *Salmonella* infections, immunity is generated through CD4$^+$ T cell responses but we and others[1] have observed that B-cell deficient mice are unable to mount a protective immune response. Following recognition by cognate B cells of their antigens, CD4$^+$ T cells activate B cells (T-dependent activation) leading to antibody production by B cells. In a co-operative process, B cells are thought to help CD4$^+$ T cells by generating essential survival signals when antigen levels become limiting, to effect a memory phase of the immune response. This antibody independent function of B cells has not been thoroughly examined in an infectious setting. Live-attenuated vaccine (LAV) strains of *Salmonella enterica* serovar Typhimurium (STm) infection in susceptible C57BL/6 mice has served as an excellent model to study the adaptive immune responses. We have demonstrated that Th1 functions of the CD4$^+$ T cells are key in the clearance of STm from the infected host[2]. In vaccinated animals, CD4$^+$ T cells are also critical in the development of protective immunity. We showed that in B cell-deficient (µMT) mice, infection with LAV is cleared but these mice fail to develop protective memory. The observed phenomenon is independent of secreted antibodies as Ab-supplementation fails to rescue the protective phenotype. During the early stages of vaccination, CD4$^+$ T cells in µMT mice exhibit apparently normal activation phenotype in terms of Th1 lineage commitment (e.g. T-bet expression) and effector function (e.g. *ex-vivo* IFNγ production) as compared to the wild-type C57BL/6 mice. However, T cell activation is not maintained through the later stages of vaccine clearance. The vaccinated µMT mice also show a reduction in the number of antigen-specific CD4$^+$ T cells in the spleen; these T cells are identified with the help of STm-specific tetramers. These data demonstrate that there is a defect in the CD4$^+$ T cell memory in the absence of B cells. Future work aims to address the mechanism by which B cells influence the development and/or maintenance of CD4$^+$ T cell memory.

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106

Characterising interacting thymocytes and thymic stromal cells for mapping physical thymocyte-stroma interactions during T cell development

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The thymic microenvironment is comprised of a range of thymic stromal cells and is a crucial requirement for T cell development. As thymocytes migrate through different microenvironments of the thymus, they receive the necessary signals for development. Although there are various populations of thymic stromal cells, the range of cell types in this compartment is not yet fully characterized. Moreover, much about their exact functions and how they guide T cell development remains poorly understood.

We hypothesised that unbiased profiling of cell types that make up the thymic stroma and how they physically interact with developing thymocytes will allow a better understanding of their roles in T cell development. To achieve this, we exploited the phenomenon of thymic rosettes, which are *in vivo* cellular complexes composed of 10-20 developing thymocytes surrounding with a central stromal cell. They represent a snapshot of the physical interactions between thymocytes and stroma and can be isolated intact from the thymus.

To characterize the composition of rosettes, we employed flow cytometry analysis and single cell RNA sequencing on the 10X Genomics platform. Thymocytes at post-positive selection stages of development were enriched in rosettes, suggesting that interactions with stromal cells in rosettes are particularly important in the later stages. Furthermore, single cell RNA sequencing revealed previously unknown complexities in the thymic B cells, dendritic cells and macrophages. They displayed gene signatures unique to the thymus, suggesting that they have specialised and unique functions for guiding T cell development. The thymic B cells in rosettes were distinct from splenic B cell subsets and were capable of differentiating into plasma cells. Monocyte and macrophage populations in rosettes represented a linear differentiation process, and the intermediate stages appeared to be dendritic cell-like. There appeared to be four functionally distinct subsets of thymic dendritic cells, instead of three as previously identified, and these subsets displayed subset-specific chemokine receptor expression.

These new insights into thymic rosettes help to establish a platform for mapping specific interactions in individual rosettes using high throughput sequencing techniques. Ultimately, delineating these interactions helps to complete understanding of T cell development.

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107

Optimizing a dual RNA-sequencing protocol for the analysis of host-pathogen interactions during neonatal sepsis

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Sepsis is responsible for >25% of neonatal deaths occurring within the first 4 weeks of life and for survivors, is often associated with morbid complications and severe long-term sequelae. Commensal bacterial species have emerged as the most common causative pathogens in neonatal late-onset sepsis (>3 days of age) and to date, it remains unclear why certain commensal bacteria appear pathogenic in the context of preterm neonatal sepsis. Furthermore, the neonatal response to invasion by commensal bacterial species is poorly characterised. Advances in next-generation sequencing may give us crucial new insights into sepsis pathogenicity by analysing the global gene expression changes occurring in both host as well as invading pathogen.

For my PhD project, I am developing a dual RNA-sequencing (dual RNA-seq) protocol and pipeline which allows me to simultaneously analyse transcriptional changes occurring in the blood cells of neonatal host as well as infecting pathogen during an episode of sepsis. I am currently optimising a clinically compatible RNA extraction protocol for the sensitive detection of low-abundance bacterial transcripts from human whole blood in an in vitro model of sepsis using clinically relevant neonatal sepsis pathogens. I aim to define a set of stereotypic and species-specific virulence and host defence genes upregulated during host-pathogen interactions in in vitro blood challenge models, and validate my findings by applying dual RNA-seq to a small set of clinical preterm neonatal sepsis samples collected from King Edward Memorial Hospital as part of a prospective, observational clinical study. The obtained transcriptional data will undergo comprehensive bioinformatic analysis and characterization to determine differentially expressed genes, enriched host/pathogen pathways, and interspecies correlation.

My work will elucidate the mechanisms of infection in the vulnerable population of preterm infants and may identify novel molecular targets for much-needed rapid sepsis diagnosis and therapy. Lastly, it will generate a universal platform for characterizing host-pathogen interactions in small volume pediatric samples in other infectious diseases.

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108

Therapeutic blockade of CXCR2 rapidly clears inflammation in Arthritis and Atopic Dermatitis models: Demonstration with surrogate and humanized antibodies

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Neutrophils are the most abundant effector cells of the innate immune system and represent the first line of defense against infection. However, in many common pathologies, including autoimmune diseases, excessive recruitment and activation of neutrophils can drive a chronic inflammatory response leading to unwanted tissue destruction. Several strategies have been investigated to tackle pathologic neutrophil biology and thus provide a novel therapy for chronic inflammatory diseases. The chemokine receptor CXCR2 plays a crucial role in regulating neutrophil homeostasis and is a promising pharmaceutical target. In this study, we evaluated the therapeutic potential of blocking this receptor using anti-CXCR2 mAb in two chronic inflammatory mouse models, atopic dermatitis (AD) and arthritis (RA). After inducing AD, anti-mouse CXCR2 mAb treatment significantly attenuated the clinical severity of the disease, including skin inflammation, dermal and epidermal thickness, and itch-evoked scratching compared to isotype-control treated animals. This mAb treatment also reduces the infiltrations of inflammatory cells to inflamed skin and ear draining lymph nodes. To test our therapeutic regiment in human, we use human CXCR2-knock-in mice. We observed similar results when human CXCR2-knock-in mice were treated with the humanized anti-human CXCR2 mAb. In RA, administration of anti-CXCR2 mAb quickly reversed arthritic clinical symptoms, such as ankle thickness, inflammatory leukocytes infiltration in the synovial space, and loss of cartilage in the ankle joint. This mAb treatment also reduces the infiltration of innate effector cells to the ankle joints and popliteal draining lymph nodes in comparison with isotype control-treated mice. Similar results were observed when human CXCR2-knock-in mice were treated with the humanized anti-human CXCR2 mAb. Our findings suggest that blocking CXCR2 will be a promising therapeutic strategy to treat AD and RA, as well as in other neutrophil-mediated inflammatory conditions where neutrophils are pathogenic, and medical needs are unmet.

109

The role of Kelch 13 protein in the malaria parasite *Plasmodium falciparum*

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Resistance to antimalarials is a recurring hurdle towards effective management and/or elimination of malaria. Decreased susceptibility to artemisinin, the most effective antimalarial currently available, has emerged and its global spread is a serious risk to malaria control. Mutations in the *P. falciparum* Kelch 13 (PfK13) protein lead to decreased susceptibility to artemisinin. The function of this protein is unknown, but recent evidence shows involvement in the parasite's haemoglobin uptake process. Digestion of haemoglobin taken up by the parasite produces free haem, which is necessary to activate artemisinin and initiates the events leading to parasite death. Using indirect immunofluorescence visualised by super-resolution microscopy, we show that PfK13 forms doughnut-shaped structures located at the parasite periphery. This indicates that PfK13 is located at the parasite's primary uptake structure called the cytostome. Furthermore, fluorescent live cell imaging of GFP-tagged PfK13 (GFP-PfK13) parasite line grown in fluorescent dextran-loaded red blood cells (RBC) show that GFP-PfK13 is closely associated with fluorescent dextran-loaded structures that form inside the parasite as it feeds from the RBC host. In mid-ring stage parasites, fluorescent dextran-loaded structures are also found associated with hemozoin pigments that form prior to the formation of the digestive vacuole. GFP-PfK13 is also seen associated with these structures, indicating involvement of PfK13 in haemoglobin digestion and hemozoin formation. As previously shown, in late ring or trophozoite parasite stage the hemozoin pigments coalesce to form a single digestive vacuole that is fed by haemoglobin-containing vesicles. We show that GFP-PfK13 is associated with such structures, indicating PfK13's involvement in digestive vacuole biogenesis. Taken together, our results show that PfK13 is closely associated with the haemoglobin uptake process and digestive vacuole biogenesis. Mutations in PfK13 have been shown to lead to a decreased abundance of haemoglobin peptides in the parasite, which can lead to decreased activation of artemisinin and, ultimately, decreased parasite susceptibility.

1. Decreased K13 Abundance Reduces Hemoglobin Catabolism and Proteotoxic Stress, Underpinning Artemisinin Resistance Tuo Yang, Lee M. Yeoh, Madel V. Tutor, Matthew W.Dixon, Paul J.McMillan, Stanley C.Xie, Jessica L. Bridgford, David L.Gillett, Michael F.Duffy, Stuart A.Ralph, Malcolm J.McConville, LeannTilley, Simon A. Cobbold
2. Digestive-vacuole genesis and endocytic processes in the early intraerythrocytic stages of Plasmodium falciparum Nurhidanatasha Abu Bakar, Nectarios Klonis, Eric Hanssen, Cherrine Chan and Leann Tilley
3. Multi-omics Based Identification of Specific Biochemical Changes Associated With PfKelch13-Mutant Artemisinin-Resistant Plasmodium falciparum Ghizal Siddiqui, Anubhav Srivastava, Adrian S. Russell, Darren J. Creek

110

Identifying novel Vgamma9Vddelta2 T cell phenotypes during human *Plasmodium falciparum* infection

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Increasing evidence shows that repeat exposures to *Plasmodium falciparum* (*Pf*) alone does not induce the development of long-lived malaria immunity, and current vaccinations that target antibody responses have short-lived protection. Vg9Vd2 T cells are an innate-like

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subset of T cells which are essential to the inflammatory immune response to malaria. Alongside inflammatory responses, we propose Vg9Vd2 cells may also act as antigen-presenting cells (APCs) or take on T follicular helper (TfH) cell like characteristics during *Pf* infection to contribute to parasite-specific antibody development. Recently, we observed the emergence of these adaptive Vg9Vd2 T cell phenotypes during a Controlled Human Malaria Infection (CHMI). In this CHMI, malaria-naive Australian adults were intravenously inoculated with 2,800 intact 3D7-*Pf* parasitised red blood cells (pRBCs), and blood drawn at days 0, 4, 8, 15 and 45 post-infection (n = 8). Whole blood staining showed increased Vg9Vd2 T cell surface expression of APC-marker HLA-DR, and Tfh-related markers CD40L and CXCR5 at 15 days post infection. To confirm these findings *in vitro*, PBMCs from malaria-naive adults were stimulated with pRBCs for 72 hours. We observed increase surface antibody frequencies of CD86, HLA-DR, CD40L and CXCR5 compared to uninfected RBC and blank media cultures. Surface phenotypes were characterised by fluorescent antibody detection using a BD LSR Fortessa 5 flow cytometer. Data generated identifies an unconventional adaptive Vg9Vd2 T cell phenotype activated during *Pf* infection. Modulation of the gd T cell adaptive immune response in malaria could have significant implications on pathogenesis and symptomatic disease

111

The role of SMG1 in regulating innate immunity

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SMG1 (Suppressor of Morphogenesis in Genitalia 1), is a member of the PIKK (phosphoinositide 3-kinase related kinase) family that includes ATM, ATR and DNA-PK. We have identified the protein kinase SMG1 as a regulator of TLR response and SMG1 knockout mice are early embryonic lethal. To further study the role of SMG1 in inflammation we have generated mice lacking SMG1 in myeloid cells (*LysMCreSmg1$^{fl/fl}$*) and control littermates (*Smg1$^{fl/fl}$*). We treated bone marrow derived macrophages (BMM) from Smg1 deficient mice and wild type mice with lipopolysaccharide (an activator of TLR4) and measured their pro-inflammatory cytokine responses. BMMs from *LysMCreSmg1$^{fl/fl}$* male mice showed less induction of the pro-inflammatory cytokines IL-1β, IFNβ, and TNFα, while increased pro-inflammatory cytokines production only can be observed of BMMs from *LysMCreSmg1$^{fl/fl}$* female mice as compared with control, which indicating Smg1 deficiency alters toll-like receptor induced inflammatory gene expression. Interestingly, loss of Smg1 also affects pro-inflammatory cytokine production between male and female mice. Characterisation of LysMCre mice showed SMG1 only knocked out in myeloid lineage cells(macrophages), but cre efficiency is based on the cre-recombination. Firstly, we found cre efficieny affects pro-inflammatory cytokine production, however, does not show effect on female mice. To test whether loss of SMG1 may be implicated X chromosome inactivation, we compared several target genes by qPCR in female and male BMMs from *LysMCreSmg1$^{fl/fl}$*(Cre mice) and *Smg1$^{fl/fl}$*(control) and found that loss of SMG1 affects pro-inflammatory cytokine production by up-regulating of Xist regulation(Eif1 and Rasa1), but not involve in mRNA degradation(Upf1). Additionally, results from qPCR and western blots showed increased TLR4 mRNA expression and protein level among BMMs from female but not male *LysMCreSmg1$^{fl/fl}$*(Cre mice) at 2 hours post-LPS treatment. In summary, loss of SMG1 may involve in the regulation of innate immunity with potential sex differences affecting pro-inflammatory cytokine production in response to LPS treatment.

112

The transmembrane TLR adaptor SCIMP scaffolds Erk1/2 to drive macrophage pro-inflammatory responses

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Innate immune and inflammatory responses are triggered by pathogen or damage activation of Toll-like (TLR) receptors. We have previously shown that the immune-specific transmembrane adaptor SCIMP is a regulator of TLR signalling and pro-inflammatory responses in macrophages[1,2]. SCIMP directly binds TLRs through a non-canonical, TIR-non-TIR interaction, scaffolding the Src family kinase, Lyn, for TLR activation and driving the selective production of IL-6 and IL-12p40, but the mechanism for the selectivity of these cytokine outputs was not known. Using mass spectrometry, affinity pull downs and co-immunoprecipitation in macrophage lysates we identify Erk1/2 as a novel binding partner of SCIMP. BMMs from SCIMP KO mice show the requirement for SCIMP in Erk-mediated production of pro-inflammatory IL-1b, IL-2 and TNF but not for anti-inflammatory cytokines or chemokines. High-resolution live imaging shows that SCIMP recruits Erk to signalling domains on cell surface ruffles in an LPS-inducible manner. SCIMP-scaffolded Erk1/2 enhances nuclear translocation of c-Fos for transcriptional activation. Thus, we identify a mechanism by which SCIMP recruits Erk1/2 for c-Fos activation in pro-inflammatory TLR signalling in macrophages. From this, SCIMP emerges as a novel, immune specific scaffold for Erk1/2 kinase with important roles in inflammation and infection. SCIMP is genetically associated with human autoimmune and chronic inflammatory diseases including SLE and Alzheimer's disease, highlighting the SCIMP/Erk/c-Fos axis as a possible therapeutic target.

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113

Characterisation of a novel type I interferon pathway and its implications in inflammatory disease

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Type I interferon (IFN) signalling is integral to eliminating infections and cancer. Conventional signalling requires the binding of IFN to both transmembrane receptor subunits, IFNAR1 and IFNAR2, to activate the classical JAK-STAT pathway [1, 2]. Our lab recently demonstrated that IFNβ can bind to the IFNAR1 subunit in the absence of IFNAR2 and activate STAT-independent signalling and unique interferon-regulated genes [3]. Activation of this pathway using *Ifnar2-/-* mice has been shown to result in lethality following lipopolysaccharide (LPS) induced septic shock and exacerbate neuronal cell death in ischaemic stroke models [3, 4]. This project aims to characterise this novel, non-canonical IFN pathway at the cellular, tissue, and molecular levels to better understand its activation and involvement in pathogenesis, as well as its potential for therapeutic intervention.

Cellular: To investigate this novel pathway *in vivo*, *Ifnar2-/-* mice are administered intraperitoneal IFNβ, the IFN selectively produced in LPS sepsis, and peritoneal exudate cells are extracted for FACS analysis. A reduction in peritoneal B cell numbers is observed, with the induction of TREM1 (a non-canonical marker) expression detected on macrophages. Furthermore, *Ifnar2-/-* B cells and macrophages were found to down-regulate IFNAR1 following IFNβ stimulation *in vitro*, indicating their potential role as the populations participating in the pathogenesis of the septic shock model. **Tissue:** To establish the potential of non-canonical signalling in mucosal tissues, we used immunohistochemistry to reveal strong IFNAR1 expression throughout the cytoplasm of the stromal cells from gastrointestinal tract (GIT) tissues with the absence of IFNAR2, indicating a potential for independent receptor activation (non-canonical signalling). Conversely, expression of the subunits is reversed in the female reproductive tract (FRT), suggesting non-canonical signalling may be tissue specific. **Molecular:** Using single-molecule microscopy to characterise the receptor dynamics of non-canonical signalling, we showed that the presence of IFNAR2 slows down the mobility of IFNAR1 upon the addition of IFNβ, whereas no change in surface dynamics was observed for IFNAR1 expressed alone, revealing a different kinetic profile for the novel IFNβ:IFNAR1 axis.

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114

The flexible usage of diverse cell death pathways ensures host protection against *Salmonella* Typhimurium infection

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Infectious diseases like typhoid fever, characterized by intracellular bacterial replication, remain a major problem of human health. Every year up to 20 million people worldwide are affected and around 150.000 individuals are killed by this life-threatening disease caused by *Salmonella*. The killing of infected cells through programmed cell death (i.e. pyroptosis, necroptosis, apoptosis) plays an important role in the host defence against intracellular pathogens. Cell suicide is thought to control pathogen replication by removing the replicative niche and re-exposing the pathogens to extracellular immune effector mechanisms. However, the relative importance of each of these pathways in infection control as well as their regulation and kinetics has not been fully elucidated.

To investigate this, we used two different strategies. We infected host cells with *Salmonella* mutants with different potential to induce distinct forms of cell death. This approach demonstrated how several bacterial factors such as flagellin or invasive protein A impact host cell death. Additionally, we infected mice and cells lacking individual or multiple cell death initiators to measure bacterial burdens and cell death kinetics. Surprisingly, the loss of pyroptosis, necroptosis or extrinsic apoptosis alone had only minor impacts on *Salmonella* control, demonstrating that host defence can employ several cell death pathways to limit intracellular infection. However, combined deficiency of these cell death pathways caused loss of bacterial control in mice and macrophages, indicating that killing of infected cells is required for the clearance of intracellular pathogens. Our current work focuses on the exploitation of this system by targeting cellular inhibitors of intrinsic apoptosis with BH3-mimetic compounds inducing host cell death of infected cells. All together, these findings not only uncover a highly coordinated and flexible backup system between several programmed cell death processes that protects the host from intracellular bacterial infections but additionally raises the possibility that BH3-mimetics can be used for *Salmonella* treatment.

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115

Intestinal microfold cells orchestrate the interactions between microbiota and immunity

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Microbiota-immune cell interactions play a vital role in host defense against potentially harmful external organisms, such as viruses and bacteria, and environmental agents including food. Microfold(M) cells are specialized epithelial cells found in the gut epithelium that are pivotally positioned to sample gut contents and importantly actively transport antigens across the gut epithelium. Critically, they link the gut lumen with the immune cell network positioning them to instruct appropriate immune responses, including the production of immunoglobin A against invading pathogens. Exactly how they orchestrate these events, however, is not clear.

Despite their critical function, to date few specific tools exist to study intestinal M cells, the molecular mechanisms that regulate their generation or how they drive mucosal immunity. To overcome this gap, we have generated novel reporter mouse strains to identify M cells allowing us to show that SpiB expressing M cells are present along the entire intestinal tract and not only localized to Peyer's Patch as previously thought. Analysis of gut epithelial cells at these different sites in the gut using single cell RNA sequencing revealed tissue-specific heterogeneity allowing us to define distinct gene expression signatures for M cells based on their location. These molecular blueprints identify novel maturation programs that are likely to be dependent on local environmental cues shaped by the microbiota and that influence induction of immune responses. Furthermore, we propose that the functional pathways of localized M cells significantly impact gut region-specific disease and plan to explore these pathways and their impact on gut integrity during homeostasis and infection.

116

Structural and functional heterogeneity in pathoadaptive FimH adhesin variants in *Escherichia coli*

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Publish consent withheld

117

An integrated analysis of myeloid cells identifies gaps in *in vitro* models of *in vivo* biology

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Macrophages isolated from different tissues have distinct niche roles that maintain tissue homeostasis, as well as providing surveillance for tissue injury or infection. The potential to model tissue residency, disease phenotypes and activation status of human macrophages using pluripotent stem cells is both exciting and a growing area of interest. Benchmarking pluripotent stem cell-derived macrophages to primary macrophages has previously been limited by the availability of data on a compatible platform and the number of comparisons carried out. By assembling an integrated transcriptome atlas of human myeloid biology representing ~1000 samples, we address the question of how well laboratory models, including pluripotent-derived cells, represent macrophage biology, and particularly whether these can model resident tissue macrophage specialisation. Through our analysis, we identified two broad classes of tissue-resident macrophages with lung, gut and tumour-associated macrophages most similar to monocytes. Microglia, Kupffer cells and synovial macrophages shared similar profiles with each other, and with cultured macrophages. In comparison, pluripotent stem cell-derived macrophages were found to sit away from *in vivo* cell types despite sharing some features with tissue-resident macrophages. Gene-set enrichment analysis of the genes that most correlated with *in vitro*-derived macrophages moving away from the tissue-resident populations revealed that the most significant pathways in these cells involved collagen synthesis and production Moreover, by single cell projection of human fetal yolk sac cell clusters onto the atlas, we further found that pluripotent stem cell-derived macrophages to not be reminiscent of fetal-derived cells. Overall, our analyses highlight that there is room for improvement in the development of *in vitro* model systems that attempt to mimic *in vivo* counterparts.

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Global 3'-UTR length changes mediated by interferon beta in murine and human macrophages

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Interferon signalling is one of the most important mechanisms shaping innate immune responses and needs to be tightly regulated to successfully fight infections and regulate immune responses while avoiding toxicity. Type-1 interferons (IFN), induce changes in cells on many different levels, such as transcriptional, translational and metabolic. In this study, many of these changes have been characterized on a global level in murine and human macrophages using multi-omics strategies.

We conducted time-course experiments treating murine bone marrow-derived macrophages or human blood monocyte-derived macrophages with IFN-beta. While confirming changes described in previous studies, we also found a large number of transcripts expressing a shortened 3' untranslated region (3'-UTR), a region heavily targeted by miRNAs and harbouring binding sites for many RNA binding proteins. Subsequently, this project has been focusing on 3'-UTR dynamics, a field that is only starting to be explored. The regulation and function of this process in an interferon context remain unknown.

Recent publications have described a role of 3'-UTRs in mediating differential protein complex formation, which can affect localization and function. This unique regulatory function of 3'-UTR length will be addressed for two candidate proteins. 3'-UTR-dependent protein-protein interactions will be identified by mass spectrometry using overexpression constructs.

We also aim to identify components involved in mediating the shortening of 3'-UTRs in response to IFN-beta treatment using a CRISPR/Cas9-based screening approach to shed light on how this novel signalling pathway is initiated.

This study describes a new aspect of interferon signalling and a novel layer of regulation through non-ISGs. It will show how differential expression of distinct 3'-UTR transcript isoforms shapes macrophage innate immune responses.

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201

Developing a high-throughput multiplex immunoassay that characterises antibody responses to StrepA vaccine antigens

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Streptococcus pyogenes (StrepA) is an important human pathogen that causes illness ranging from mild skin and throat infections, to life-threatening invasive disease. Untreated StrepA infections can also lead to the autoimmune sequelae acute rheumatic fever (ARF), which can progress to permanent heart damage. Globally, these diseases cause significant morbidity and mortality and despite years of research, there is still no vaccine available to prevent StrepA disease. Several vaccines that target conserved antigens are currently in the early stages of development. Measuring antibodies specific to these antigens at a population level is essential to understanding immune status and establishing correlates of protection. The technology to do this for StrepA has been limited however, and rapid and sensitive assays for measuring serum antibody levels are needed. We have consequently developed a multiplex bead-based immunoassay that can detect and quantify antibody responses to leading StrepA vaccine antigens. This builds on our existing triplex assay whereby antigens used in clinical serology (SLO, DNase B and SpnA) together with five additional vaccine antigens (C5a Peptidase, Spy_0843, SpyCEP, SpyAD and the Group A carbohydrate), have been coupled to spectrally unique beads to form an 8-plex antigen panel. The assay has been optimised so that a broad range of antibody titres can be quickly and efficiently determined from a single serum dilution. Validation studies using human sera are now underway. This assay will be a powerful tool for measuring the prevalence of antibodies in patients with StrepA disease. In doing so, we can profile the natural immune response in high-risk populations to inform vaccine coverage.

202

CD8+ T cell cross-reactivity across conserved Influenza A and B epitopes

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In 2018, the World Health Organisation listed influenza pandemics as one of the top threats to global health. Influenza pandemics occur as a result of the emergence of antigenically distinct strains, which arise due to genomic re-assortment or through the acquisition of mutations. Despite Influenza having a high mutation rate, amino acid positions 265-273 of the Influenza A virus (IAV) nucleoprotein (NP) is a conserved immunodominant epitope presented by the Human Leukocyte Antigen (HLA) A*03:01. We investigated the breadth of CD8+ T cell response towards this epitope and identified cross-reactive responses to a novel Influenza B virus (IBV) epitope.

We investigated the level of cross-reactivity of responding CD8+ cells by stimulating T cells from donors expressing HLA-A*03:01 with the FluA and FluB epitopes derived from the NP protein. We also determined the crystal structure of the HLA-A*03:01 in complex with both FluA and FluB epitopes, and provided the molecular basis for T cell crossreactivity. This study contributes unprecedented cross-reactivity between two conserved epitopes derived from Influenza A and B strains, identified a new FluB epitope, and is a key finding for the design of universal vaccines.

203

Age related epigenetic changes associated with and causative of intrinsic CD8+ T cell dysfunction

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A quarter of the world's population will be >60 years of age by 2050. More must be done to understand the intricacies associated with age-related pathology as we rapidly approach an aged society. With aging there is decrease in peripheral naïve CD8+ T cells with a simultaneous increase in CD44hi memory CD8+ T cells. Virtual-Memory T (Tvm) cells are antigen-naïve T cells that present a classical memory phenotype whilst also possessing innate-like immune function. Unlike other CD8 subsets, Tvm cell homeostasis is highly dependent on cytokines, as shown by their selective depletion in IL-15$^{-/-}$ mice. Tvm cells acquire TCR-associated defects in aged mice and humans, which could contribute to the overall decline in CD8 T cell function seen with aging. Tvm cells make up a large proportion of the naïve CD8+ T cell pool making them ideal targets for age specific therapeutic interventions. Here, we have performed ATAC-Seq analysis on young and aged CD8+ T cell subsets and have identified several key signatures selectively associated with age-related dysfunction. The aged-specific Tvm cell signature will be validated and the role of candidate genes involved in mediating the dysfunction assessed using a range of molecular and cellular techniques. Validated targets will be investigated for the capacity to block their inhibitory effects to mediate functional recovery in dysfunctional T cells. Collectively, this study will provide fundamental information on biological changes associated with advanced age in CD8+ T cells, which is essential for informed targeting to rejuvenate cellular immunity in the elderly.

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204

Humoral immune responses during acute bacterial urinary tract infection in mice

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Urinary tract infections (UTIs) are the second most common bacterial infection with high recurrence rates in women[1]. With the rise of multi-drug resistant (MDR) bacteria, UTI treatment is becoming increasingly difficult, at great cost to healthcare systems[2]. Here we investigated whether protective adaptive immune responses occur in a mouse model of MDR UTI.

C57BL/6J wild-type or *rag1*$^{-/-}$mice were transurethrally inoculated with an MDR UTI strain of Gram-negative uropathogenic *Escherichia coli*, depositing bacteria directly into the bladder[3]. Flow cytometry was used to characterise immune cell populations in bladder-draining lymph nodes of wild-type mice. Bladders and urine were also collected at various time-points to assess bacterial numbers.

In bladder-draining lymph nodes, germinal centre (GC) B cell responses had developed by 4 weeks post-infection, with immunoglobulin class switching towards IgG. GC B cell responses varied between individual infected wild-type mice. 30% of wild-type mice cleared bladder infection by 4 weeks, but we noted no clear correlation between the magnitude of GC B cell responses and bacterial load. At this timepoint, *rag1*$^{-/-}$mice had significantly higher loads of bacteria in their bladders compared to wild-type mice, suggesting a role for adaptive immune responses in protecting against UTI. In conclusion, our data suggests for the first time in a mouse model, that Gram-negative bacterial UTI induces humoral immune responses in local lymph nodes draining the bladder, and that these responses could potentially help clear infection.

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205

Modeling the immune complex cycling in follicular dendritic cells

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Follicular dendritic cells (FDCs) are stromal cells residing in the primary B cell follicles and germinal centers (GCs) of the secondary lymphoid organs. FDCs are specialized for capturing native antigen in the form of immune complexes (ICs) and retain it for a long period of time in a stable form. As the B cell affinity maturation process that occurs in the GCs rely on the antigen uptake of B cells, FDCs are considered critical for supporting the GC reaction by providing ICs to the B cells although other roles of FDCs have also been recognized. Heesters et al., discovered that antigen in FDCs undergo periodic cycling using an *ex-vivo* culture system and suggested that this mechanism might be the reason behind the enhanced stability of ICs in the FDCs [Heesters et al., Immunity 2013].

To further characterize the antigen cycling in FDCs and understand its potential implications in GC reactions, we estimated the time scale of antigen cycling using the data of Heesters et al., by performing *in-silico* simulations. Simulations using an agent-based model of GC reaction [Meyer-Hermann 2012, Binder and Meyer-Hermann 2016] modified to incorporate the dynamics of antigen on FDCs suggested that antigen cycling could impact the GC dynamics by redistributing the antigen on FDC surface and by protecting the antigen from degradation. We also found that the dynamics of antigen cycling has an impact on the extent of antigen protection and GC B cell antigen uptake. Consequently, changes in the antigen cycling dynamics can potentially alter the trade-off between antigen protection from degradation and GC B cell antigen uptake. Further, we predicted that blocking antigen cycling can terminate the GC reactions suggesting that antigen cycling could be a therapeutic target for disrupting chronic pathologic GCs. These findings extend our knowledge of antigen cycling in FDCs and suggest a need to better understand the mechanism of antigen cycling to fully exploit the potential therapeutic opportunities.

206

Targeting SARS-CoV-2 using stealth nanoparticles loaded with siRNAs

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SARS-CoV-2 has emerged on the world stage as a highly infectious agent that can spread rapidly geographically and has a case fatality rate of ~2-3.0%. Several vaccine candidates against SARS-CoV-2 are being developed. However, these may take a year or more to generate and to undergo field testing for safety and efficacy, and will not help those already infected with the virus. There are a number of studies looking at repurposing of old drugs and aim to reduce viral growth in order to alter clinical outcomes in the very sick, or to prophylactically treat front line workers. None are coronavirus specific and their effectiveness at this time is at best controversial. SARS-CoV-2 siRNAs can be developed rapidly due to our previous work on respiratory viruses and drug delivery. Moreover, siRNAs can be used both prophylactically and therapeutically. To date, there is nothing reported on the use of siRNAs to treat SARS-CoV-2. Hence, we carried out a screen of siRNAs targeted SARS-CoV-2. We showed that a number of siRNAs could prophylactically reduce viral infectivity in Vero E6 cells. Moreover, our siRNAs exhibited little to no immmuno-stimulatory effect on THP-1 macrophages. Using our previously

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developed stealth nanoparticle delivery system, which is able to deliver siRNAs to the lung via the bloodstream, we are currently conducting experiments to deliver our top candidate anti-SARS-CoV-2 siRNAs intoSARS-CoV-2-infected hACE2 mice. We predict that our encapsulated SARS-CoV-2 siRNAs can provide robust inhibition of viral infectivity *in vivo*. These *in vivo* studies will establish the proof-of-concept for our strategy to deliver anti-SARS-CoV-2 siRNAs to the lungs of infected individuals to repress virus expression and COVID-19 disease. The innovative approach we propose here will target SARS-CoV-2 with single or even multi-targeted siRNAs which enhances potency and limits the potential for the virus to evolve resistance.

207

A novel purification procedure for active recombinant human DPP4 and the inability of DPP4 to bind SARS-CoV-2

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The protease, dipeptidyl peptidase 4 (DPP4), is a pharmacological target in type 2 diabetes therapy. DPP4 also has roles in steatosis, insulin resistance, cancers and inflammatory and fibrotic diseases. In addition, DPP4 binds to the spike protein of MERS virus, causing it to be the human cell surface receptor for that virus. DPP4 has been identified as a potential binding target of SARS-CoV-2 spike protein, so this question requires experimental investigation. Understanding protein structure and function requires reliable protocols for production and purification. The current study aimed to develop such strategies for baculovirus generated soluble recombinant human DPP4 (residues 29-766) produced from *Spodoptera frugiperda* 9 (Sf9) insect cells. Purification used differential ammonium sulfate precipitation, hydrophobic interaction chromatography, dye affinity chromatography in series with immobilised metal affinity chromatography, and ion exchange chromatography. The binding affinities of DPP4 to the SARS-CoV-2 full-length spike protein and its receptor binding domain (RBD) were measured using surface plasmon resonance and using ELISA. This optimised DPP4 purification procedure yielded 1 to 1.8 mg of pure fully active soluble DPP4 protein per litre of insect cell culture with specific activity >30 U/mg, indicative of high purity. This DPP4 bound to MERS-CoV spike. No specific binding between DPP4 and CoV-2 spike or spike RBD was detected. In summary, a procedure for high purity high yield soluble human DPP4 was achieved and used to show that, unlike MERS, SARS-CoV-2 does not bind human DPP4.

208

SARS-CoV-2 infects but does not replicate in human macrophages and triggers pro-inflammatory and anti-viral cytokine responses.

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Severe inflammation is a hallmark of viral disease including COVID-19. The cellular source of the initial and ongoing inflammation in COVID-19 is unknown. Macrophages, as sentinel innate immune cells, are specialised for detecting infection and releasing potent pro-inflammatory (IL-6, TNF, IL-1b) and anti-viral cytokines (e.g. IFNb). They are recruited to the lung in large numbers during severe COVID-19. However, whether these macrophages are directly infected with SARS-CoV-2 and whether they are the source of pathogenic inflammation is unclear. Primary human monocyte derived macrophages (HMDM) are a suitable in vitro model for investigating human macrophage inflammatory responses to viral infection. HMDM did not support SARS-CoV-2 replication, as determined by viral RNA accumulation and plaque assay at MOI 5 or MOI 0.5. In contrast, pandemic H1N1 (pH1N1) 2009 Influenza A Virus (IAV) infected and replicated in HMDM. While IAV induced strong pro-inflammatory (IL-6, TNF, IL-1b) and anti-viral (IFNb, CXCL10) responses over time, indicative of detection of replicating viral RNA, SARS-CoV-2 only induced macrophage pro-inflammatory and anti-viral responses at MOI 5. Therefore, HMDM sense incoming SARS-CoV-2 virions to trigger inflammation, unlike IAV where both incoming virions and replicating virus are sensed. Blocking macrophage specific sensing pathways to block inflammation may prevent pathologic inflammation in severe COVID-19 and IAV infections.

210

Antibodies to neutralizing epitopes synergistically block the interaction of the receptor binding domain of SARS-CoV-2 to ACE 2

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A vaccine to prevent infection with SARS-CoV-2 is regarded as the most effective way to halt the COVID-19 pandemic. A major vaccine strategy is to prevent interaction between the receptor binding domain (RBD) of the Spike protein and the angiotensin converting enzyme-2 (ACE2) receptor on lung epithelial cells and on other cells. These vaccines will also induce T-cell responses, which may play a role in protection. However, concerns were raised that aberrant vaccine-induced immune responses may exacerbate disease, as has been shown to occur in other viruses, including some coronaviruses. We identified minimal epitopes on the RBD that would induce antibody responses that block the interaction of the RBD and ACE2 as a strategy that would lead to an effective vaccine with reduced likelihood of inducing immunopathology.

We tested convalescent plasma for their ability to neutralize SARS-CoV-2. We procured a series of overlapping linear peptides spanning the RBD and asked which of these were recognized by plasma from COVID-19 convalescent patients. Identified peptide epitopes were then conjugated to a carrier protein and used to vaccinate mice. Immune sera were tested for binding to the RBD and for their ability to compete with the interaction of the RBD and ACE2. We identified seven putative peptide epitopes of which three induced antibodies that could partially block the interaction of the RBD and ACE2 individually. Antibody titres did not diminish over 3 months. Two of the peptides were located in the two main regions of the RBD known to contact ACE2. Epitope-specific memory B-cells (MBCs) found in the blood of convalescent patients corelated and with epitope-specific antibody responses. Taken together, the data demonstrate that COVID-19 convalescent patients have SARS-CoV-2-specific antibodies and MBCs, the specificities of which can be defined with short peptides. This approach will aid the design of a vaccine containing only minimal antigenic material, thus improving the vaccine's safety profile.

211

Structure of SARS-CoV-2 Nsp1/5′-UTR Complex and Implications for Potential Therapeutics, Vaccine & Virulence

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Abstract

The SARS-CoV-2 is the cause of the ongoing Coronavirus disease 19 (COVID-19) pandemic around the world causing pneumonia and lower respiratory tract infections. Understanding the SARS-CoV-2 pathogenicity and mechanism of action, it is essential to depict the full repertoire of expressed viral proteins. The recent biological studies have highlighted the leader protein Nsp1 of SARS-CoV-2 importance in shutting down the host protein production. Besides it still enigmatic how Nsp1 regulate for translation. Here we report the novel structure of Nsp1 from SARS-CoV-2 in complex with SL1 region of 5′UTR of SARS-CoV-2 and its factual interaction is corroborated with enzyme kinetics and experimental binding affinity studies. The studies also address how the leader protein Nsp1 of SARS-CoV-2 recognises its self RNA towards the translational regulation by further recruitment of 40S ribosome. With the aid of molecular dynamics and simulations, we also modelled the real-time stability and functional dynamics of Nsp1/SL1 complex. The studies also report the potential inhibitors and its mode of action to block the viral protein/RNA complex formation. This built the fundamental in understanding the mechanism of first viral protein synthesised in the human cell to regulate the translation of self and host. Understanding the structure and mechanism of SARS-CoV-2 Nsp1& its interplay with the viral RNA and ribosome will open the arena of exploring the development of live attenuated vaccines and effective therapeutic targets for this disease.

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212

The alternative sigma factor RpoE2 is involved in the stress response to hypochlorite and in vivo survival of Haemophilus influenzae

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Extracytoplasmic function (ECF) sigma factors underpin the ability of bacteria to adapt to changing environmental conditions, a process that is particularly relevant in human pathogens that inhabit niches where human immune cells contribute to high levels of extracellular stress. Here we have characterized the previously unstudied RpoE2 ECF sigma factor from the human respiratory pathogen *Haemophilus influenzae* (Hi) and its role in hypochlorite induced stress. Exposure of Hi to oxidative stress (HOCl, H_2O_2) increased *rpoE2* gene expression, and activity of RpoE2 was controlled by a cytoplasmic, 67aa antisigma factor, HrsE. RpoE2 regulated expression of the periplasmic MsrAB peptide methionine sulfoxide reductase that in Hi is required for HOCl resistance, thus linking RpoE2 to HOCl stress. Interestingly, a HiD*rpoE2* strain had wild-type levels of resistance to oxidative stress *in vitro*, but HiD*rpoE2* survival was reduced 26-fold in a mouse model of lung infection, demonstrating the relevance of this sigma factor for *Hi* pathogenesis.

The Hi RpoE2 system has some similarity to ECF sigma factors described in *Streptomyces* and *Neisseria* sp. that also control expression of *msr* genes. However, HiRpoE2 regulation extended to genes encoding other periplasmic damage repair proteins, an operon containing a DoxX-like protein and also included selected OxyR-controlled genes. Based on our results we propose that the highly conserved Hi RpoE2 sigma factor is a key regulator of Hi responses to oxidative damage in the cell envelope region that controls a variety of target genes required for survival in the host.

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213

Molecular insights into secreted toxins from *Escherichia coli* pathogens reveal approaches to combat diarrheal infections

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Introduction:

Diarrheal disease is a major health problem especially in developing countries where pathogenic *Escherichia coli*cause 1.5 million deaths annually. Secretion of high-molecular-weight toxins known as serine protease autotransporters of *Enterobacteriacea*(SPATES) is a common virulence trait of enteropathogenic *E. coli*(EPEC) and enteroaggregative *E. coli*(EAEC), two major causative agents of diarrheal disease. EspC and Pet are two of these SPATEs which bind and enter epithelial cells to cause tissue destruction during infection. However, the molecular details of how these SPATEs recognize and enter epithelial cells are unknown.

Hypothesis & Aim:

To determine the structures and define the mechanisms of action of the EspC and Pet toxins for the development of antimicrobials against diarrheal disease.

Methodology:

A cross-disciplinary approach by combining cutting edge techniques in structural biology, microbiology and molecular biology.

Results & Conclusions:

We determined the crystal structure of EspC which was found to encompass a large 3-stranded b-helix with an N-terminal chymotrypsin-like serine protease domain. The large b-helix contains a number of loops thought to be responsible for epithelial cell binding and internalisation. Using the EspC structure we have designed deletion mutants of these protruding loops to uncover how this toxin hijacks the bacterial type III secretion system to specifically enter intestinal enterocytes. Unexpectantly, we found that these loops are also required for the protease activity & overall stability of these toxins. Detailed understanding of the structural-function relationships of this toxin will inform the design of inhibitors to block EspC from entering host cells and cause tissue destruction. The EspC structure is also a means to design anti-microbials that block the protease active site using small molecules. To this end we have also determined the EspC & Pet structures in complex with inhibitor PMSF and confirmed their *in vitro* inactivation. Collectively this work is revealing all important molecular details of the SPATEs mechanism of action and laying a foundation towards the structure-based drug discovery to combat diarrheal disease.

214

Hijacking of lipid synthesis and storage during flavivirus infection

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Flaviviruses are positive sense single strand RNA viruses (+ssRNA), which include several clinically important and widespread mosquito-borne viruses such as dengue, Zika and yellow fever viruses. These viruses have a demonstrated ability to invade new environments with ease and mutate to cause increasingly severe disease symptoms, and the imminent threats of climate change and urban development have the potential to greatly alter the epidemiology of these viruses. Upon entry into a cell, flaviviruses cause a drastic rearrangement of the host cell lipid landscape, sequestering and upregulating lipid synthesis to provide substrates for increased metabolism and the formation of membranous replication complexes. Perturbing the synthesis of certain lipid classes has been demonstrated to attenuate the replication of some viruses and could therefore be a potentially effective antiviral target. Here we investigate the role and manipulation of fatty acid synthase (FASN) by West Nile and Zika viruses, and the relationship between viral replication and lipid droplet content. We used several chemical inhibitors of FASN targeting different enzymatic domains, to determine which domains and metabolites contribute to replication. We found that inhibitors, orlistat and C75, had a profound effect on replication and infectious virus production. Additionally, we found that treating with orlistat caused the induction of intracellular lipid droplets not seen with other treatments, and that exogenously adding fatty acids did not rescue the inhibitory effect or orlistat but did for c75. Collectively our data suggests that blocking this domain is attenuating viral replication via a mechanism other than reducing the bioavailability of lipids to the virus, and this is something we are currently investigating.

215

Inferior outcomes in lung transplant recipients with serum *Pseudomonas aeruginosa* specific cloaking antibody

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Lung transplantation is a well-accepted treatment option for patients with end-stage lung disease, however survival is limited by the development of chronic lung allograft dysfunction (CLAD) and infection. *Pseudomonas aeruginosa* is a nosocomial pathogen that is

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particularly problematic in lung transplant recipients. Infection is difficult to eradicate once established, associated with chronic inflammation, deteriorating lung function and mortality.

A paradoxical role for antibodies in patients with chronic bacterial infections have been demonstrated. Termed cloaking antibodies (cAb), O-antigen specific IgG2 and IgA antibodies in the host have been found to block complement-mediated serum killing of the infecting bacterium, and were associated with worse disease severity. Our lab has demonstrated the clinical benefits of therapeutic plasmapheresis on three separate occasions to treat cAbs. Here, we assessed the prevalence, risk factors for, and clinical impact of cAbs in a lung transplant cohort. 123 transplant recipients with 425 archived sera were screened for cAbs against a panel of three O-antigen serotypes of *P. aeruginosa* commonly reported in clinical infections, and confirmed by serum bactericidal assays or dilution ELISA.

cAbs were detected in the sera of 40.7% of lung transplant recipients. Cystic fibrosis and younger age were associated with increased risk of serum cAbs (CF diagnosis, OR 7.03, 95% CI 3.07 – 17.07, $p < 0.001$; age at transplant, OR 0.93, 95% CI 0.90 – 0.96, $p < 0.001$). Serum cAbs and CMV mismatch were both independently associated with increased risk of CLAD (cAb, HR 4.34, 95% CI 1.91 – 9.83, $p < 0.001$;CMV mismatch (D+/R-), HR 5.40, 95% CI 2.36 – 12.32, $p < 0.001$) and all-cause mortality (cAb, HR 2.75, 95% CI 1.27 – 5.95, $p = 0.010$, CMV mismatch, HR 3.53, 95% CI 1.62 – 7.70, $p = 0.002$) in multivariable regression analyses.

These findings demonstrate that cAbs are prevalent after lung transplantation, and in part explain the known association between *Pseudomonas aeruginosa* colonisation and CLAD, and independently predict poor outcomes. Understanding the role of these antibodies in the pathobiology of chronic pulmonary infection and CLAD will be important in developing strategies to reduce irreversible allograft injury and improve outcomes post-transplant.

216

Genetic diversity and antibiotic resistance rates amongst recent Australian NTHi clinical isolates

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Antimicrobial resistance is an increasing concern for the treatment of many human pathogens, including the respiratory tract pathobiont, *Haemophilus influenzae* (NTHi), which causes upper and lower respiratory tract infections. NTHi isolates are increasingly resistant to common antibiotics, with reported ampicillin resistance rates ranging from 7.4% to 69.4% [1], and resistance to trimethoprim-sulfamethoxazole also increasing [2].

Here we have determined the genome sequences of 49 recent NTHi clinical isolates from Australia. The isolates had an average genome size of 1.85Mb ± 0.05Mb with ~1763 protein encoding genes. Comparison of gene contents revealed a small core genome of 1315 protein coding genes, with 1342 genes classified as cloud genes in keeping with previously reported values [3] and the ability of NTHi to acquire genetic material through natural competence.

No prevalent MLST classes were detected in the group of isolates, but 19 strains contained genes encoding AMR determinants. As expected, genes encoding β-lactamases were most common, but determinants for resistances to aminoglycosides, chloramphenicol, sulfonamides and tetracyclines were also present. Interestingly several strains encoded multiple resistance determinants, with 2 being potential multi-drug resistant. Analysis of the PBP3 genes for mutations conferring intrinsic resistant to β-lactam antibiotics identified 28 strains carrying relevant mutations, but only 11 strains contained the combinations of mutations associated with β-lactamase negative ampicillin resistant NTHi.

Phenotypic verification of expected AMR phenotypes using disk diffusion assays for 15 strains confirmed the expected phenotypes for 8 out of 15 strains, but also uncovered additional, unexpected resistances. Overall, 60% of these strains were ampicillin resistant, and 3 strains were classified as multi-drug resistant after showing resistance to ≥3 antibiotics.

These results indicate a clear increase in antibiotic resistance in Australian NTHi isolates since the last published survey in 2006 [4], highlighting the need for closer monitoring of these isolates across Australia.

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217

Shape-shifting bacteria are key to infection

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Bacteria naturally form a variety of shapes and sizes to survive different environments. This phenomenon is assumed to be important for infection; however, its impact is still largely unknown. One shape change is filamentation; a morphology where bacteria stop dividing and grow into long “spaghetti-like” cells. A number of infections involve bacterial filamentation, including tuberculosis, legionellosis, and the well-studied urinary tract infections (UTIs). UTIs are extremely common, affecting around 150 million people globally every year, with uropathogenic *Escherichia coli* (UPEC) causing over 80% of these infections. Filamentation is an essential part of the infection cycle of UTIs and filaments have been isolated from the urine of acute and chronic patients. While this morphology has been observed, how it allows UPEC to successfully survive the immune response is not well understood.

Most studies focus on one parameter being important for macrophage engulfment but we studied a range of these with rigorous controls to provide a more holistic view. Employing different antibiotics used to treat UTIs, we have manipulated the size and shape of the UPEC strain UTI89 to study the interaction and responses of live bacteria with THP-1 macrophages. First we used end-point assays to quantify

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the engulfment ability of macrophages. We found that engulfment is a multifactorial mechanism where bacterial size, shape and surface all matter. We further investigated the interaction between macrophages and filaments using novel microscopy analysis and found that rods and filaments display different engulfment dynamics. We identified that the engulfment of filaments takes longer and is overall less efficient. We are currently performing similar experiments using filaments isolated from a human *in vitro* bladder model. With several strains of UPEC now resistant to current antibiotics, our work identifies the importance of bacterial morphology during infections and may provide new ways to prevent or treat these infections.

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218

Spatiotemporal quantification of host membrane lipid order during Mycobacterium tuberculosis infection

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Mycobacterium tuberculosis (Mtb) is known to modulate many aspects of its host cell during infection, including the utilisation and modulation of lipids. Some lipids are targeted for pathogen entry and for use as a carbon source during replication. However, lipid utilisation and host membrane micro domain composition is poorly understood. Quantifying lipid composition of host cell membranes during Mtb infection will provide further evidence of how the pathogen is utilising lipids for survival and replication.

Through the use of high spatial and temporal resolutions afforded by confocal and lattice-light sheet imaging, micro- and macro-scale changes in lipid order of the host cell membrane can be captured. Lipid order is quantified through the use of environmentally sensitive dyes incorporated into cellular membranes. This quantification can be further extended through time to measure dynamic changes to the host cell plasma membrane, and internal membranes which include organelles and vesicles.

These imaging modalities will capture any changes in lipid order within the membranes of infected cells. The high spatiotemporal resolution these imaging techniques permit will allowing further clarification of the pathogens modulation of the hosts membranes through the infection cycle. Crucially, this includes the plasma membrane, potentially leading to lipid-specific biomarkers of disease state, an understanding of the metabolic processes during infection and scope to uncover lipid-based therapies to infection.

219

H. influenzae nutritional virulence determines outcomes of interactions with human host cells during intra- and extracellular growth

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Haemophilus influenzae (Hi) is a highly prevalent human respiratory pathobiont that is associated with recurring, acute, and chronic diseases in children and adults such as otitis media, pneumonia, chronic obstructive pulmonary disease, and asthma. The molecular basis for persistence and recurrence of Hi infections is not well understood but is likely linked to Hi metabolic properties and its increasingly recognized ability to survive intracellularly in host cells. Here we have investigated the role of nutritional virulence for Hi persistence and access to different host cell niches.

Hi preferred growth substrates were shown to be highly adapted to human epithelial surfaces and included lactate, pentoses and nucleosides, but not glucose that is typically used for Hi *in vitro* growth. Lactate utilization in Hi involved three enzymes, with the LldD L-lactate dehydrogenase driving use of lactate as a carbon source, while the two D-lactate dehydrogenases, Dld and LdhA, were involved in redox balancing. Colonization and survival of Hi in the intracellular space was directly linked to substrate utilization, with L-lactate being required for intracellular survival, while guanine de novo biosynthesis affected both extra- and intra-cellular survival in several models of infection, including mice and primary normal human nasal epithelia.

Pathogenic bacteria can also affect host responses to infection via the excretion of particular metabolites, and accumulation of major metabolic end-products such as the immunometabolite acetate in the case of Hi. Acetate had anti-inflammatory effects on cultured human tissue cells in the presence of live but not heat-killed Hi. Our work provides evidence of the critical role of metabolic processes for persistence of Hi inside host cells and the immunomodulatory potential of Hi metabolic end-products.

220

Age-dependent innate and adaptive cellular immune responses in malaria-naive children and adults.

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Children are the most at-risk group for severe and symptomatic malaria, with age and exposure to malaria independently impacting the ability of children to gain protective antibodies. To improve outcomes for children, an increased understanding of the impact of age on innate and adaptive cellular immune responses is necessary. We investigated novel antigen-presenting cell subsets (monocytes and Vδ2 γδ T cells) and T-follicular helper (Tfh) cell activation in malaria-naive children and adults after in-vitro stimulation with *Plasmodium*-infected RBCs (iRBCs). After iRBC stimulation, we show age-dependent differences in monocytes from malaria-naive adults and children. Children produce more cytokines including IL-10, IL-1β, and IL-6 compared to adults and children have more polyfunctional (multiple-cytokine) producing monocytes. Further, adults had a higher proportion of Vδ2 γδ TNF/IFNγ producing cells compared to children. In contrast, Tfh cell subsets were similarly activated after in-vitro iRBC stimulation in both malaria-naive children and adults. This finding contrasts our previous data of age-dependent differences to Tfh activation in children and adults with symptomatic naturally acquired malaria and reveals intrinsic differences in innate cell subsets between children and adults in response to iRBCs. Together these data inform our understanding of age-dependent differences that contribute to the development of protective anti-malarial immunity.

221

Elucidating genes that are required for chronic infections of *Salmonella enterica*

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Pathogenic bacteria have evolved complex mechanisms to cause infection and to evade the host immune system. The facultative intracellular pathogen *Salmonella enterica* is one such organism that has adapted a range of genes and pathways that enable host colonisation and persistence. *S. enterica* can be broadly be divided into two groups; serovars that are host specific and serovars that are generalists able to infect many different hosts. These different serovars often have specific genes that are important for survival in serovar specific replicative niches. However, one universal and important aspect of *S. enterica* infections is the ability of the infecting strain to cause chronic asymptomatic infections. It is estimated that up to 5% of people infection with *S. enterica* serovar Typhi become chronically infected, and there are examples of chronic carriers in other human, pig, chicken and mouse infections with *Salmonella*. We hypothesized that the genes required for the development of chronic *Salmonella* infections would be highly conserved. Using a bioinformatics approach, we identified conserved *Salmonella* genes that are not present in other closely related Enterobacteriaceae. From our list of conserved genes, we identified genes that were present on specific *Salmonella* pathogenicity islands, and those that encoded for known virulence factors, such as type three secretions system apparatus and effectors. We also identified a gene of previously unknown biological function, ApeE, which had not been linked to *Salmonella* virulence, despite being conserved in all currently sequenced *Salmonella.* Here, through a combination of molecular *in vivo,* omics and enzymatic techniques, we demonstrate a tissue-specific role for ApeE and provide evidence that ApeE is important during *Salmonella* chronic infections.

222

The *Salmonella* Effector SseK3 Targets Small Rab GTPases

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Salmonella species are among the most common foodborne pathogens that incur significant burden on the healthcare system worldwide. As an intracellular pathogen, *Salmonella* utilises two Type III secretion systems (SPI-1 and SPI-2) to inject virulence effector proteins into host cells to mediate invasion and subsequent intracellular replication via subverting a series of critical host cell biochemical and physiological events. It has become clear that one strategy employed by *Salmonella* to survive intracellularly is the subversion of key intracellular vesicle transport regulators, the Rab GTPases.

Within the *Salmonella* SPI-2 effector cohort, SseK1, SseK2 and SseK3 have been identified as three homologues of NleB1, an arginine *N*-acetylglucosamine (GlcNAc) transferase of enteropathogenic *Escherichia coli* that blocks protective cell death by modifying death domain containing proteins in host cells. Although these effectors have been studied previously, detailed virulence mechanisms and host targets of these SseK proteins still remain to be explored and verified.

Work from our laboratory revealed that SseK3 modifies several Rab GTPases during *Salmonella* infection of murine macrophages. We confirmed the modification of Rab1, Rab5 and Rab11 by SseK3 in immunoprecipitation experiments. Interestingly, SseK3 targeted three arginine residues (R74, R82, R111) for modification on Rab1, while R74 and R82 localized to the catalytically important switch II region. Rab1 mediates vesicle transport from endoplasmic reticulum to the Golgi apparatus, and SseK3 co-localized with Rab1 on the Golgi of host cells. The expression of SseK3 resulted in reduced secretion of secreted embryonic alkaline phosphatase (SEAP) in transfected cells, which is largely dependent on its glycosyltransferase activity. However, SseK3 only exhibited a modest effect on SEAP secretion during infection on a HeLa229 cell line. The cytokine secretion profile was examined on infected Raw264.7 cells, and inhibition of IL-1 and GM-CSF was only observed when SseK3 was over-expressed. Our results suggest that SseK3 may contribute to *Salmonella* infection by targeting the activity of key Rab GTPases.

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223

Novel serum-resistance mechanism in patients with *P. aeruginosa* bacteraemia

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Pseudomonas aeruginosa is an opportunistic and highly virulent pathogen with an increasing prevalence of multi-drug resistant strains. In bacteraemia, *P. aeruginosa* is associated with significantly increased patient morbidity and mortality and poses a major risk for immunocompromised individuals and patients in intensive care units. The high incidence of intrinsic antibiotic resistance in *P. aeruginosa* and the rapidly growing number of extensively resistant strains necessitate the development of new treatments and drugs. Recently, a novel mechanism of serum resistance for *P. aeruginosa* was described which is mediated by the presence of 'cloaking antibodies' (cAb). In this mechanism, high titres of IgG or IgA specific for the O-antigen polysaccharide can actually prevent serum-mediated killing of *P. aeruginosa.* Although this phenomenon is well established in chronic *P. aeruginosa* lung infections, at present its role in acute bacteraemia is unknown. To investigate this, we collected 75 matched serum and bacterial blood isolates from patients with *P. aeruginosa* bacteraemia and screened for the presence of cAb. Strains were serotyped via PCR and the titre of antibody specific for their matched serotype O-antigen determined. Despite being an acute infection 46% of patient sera had significantly high antibody responses to their matched O-antigen serotype. To determine if this sera could inhibit serum-mediated killing of their *P. aeruginosa,* serum bactericidal assays were performed against their cognate strain (if sensitive to healthy control serum killing) or serotype-matched lab strains. Twenty-one of the 75 patient sera could inhibit healthy control serum killing of a *P. aeruginosa* strain, however as the majority of bacterial isolates were innately serum resistant, only four could inhibit their cognate strain. These results are the first to demonstrate the presence and clinical relevance of this cloaking mechanism in *P. aeruginosa* bacteraemia.

224

Defining the glycointeractome of the multidrug resistant pathogen *Acinetobacter baumannii*: towards novel treatments and therapeutics

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Acinetobacter baumannii is a major hospital-acquired pathogen, and responsible for a range of opportunistic infections. Over half of *A. baumannii* isolates are resistant to nearly all antibiotics, costing >$700million per year. Multidrug resistance, and a dearth of new antibiotic development, mean novel ways to treat infections are needed. Although many *A. baumannii* virulence factors have been studied, very little is known about the host receptors *A. baumannii* adhesins interact with. Nearly all proteins and cells are decorated by glycans (sugars), and the significance of glycans in microbial:host interactions is emerging. Recent studies have identified critical roles for glycan-binding adhesins in many pathogens including NTHi and UPEC. Many *A. baumannii* adhesins bind fibronectin and collagen, found throughout the body, and both are heavily glycosylated. Therefore, by defining the host glycans bound by *A. baumannii*, i.e., the 'glycointeractome', we can develop new ways to block host cell interactions.

As a proof of concept, we over-expressed the *A. baumannii* adhesin Ata in *E. coli.* This allowed us to determine the glycan binding specificity using glycan arrays, and determine affinity using surface plasmon resonance (SPR). Over-expression of the Ata 'head domain' allowed us to confirm that this is the receptor binding region of Ata. Glycan arrays identified a subset of ~20 glycans bound by Ata, with these glycans all containing a galactose (Gal) linked to N-acetyl-glucosamine (GlcNAc) or an N-acetyl-galactosamine (GalNAc). SPR using these glycans showed a preference for Gal-GlcNAc. These structures are common glycan decorations on both fibronectin and collagen. Carrying out SPR with the Ata-head domain using fibronectin that had been treated with glycosidases showed a complete loss of binding compared to untreated fibronectin.

This approach has shown that a key *A. baumannii* adhesin, Ata, specifically interacts with host glycans, and that this interaction is required for high-affinity binding to the human protein fibronectin. In future, we will characterise additional *A. baumannii* adhesins to define the complete glycointeractome, and use this knowledge to develop ways to block interactions with host cells. This will allow us to augment traditional antibiotics, providing a much-needed alternative to treat a major drug resistant pathogen.

225

Induction of stable human FOXP3+ Tregs by a parasite-derived TGF-β mimic

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Background & Aims. Immune homeostasis in the intestinal tract is tightly controlled by FOXP3+ regulatory T cells (Tregs), with loss of this Treg-mediated control linked to development of chronic conditions, such as inflammatory bowel disease (IBD). As a mechanism of immune evasion, several species of intestinal parasites can strengthen intestinal Treg activity, leading to the notion that parasite-derived products could be harnessed and used as an immune regulatory therapy for IBD. It has been previously demonstrated that the parasite *Heligmosomoides polygyrus* secretes a molecule (*Hp*-TGM) which mimics the ability of TGF-β to induce FOXP3 expression in CD4+ T cells. Our aim was to investigate whether *Hp*-TGM could induce human FOXP3+ Tregs as a potential therapeutic approach.

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Methods. Human CD4⁺ T cells from healthy donor blood were expanded in the presence of *Hp*-TGM or mammalian TGF-β. The induction of Tregs was measured by flow cytometric detection of FOXP3 and other Treg markers, such as CTLA-4 and CD25. Epigenetic changes were detected using CHIpSeq and pyrosequencing of *FOXP3.* Treg phenotype stability was assessed following inflammatory cytokine challenge and Treg function was assessed by cellular co-culture suppression assays and secreted cytokines measured by cytometric bead array.

Results. *Hp*-TGM efficiently induced FOXP3 expression (>60%), in addition to another Treg functional marker CTLA4, and caused epigenetic modification of the *FOXP3* loci to a greater extent than TGF-β, indicative of stable lineage differentiation. *Hp*-TGM-induced FOXP3⁺ Tregs also had superior suppressive function and retained their phenotype following exposure to inflammatory cytokines. *Hp*-TGM also induced a Treg phenotype in *in vivo* differentiated Th1 and Th17 cells, indicating its potential ability to re-program memory cells to enhance immune tolerance.

Conclusions. These data indicate *Hp*-TGM has the potential to be used to generate stable human FOXP3⁺ Tregs in the treatment of IBD.

226

Antimicrobial activity of outer membrane vesicles against Gram-negative and Gram-positive bacteria is altered by their mechanism of biogenesis

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Outer membrane vesicles (OMVs) are nanoparticles naturally produced by all Gram-negative bacteria. OMVs are produced by budding from the cell membrane during bacterial growth or by prophage-induced explosive cell lysis resulting in bacterial membrane fragments ligating to form OMVs. Depending on their cargo composition, OMVs have numerous roles including the lysis of competing bacterial species as an antimicrobial defence mechanism. Although bacteria can regulate the cargo composition and functions of OMVs throughout bacterial growth, it is unknown whether the mechanism of OMV biogenesis can also affect their composition and functions. Therefore, in this study we examined whether different mechanisms of OMV biogenesis can regulate OMV cargo composition and their subsequent antimicrobial functions.

We isolated OMVs from three *Pseudomonas aeruginosa* strains; PAO1 which releases OMVs naturally by budding, PAO1*Δlys* which cannot undergo explosive cell lysis, and PAO1*Δlys* pJN105*lys* which produces OMVs via the inducible expression of explosive cell lysis. The antimicrobial activity of these OMVs was tested against *P. aeruginosa* and *Staphylococcus aureus.* OMVs generated naturally from PAO1 and PAO1*Δlys* were unable to inhibit the growth of *P. aeruginosa.* However, OMVs produced via explosive cell lysis could inhibit *P. aeruginosa* growth. Interestingly, OMVs isolated from all *P. aeruginosa* strains were able to significantly inhibit the growth of *S. aureus.* These results suggest there are differences in the antimicrobial functions of OMVs against Gram-negative and Gram-positive bacteria.

Collectively, our data shows that *P. aeruginosa* OMVs generated by explosive cell lysis can inhibit both *P. aeruginosa* and *S. aureus* growth whilst OMVs generated via budding from the cell membrane can only inhibit *S. aureus* growth and not that of their parent bacterium. These findings provide insight into the regulation of OMV cargo composition and will enable us to advance our understanding of how OMV biogenesis dictates OMV content and their subsequent antimicrobial functions.

227

Characterisation and inhibition of bacterial virulence factors

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Autotransporters are the largest family of outer membrane/secreted proteins in Gram-negative bacteria. Many autotransporters are virulence factors which contribute to the pathogenesis of bacteria responsible for infectious diseases such as urinary tract infections, diarrhoea, whooping cough, meningitis and sepsis. These secreted proteins can be broadly divided in two groups, adhesins and proteases, which aid in establishing infections and contributing to disease by mediating host colonisation, cell invasion, biofilm formation and cytotoxicity. We have expressed and purified multi-milligram quantities of autotransporters belonging to different classes with the aim to characterise their unique structural properties that determine their specific function in pathogenesis. In addition, we are using antibody-based strategies to inhibit the activity of these proteins with aims to develop novel therapies targeting bacterial virulence.

228

Salmonella Typhimurium induces cIAP1 degradation to promote death in macrophages

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Salmonella Typhimurium is a gastrointestinal pathogen that infects both humans and animals. *S.* Typhimurium infects epithelial cells and macrophages intracellularly, enabled by two specialised Type III Secretion Systems (T3SSs) which translocate effector proteins directly into the host cell cytosol. These effector proteins exert a range of pathogenic activities, including manipulation of innate immune signalling and programmed cell death processes. Our preliminary research shows that infection of immortalised murine macrophages with wild type *S.* Typhimurium induces the degradation of cellular inhibitor of apoptosis protein 1 (cIAP1), an important host cell adaptor of tumour necrosis factor receptor 1 (TNFR1) signalling and inhibitor of apoptotic cell death. Degradation of cIAP1 was associated with functional

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Salmonella Pathogenicity Island 1 (SPI-1) T3SS effector translocation, and was not prevented by pan-caspase, proteasomal or lysosomal inhibitors. Consistent with cIAP1-mediated inhibition of apoptosis, we observed strong association between loss of cIAP1 and increased cellular cytotoxicity. Anti-cIAP1 immunoblot detected a low molecular weight peptide following *S.* Typhimurium infection, suggesting that a SPI-1 effector may cleave cIAP1 during infection. Current work combines several molecular and *in vitro* techniques, aiming to elucidate the cIAP cleavage mechanism, and determine the responsible SPI-1 effector protein. Future work will assess the involvement of cIAP proteins in overall susceptibility to *Salmonella* infection *in vivo*. Together, these data provide early support for our hypothesis that cIAP1 depletion was induced by a *S.* Typhimurium SPI-1 effector in order to promote host cell death, and potentially dissemination of the bacterium.

229

'Unleishing' host cell death pathways to promote clearance of *Leishmania donovani*

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Leishmaniasis, a disease caused by the *Leishmania spp.* parasite, affects 1 million people annually worldwide. Specific *Leishmania* species*,* such as *Leishmania donovani,* cause chronic spleen, liver and bone marrow infection which if left untreated leads to a fatal visceral infection resulting in 25,000-65,000 deaths globally each year. Current visceral leishmaniasis treatments are expensive, have severe side effects, and with rising drug resistance and no vaccine, there is an unmet need for novel therapeutics.

Intracellular pathogens, such as *Leishmania spp.* manipulate host cell survival and death signalling pathways to survive, replicate and disseminate. We hypothesise that identifying the exact cell death modalities hijacked by the pathogen informs specific therapeutic targeting to reduce parasite burden and ultimately, treat visceral leishmaniasis.

To this end, we used *L. donovani in vivo* and *in vitro* infection models including gene-targeted mice and therapeutic compounds targeting host cell apoptotic and pyroptotic machinery. We determined the parasitic burden, cell death and protein expression through microscopy, live-cell imaging, flow cytometry, immunohistochemistry and immunoblotting upon infection with *L. donovani*.

Our results suggest there is no role for pyroptosis as mice deficient in either Gasdermin-D, the executioner protein for pyroptosis, showed no difference in parasite burden compared to C57Bl/6 controls. Additionally, targeting intrinsic apoptosis using BCL-2, MCL-1, BCL-X_L inhibitors also did not affect parasite burdens *in vitro*.

However, inducing extrinsic apoptosis of infected primary macrophages with IAP inhibitors resulted in both host cell and concurrent parasite death. *L. donovani* infected mice treated with IAP inhibitors displayed reduced leishmaniasis symptoms, and the splenic parasite reservoir was decreased due to apoptosis of macrophages - the parasite reservoir cells. Importantly, combining IAP inhibitors with the standard therapy for visceral leishmaniasis, Amphotericin-B, enabled the dosage and thus toxicity of both therapies to be reduced while maintaining significant reduction in splenic parasite burden.

Taken together, our data indicates that targeting host extrinsic apoptotic pathways using clinical stage IAP inhibitors may be a valid therapeutic option for visceral leishmaniasis.

230

Maurer's cleft tethers – is tethering important for malarial adhesion?

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The malaria parasite *Plasmodium falciparum* modifies the host red blood cell (RBC) for survival. This remodelling is facilitated by the export of many parasite proteins into the RBC cytoplasm. Some of these proteins, including the major virulence protein *Pf*EMP1, are exported via an intermediate trafficking structure called Maurer's clefts. The final steps in the transfer of *Pf*EMP1 to the RBC membrane from the cleft are poorly understood. Previous electron tomography studies of the parasite have revealed tether-like structures connecting Maurer's clefts to the RBC membrane. These tethers have been hypothesised to play a role in immobilising the Maurer's clefts at the RBC membrane, where they may function in the final steps of *Pf*EMP1 trafficking and surface display. MAHRP2 is the only protein currently known to localise to the tether. Immunoprecipitation studies of MAHRP2-GFP revealed several new proteins. Here we report on one of these proteins termed the MAHRP2 interacting protein (MIP). MIP localises to the Maurer's clefts and conditional knockdown studies show that MIP is required for parasite adhesion to endothelial receptors and that deletion of MIP leads to a delay in the immobilisation of the Maurer's clefts to the RBC membrane.

232

Host and pathogen genetic determinants that contribute to Buruli ulcer severity

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Buruli Ulcer is a neglected tropical disease caused by the flesh-eating *Mycobacterium ulcerans* which results in a subcutaneous necrotic skin infection. If untreated at its early stage, the disease could result in prolonged morbidity. The transmission of the disease is currently unknown, but it is assumed that micropunctures in the skin, as well as insect vectors such as mosquitoes, could contribute to its transmission. The exponential growth of Buruli ulcer cases in Victoria has called for an urgent scientific response. The reasons for the Victorian epidemic are unclear but may relate to genotypic changes in the organism that have made it more transmissible or pathogenic, and/or genetic variation in the human host. However, there is no information available on the relationship between the genotype of an

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isolate or that of the patient (or both) and the clinical severity of the disease. Such knowledge may provide useful information improve understanding of interactions between *M. ulcerans* and pathogenesis in its human host. Using samples collected from a Barwon Health cohort (n=120), the overall aim of this project is to perform metagenomic sequencing to identify host and microbial factors contributing to the disease. Next-generation sequencing and bioinformatics approaches are being developed to extract host, pathogen and ulcer microbiome genomes. Using these data, host genetic variations associated with disease severity, pathogen strains, microbiome and their contribution to disease severity will be explored. Analysis pipelines have been developed, and genomic sequence analysis is currently underway. Results of the preliminary analysis of 120 samples will be discussed in context with disease severity.

233

Complement and Fcγ-receptor mediated antibody effector functions target merozoites and are associated with protection from severe malaria in children

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Clearance of malaria parasites in the blood may involve complement proteins that can lead to merozoite lysis and inhibition of RBC invasion and replication. Opsonization of malaria blood-stages by antibodies and complement, which interact with complement receptors (CR) and Fcγ-receptors, can promote phagocytic clearance by monocytes and neutrophils. However, complement dysfunction and excessive activation of immune cells can also potentially contribute to pathogenesis of severe disease. Currently, there are limited data on complement and Fc-gamma receptor (FcγR) mediated responses in severe malaria. In this study we investigated, for the first time, antibodies that fix and activate complement or interact with different Fcγ-receptors to promote phagocytosis in a case-control study of severe malaria in 383 young children in Papua New Guinea (PNG). Our results show that IgG and complement (C1q) fixing antibodies were significantly lower in children with severe malaria compared to children with mild uncomplicated malaria (p=0.04 and p=0.02 respectively). IgG strongly correlated with functional antibodies in both severe and uncomplicated malaria. Further, children with broad antibody multi-functional activity had a significantly reduced odds of severe disease. Our results show differences in response types between severe and uncomplicated disease among children that provide new insights into mechanisms that could be exploited in vaccine development to provide protection against severe disease.

234

Pathological and immune responses to chronic *Mycoplasma gallisepticum* infection and protective immunity of the ts-304 vaccine in chickens

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Mycoplasma gallisepticum causes chronic respiratory disease in chickens, but the molecular pathways underlying the chronic pathological changes caused by *M. gallisepticum* in the tracheal mucosa are poorly understood, and the types of immune responses induced after infection or vaccination are not well differentiated. *M. gallisepticum* ts-304 is a novel live vaccine strain that is safe and more efficacious than the current commercial vaccine strain ts-11. The transcriptional profiles of the tracheal mucosa were analysed in chickens vaccinated with ts-304 at 4 and 57 weeks after vaccination and compared with those of unvaccinated chickens after experimental infection with *M. gallisepticum.* Pathological changes and immune cells accumulating in the mucosa were assessed by histopathological examination and indirect immunofluorescence staining. The levels of transcription of a panel of chicken cytokine and chemokine genes were quantified using RT-qPCR. Two weeks after challenge, genes, pathways, gene ontologies and protein classes involved in inflammation, cytokine production, and signaling and cell proliferation were upregulated, while those involved in formation and movement of cilia and formation of the cytoskeleton and intercellular junctional complexes were downregulated in the challenged-only birds compared to vaccinated-only, vaccinated-and-challenged, and negative-control birds. There was no significant difference in gene expression between vaccinated birds and the negative control group. After infection, there was a significant increase in the tracheal mucosal thickness and transcription of genes for cytokines and chemokines, including those for IFN-γ, IL-17, RANTES and CXCL-14, and B-cells, T-cells and macrophages accumulated in the mucosa of challenged-only birds. There were indications that acquired immunity was developing towards the end of the second week after infection - predominantly a T_{helper}-1 cell-dependent B cell response. The new ts-304 vaccine protects the birds against the adverse effects caused by *M. gallisepticum* in the tracheal mucosa for at least 57 weeks after vaccination.

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235

Characterization of Sec13 as a host-interactor in influenza using a Yeast-based system and Mammalian cells

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Background: Annually, the influenza virus causes 500,000 deaths worldwide. Influenza -associated mortality and morbidity is especially high amongst the elderly, children and patients with chronic diseases. While there are antivirals available against influenza such as neuraminidase inhibitors and adamantanes, there is growing resistance against these drugs and there are no good drugs against influenza. Thus, there is a need for novel antivirals for resistant influenza strains. Host-directed therapies are a potential strategy for influenza as host processes are conserved and are less prone mutations as compared to virus-directed therapies.

Methods: A Pubmed search was performed for papers that performed NS1-interaction screens and the Reactome pathway database was used for the bioinformatics analysis. DAVID analysis, STRING interaction analysis was performed. YeastMine was used to determine yeast homologs. PR8 NS1 and NS1-GFP was cloned into a constitutive yeast expression vector and the Lithium Acetate method was used to transform yeast. Fluorescent microscopy was performed to visualize the colocalization in yeast. A549 cells treated with R848, a TLR7 agonist, followed by qPCR analysis.

Results: Bioinformatic analysis of NS1 interactome papers and the Interactome pathway has found that there were 167 proteins that had known yeast homologs. Of which, Sec13 was found to have one of the highest homology (66.2%) between mammalian cells and yeast. When NS1-GFP was expressed in yeast, it was found that NS1-GFP colocalized with Sec13. This is also observed in NS1 expressing stably transfected A549 cells. In A549 cells, Sec13 was found to be increased at mRNA level after 24 hours of R848 treatment.

Conclusion: Sec13 may be a key host protein in the influenza life cycle and in cytokine production.

236

Super-resolved view of PfCERLI1, a rhoptry associated protein essential for *Plasmodium falciparum* merozoite invasion of erythrocytes

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The disease-causing blood-stage of the *Plasmodium falciparum* lifecycle begins with invasion of human erythrocytes by merozoites. Many vaccine candidates with key roles in binding to the erythrocyte surface and entry are secreted from the large bulb-like rhoptry organelles at the apical tip of the merozoite. Here we utilise high- and super-resolution imaging and identify an essential role for the conserved protein *P. falciparum* Cytosolically Exposed Rhoptry Leaflet Interacting protein 1 (PfCERLI1) in rhoptry function. Using biochemical techniques and quantitative super-resolution microscopy, we show that PfCERLI1 localises to the cytosolic face of the rhoptry bulb membrane and knockdown of PfCERLI1 inhibits merozoite invasion. While schizogony and merozoite organelle biogenesis appear normal, reduction in PfCERLI1 expression appears to block secretion of key rhoptry antigens that coordinate merozoite invasion. While further studies need to be undertaken to determine the fine detail of how PfCERLI1 knockdown causes these changes in rhoptry function, identification of PfCERLI1's direct association with release of rhoptry antigens is a key step in understanding the complex molecular events that control rhoptry secretion during invasion. This study makes extensive use of semi-automated quantitative immunofluorescence microscopy and highlights how this powerful tool can be used to study the process of invasion.

237

Drug addiction in the human malaria parasite *plasmodium falciparum*

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Resistance against all existing antimalarial drugs is becoming a major concern and highlights the need for the development of novel treatment strategies. Host-directed therapy (HDT) has potential for combatting malaria, and has the advantage of depriving the parasite of the most direct pathway to resistance, namely the selection of mutated targets under drug pressure. Previous studies have revealed that *Plasmodium falciparum* relies on the activation of host erythrocyte protein kinases for its own proliferation and survival. One host kinase in particular, the mitogen activation protein kinase kinase 1 or MEK1, has been shown to be activated in infected erythrocytes and hepatocytes. Trametinib is a highly selective MEK1 inhibitor that is approved for clinical use against melanoma. We showed that Trametinib inhibits parasite proliferation *in vitro* with low nanomolar potency, suggesting that MEK activation is required for parasite survival. Surprisingly, we were able to generate parasite lines that are resistant to Trametinib, showing a 30-fold increase in the IC_{50}. Intriguingly, some of these parasites display dependency to the drug, and show optimal proliferation in the presence of approximately one micromolar Trametinib. Whole genome sequencing of clones obtained from these lines identified candidate mutations associated with the dependency phenotype. Our work provides novel insights into the molecular mechanisms of host-pathogen interactions between human erythrocytes and *P. falciparum*, and highlights human kinases as targets for the development of novel anti-malarials with an untapped mode of action.

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238

Investigating the role of *Plasmodium falciparum* exported proteins that bind the new permeability pathway complex protein RhopH2

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Every day over 1000 people die of malaria, most of who are children under the age of five. The emergence of drug resistance in *Plasmodium falciparum*, the causative agent of severe malaria, is alarming and we need new antimalarial drugs. A crucial part of this process is to understand the basic biology of the parasite and elucidate targetable proteins/pathways. The ability of *P. falciparum* to survive within the erythrocyte is dependent on the new permeability pathways (NPPs) which provide import of vital nutrients, making them an attractive drug target. The NPPs have been affiliated with the activity of three parasite proteins: RhopH1, RhopH2 and RhopH3. We previously showed that RhopH2 was associated with 30 additional proteins predicted to be exported into the erythrocyte. We therefore hypothesised that some of these exported proteins might be important for NPP function, as protein export is a prerequisite for NPP activity. Here we tagged 13 of the 30 proteins with HA*glmS* to conditionally knockdown individual proteins and study their role in NPP activity. An exported nanoluciferase reporter was introduced into each parasite line to observe changes in sorbitol lysis (NPP uptake) during protein knockdown. We show that none of the 13 proteins are individually required for NPP function. Reciprocal immunoprecipitation assays further showed that only six proteins were able to co-precipitate RhopH2. Majority of these six proteins are known cytoskeletal proteins and therefore likely interacting indirectly with RhopH2 due to cytoskeletal binding and not due to direct NPP function. Although the 13 proteins studied do not appear to be essential for the NPPs, this study greatly expands our current knowledge of exported proteins. We reveal for the first time the location of five new proteins, hypothesised to be exported, as well as providing a clearer picture of protein-protein interaction within the *P. falciparum* infected erythrocyte.

239

The gastrointestinal tract is a central site of dengue virus pathology and possible key to severe disease

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Dengue virus (DENV), a mosquito-borne flavivirus, is increasing in prevalence and a major health care burden in tropical regions. Infections range from asymptomatic through to life-threatening haemorrhagic fever or dengue shock syndrome. The severe complications of DENV disease have a rapid onset, at a time when the viremia is decreasing and fever has abated, and critically involve endothelial leak and thrombocytopenia. Gastrointestinal (GI) symptoms are more frequently seen in severe DENV infection, and can include diarrhoea, vomiting, bleeding, and abdominal pain. Bacterial LPS is found to be elevated in patient blood, correlating with disease severity, and GI barrier breakdown has been suggested to contribute to the onset of severe disease. However, there is little to no information about GI tract pathology. We established mouse models of DENV infection that mirror the vascular leak and diarrhoea seen in human disease. We have examined the GI tract during DENV infection of AG129 and IFNAR1 KO mice and find inflammation progressing down the length of the intestine, with intensifying pathology in the large intestine later in infection, including edema and mucus depletion, cytokine and chemokine expression as well as mononuclear cell and neutrophil infiltration. Some mice show GI tract permeability and bacterial penetration into tissues, and inflammatory pathology is reduced with a Toll-like receptor 4 inhibitor. We propose that gut barrier breakdown is a key tipping point leading to the rapid onset of severe disease, at a time when patients are otherwise seeming to recover. Therapies aiming to maintain the integrity of the gut barrier may help prevent severe disease.

240

T follicular helper (Tfh)-germinal centre (GC) B cell response is required for sterile immunity during enteric helminth infection

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CD4 T follicular helper (Tfh) cells are an important component of germinal centres (GCs), whose overarching goal is to produce an effective humoral immunity. However, the protective roles for Tfh-GC response during helminth infections remain controversial and poorly understood. Here, we demonstrate that diminished Tfh-GC B cell response is associated with chronic infection of the helminth parasite *Trichuris muris* (*Tm*). We show that a high-dose (HD) of parasite eggs in mice that leads to a Th2 cell-biased acute infection and worm clearance, results in a significant increase in Tfh and GC B cells, which is typified by the selection of parasite-specific IgG1 class switching. In contrast, a low-dose (LD) infection that results in a Th1 cell-biased chronic infection fails to induce a potent Tfh-GC response. Using the reporter mouse, we further demonstrate that IL-4-producing Th2 cell response predominantly occurs during the first stages of infection and are likely to initially mediate worm expulsion. Conversely, a potent Tfh-GC response is prominent during the last stages of worm expulsion. Strikingly, blockade of Tfh-GC interactions via anti-CD40L treatment during HD infection promotes chronic infection, suggesting Tfh and GC B cells are enablers of complete worm clearance and are thus required for sterile immunity. Overall, these data provide cellular and kinetics insights into the roles of Tfh-GC response during helminth infection and identify a potent Tfh-GC response as a protective component of the type 2 immunity to intestinal helminths.

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241

Depletion of alveolar macrophages by cigarette smoke causes delayed clearance of *Legionella pneumophila* infection

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Legionella pneumophila is an intracellular pathogen that is the dominant aetiological agent of Legionnaires' Disease (LD), a particularly severe form of pneumonia. Upon inhalation of aerosols containing the bacteria, alveolar macrophages (AM) engulf the pathogen and initiate an inflammatory response. Despite this, *L. pneumophila* survives within AM by establishing a replicative vacuole through the injection of more than 300 bacterial virulence proteins termed Dot/Icm effector proteins into the macrophage cytosol.

Cigarette smoke (CS) is strongly linked to increased susceptibility to LD as well as many other infections. Here, we aimed to identify why cigarette smoking renders individuals more susceptible to LD. We characterised a model of concurrent acute CS exposure and *L. pneumophila* infection. CS-exposed mice displayed increased pulmonary bacterial loads and developed more severe disease when infected with *L. pneumophila*. Using flow cytometry, we found that one of the main effects of CS on lung leukocytes was a marked reduction of AM numbers, in contrast to the current belief that CS exposure causes expansion of AM. Therefore, we confirmed this observation using light-sheet microscopy to image whole lungs in order to avoid any potential artifacts caused by extraction of leukocytes. Although it was expected that AM depletion would ameliorate disease, as they are the cellular niche for *Legionella* replication, depletion of AM in mice with clodronate liposomes mimicked the effects of CS exposure.

In summary, our results show that despite being the cellular niche for *Legionella* replication, AM contribute towards clearance of infection. We hypothesize that one of the primary ways by which CS causes more severe LD is via depleting AM. Hence, targeting AM during LD may be detrimental to disease severity, and restoration of AM numbers or function in cigarette smokers may be a potential avenue of investigation for therapeutic intervention.

242

Stabilization of tristetraprolin modulates inflammation during *Mycobacterium tuberculosis* infection

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Tuberculosis (TB) remains one of the world deadliest diseases. TB infection causes significant inflammation, often inducing irreversible tissue damage. New options to control TB-associated inflammation are urgently required. This study examined if regulating the expression of Tristetraprolin (TTP) could aid control of this inflammation. TTP inhibits pro-inflammatory responses by degrading target mRNAs including TNF and IL-1b. TTP is active when unphosphorylated but it often remains inactive inside the body. To keep TTP in its active form, murine serines at positions 52 and 178 in TTP were replaced by non-phosphorylatable alanine residues (TTP^{aa}). Macrophages from TTP^{aa} mice displayed a reduced pro-inflammatory response when infected with *Mycobacterium tuberculosis* (*M. tb)* while maintaining control of infection. TTP^{aa} mice were then infected with *M. tb.* Infection was accompanied by an early reduction in inflammatory cells recruited to the lungs of the TTP^{aa} mice, though this was then followed by a transient increase in inflammatory cells and bacterial growth in the lungs. Similarly to *in vitro* analysis, lung cells from TTP^{aa} mice showed a decrease in secretion of pro-inflammatory cytokines and chemokines, but this was not accompanied by sustained reduction in inflammation and granuloma formation. While the presence of TTP in a permanently active form reduced early inflammation, it did not prevent mice from activating T cells, recruiting inflammatory cells to form granulomas and eventually controlling bacterial growth in an equivalent manner to wild-type mice. These data suggest that other pathways might compensate for the effects of active TTP, limiting the capacity of this modification to permanently reduce *M.tb*induced inflammation.

243

Functional cure of chronic hepatitis B is associated with co-occurrence of HBsAg/anti-HBs immune complex peaks with ALT flares, and seroconversion to potently neutralising anti-HBs

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Background and Aims: HBsAg specific antibody responses during chronic hepatitis B infection are poorly defined due to the excessive amount of sub viral particles produced during infection and lack of standardised detection methodologies. The aim of this study was to investigate the nature of the anti-HBs responses in chronically infected HBV patients during the process of functional cure.

Methods: Longitudinal samples from 25 genotype A CHB patients undergoing nucleos(t)ide analogue (NA) treatment were examined, 14 of these patients achieved functional cure (FC) while 11 patients remained infected. HBsAg/anti-HBs Immune complexes (HBs-IC) were quantified using an in-house modification of a commercial diagnostic assay. Epitope-specific anti-HBs responses were detected using a 19-plex assay and neutralization efficacy of anti-HBs was measured using an *in vitro* HBV infection model.

Results: HBs-IC was detected in all patient's serum samples at fluctuating levels. In the 14 patients who achieved functional cure, 10 had an ALT flare immediately prior to HBsAg loss, and of these 10 patients, 9 had co-occurring HBs-IC peak responses. This co-occurrence was not observed in non-functionally cured patients (non-FC). Non-FC patients had lower anti-HBs responses at week 12 of NA treatment compared to FC patients. Anti-HBs derived from FC patients after seroconversion was more potent in neutralizing HBV infection *in vitro*, and recognised more anti-HBs epitopes compared to anti-HBs from vaccinees.

Conclusions: Anti-HBs responses are present and fluctuate during chronic hepatitis B infection. Functional cure in the analysed cohort was associated with co-occurring HBs-IC peak and ALT flare prior to clearance, and was followed by seroconversion to anti-HBs with broad HBsAg epitope recognition, which potently neutralize HBV infection. Failure of virus clearance was associated with lower anti-HBs responses in the early phase of NA treatment. These results demonstrate the presence and importance of broadly-reactive anti-HBs responses in clearing HBV infection.

244

TLR4 signalling pathway in the gut attributes to the intestinal inflammation during dengue infection

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Background: Dengue virus, a mosquito-borne flavivirus, is endemic in the equatorial area, infecting 390 million people out of .9 billion living in the region annually. There have been many efforts to reverse its spread, and reduce mortality and morbidity, however, the number of new cases is not decreasing. Thus, dengue pathogenesis needs to be studied for development of treatments to reduce the severity of the disease. Published work has shown LPS is elevated in dengue patients, and it is possible that influx of bacterial products contributes to severe dengue disease.

Aim: To investigate the contribution of the microbiota to disease in a mouse model of dengue virus infection, using an antibiotic cocktail (ampicillin, vancomycin, neomycin, metronidazole) and germ-free (GF) mice.

Results: Antibiotic treatment greatly decreased bacterial load. In dengue virus-infected mice, antibiotics led to decreased diarrhoea as well as inflammatory pathology and cytokine/chemokine mRNA in the colon but not ileum, whilst having no clear effect on morbidity or viral titre. When GF mice were infected with dengue virus, they still got substantial gut inflammation. The re-establishment of normal gut flora in the GF mice did not affect viremia, serum NS1 level, morbidity, ileum and colon pathology scores, but GF mice with flora had more severe diarrhoea whilst having less inflammation in the stomach.

Conclusion: Results of antibiotic treatment support the hypothesis that bacteria or bacterial products contribute to colon inflammation and diarrhoea during dengue infection. Surprisingly, GF mice did not have lower colon inflammation, and in fact showed an elevated inflammatory response in the stomach relative to GF mice recolonised with bacteria. The discrepancy between antibiotic and GF results may be a function of abnormal development of the immune system in GF mice, with exacerbated inflammatory responses. The effect of antibiotics most likely indicates a role for bacteria rather than a non-specific anti-inflammatory effect, since inflammation was not reduced in the ileum, which has a much lower bacterial load.

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245

Missense mutations in the gene encoding the cell death effector MLKL lead to lethal neonatal inflammation in mice and are present in high frequency in humans.

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Humans have highly variable innate immune responses to infection and tissue damage. This variability is a key factor in explaining why the same pathogen can be cleared with few or no symptoms by one individual, but can trigger a potentially lethal severe acute inflammatory response syndrome akin to sepsis in another. Mixed Lineage Kinase Domain Like (MLKL) is the essential effector of the inflammatory, lytic form of cell death called 'necroptosis'. Necroptosis has evolved primarily to defend against infection. The cellular signaling pathways that culminate in MLKL activation and necroptosis can be induced directly by viral and bacterial components, or as part of the ensuing immune response to pathogens or injury. Left unchecked, MLKL activation can lead to catastrophic cycles of tissue damage and inflammation in mice and humans.

We have isolated a mouse strain with a single missense mutation, *Mlkl*^{D139V}, that confers constitutive killing activity to MLKL. Homozygous *Mlkl*^{D139V} mutant mice are histologically normal at birth, but develop lethal postnatal inflammation of the salivary glands and around the

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thymus and heart by 2-3 days of age. The overtly normal embryonic and fetal development of *Mlkl*^{D139V} homozygotes, and the absence of any obvious phenotype in *Mlkl*^{D139V} heterozygotes provides important *in vivo* precedent for the capacity of cells to clear activated MLKL below a threshold. These observations also offer an important insight into the potential disease-modulating roles of a cluster of closely situated human *MLKL* gene variants (which are carried by an estimated 1 in 10 individuals). Have these human *MLKL* gene variants achieved such high frequencies by chance due to population bottlenecks, or have they conferred a selective survival advantage to one or more pathogens at some point in human history? What are the consequences of carrying these *MLKL* gene variants in the present day? We have recently shown that combinations of these variants are found at up to 12-fold the expected frequency in patients that suffer from a pediatric auto-inflammatory disease, chronic recurrent multifocal osteomyelitis (CRMO), and are actively investigating the contribution of both common and rare *MLKL* gene variants to human disease and host defense.

1. Hildebrand and Kauppi et al (2020) Nature Communications. DOI: 10.1038/s41467-020-16819-z

246

Neonatal sepsis: exploring the plasma proteome

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Sepsis is a frequent complication among newborns and accounts for >400,000 deaths globally. Infection-related inflammation contributes to long-term adverse neurodevelopmental outcomes in infants that survive sepsis. Preterm infants, particularly those born <28 weeks gestational age, are at the highest risk, affecting up to 50%, for developing late-onset sepsis (LOS; >72 hours of age). A rapid and accurate diagnosis of LOS is critical to minimise inflammation and antibiotic therapy, but early diagnosis is complicated by slow (up to 36 hours) and inaccurate diagnostic tests. Consequently, 2/3 of infants receive unnecessary antibiotic therapy, which is associated with adverse outcomes, including mortality, and contributes to antibiotic resistant organisms in the community. Innate immune development and response to infection are critical for fighting pathogens, yet are incompletely characterised in preterm infants. It is unclear if immune defence pathways differ between infants with and without sepsis and if differences are related to changes in the proteome. Proteome differences can be used to identify biomarkers that can improve the current diagnostic approach and potentially identify novel immune modulators for the prevention and treatment of LOS. We're using targeted and untargeted approaches to explore and quantify the plasma proteome of preterm infants with and without LOS. To date, our targeted immunoassay approach has revealed a number of cytokines are elevated and favour a regulatory response in infants with LOS. We have begun our untargeted quantification of the proteome using label-free data-independent acquisition mass spectrometry-based proteomics, and data analysis is in progress.

247

Antimicrobial susceptibility of *Staphylococcus aureus* from different ocular conditions

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Background*:* *Staphylococcus aureus* is a frequent cause of eye infections, with isolates exhibiting increased antimicrobial resistance to commonly prescribed antibiotics. *S. aureus* can target the cornea, tear ducts, eyelids and conjunctiva. The aim of this study was to analyse the antimicrobial susceptibility patterns of *S. aureus* strains from USA and Australia derived from infectious and non-infectious ocular surface adverse events.

Methods: 51 strains of *S. aureus* from different ocular conditions (11 microbial keratitis [MK] strains, 26 conjunctivitis strains and 14 strains from non-infectious contact lens corneal infiltrative events (niCIEs), were analysed for susceptibility to antibiotics commonly used to treat these conditions. Strains isolated from niCIEs were also tested for susceptibility to multipurpose disinfectant solutions. The broth dilution method was used for all testing.

Results: All the strains in study were sensitive to vancomycin (100%) and gentamicin (98%). The susceptibility to other antibiotics decreased in the following order chloramphenicol (80%), oxacillin (70%), ciprofloxacin (45%), ceftazidime (13%), azithromycin (10%) and polymyxin B (2%). All MK strains from Australia were susceptible to ciprofloxacin and oxacillin whereas only 11% MK strains from the USA were susceptible to these antibiotics ($p = 0.107$). 75% of conjunctivitis strains from Australia were susceptible to ciprofloxacin compared to 37% of conjunctivitis strain from USA ($p=0.278$). Most (90%) Australian niCIE and USA conjunctivitis (96%) strains were susceptible to chloramphenicol. Only 55% of MK strains from USA were susceptible to chloramphenicol and this was significantly different to the frequency for USA conjunctivitis strains ($p = 0.0036$). 84% of all strains were multi-drug resistant. All niCIE strains were sensitive to all four MPDSs at 100% concentration. The MPDS OPTI-FREE PureMoist was most active against all strains, followed by Renu advanced formula, Complete Revitalens and Biotrue.

Conclusions: All *S. aureus* strains remained sensitive to vancomycin and gentamicin. However, MK strains from USA were more likely to be resistant to oxacillin and ciprofloxacin. As gentamicin and ciprofloxacin are commonly used to treat keratitis, these results should be borne in mind when therapeutic decisions are made.

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248

Synergistic activity of ciprofloxacin and azithromycin against staphylococci and streptococci

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Aim: Antibiotics can control bacterial infections, but their overuse can lead to resistance development in bacteria. Treatment of multi-drug resistant bacteria is always challenging. This study investigated whether a combination of antibiotics could increase the susceptibility of ocular isolates of *Staphylococcus aureus* and *Streptococcus viridans* strains.

Methods: The minimum inhibitory concentration (MIC) of quinolone ciprofloxacin and macrolide azithromycin were determined by the micro broth dilution method against the multi-drug resistant S. *aureus* 117 and ciprofloxacin-resistant *S. viridans*. Based on their MICs, combinations of these two isolates were analyzed in a checkerboard assay which provided fractional inhibitory concentration index (FICI). FICIs of ≤ 0.5 indicates synergism; 0.6-1 indicate additive effect; >1-4 - no interaction and ≥4 - antagonism.

Results: Both ciprofloxacin and azithromycin had a MIC of 8µg/ml for the multi-drug resistant *S. aureus* strain 117. The FICI for the combination was 0.2 for *S. aureus* 117 indicating synergism and 1 for *S.* "*viridans*" indicating additive effect. When antibiotics were combined, only 1.25µg/ml of ciprofloxacin and 0.39 µg/ml of azithromycin were needed to kill *S. aureus* 117, whereas 2.5µg/ml ciprofloxacin and 0.781µg/ml needed to kill *S. viridans* SV04.

Conclusion: The combination of ciprofloxacin and azithromycin enhanced their antimicrobial activity producing synergism for the multidrug-resistant strain of *S. aureus*. This may be useful for clinicians who are treating bacterial infections. Future research will be extended to include more pathogenic strains and investigate the mechanism of interactions.

249

Essential protective role for miR-652 during intracellular bacterial infection

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The host response to bacterial infection is influenced by many miRNAs, and there is great interest in miRNAs for use as biomarkers and treatments of infectious disease. Previous studies in our group have identified an association with miR-652 and intracellular infection. Using a miR-652$^{-/-}$ mouse model, we investigated the impact of miR-652 on the immune response to infection with the acute intracellular pathogen *Listeria monocytogenes*.

Wild type and miR-652$^{-/-}$ mice were subject to intraperitoneal infection with *L. monocytogenes* and the host immune response were quantitated through flow-cytometric analysis and visualised by H&E histology.

miR-652$^{-/-}$ mice showed significantly increased mortality 3 days after low-dose *L. monocytogenes* infection, with 38% survival after 7 days, compared to 93% survival in wild type mice. Mice euthanised for ethical reasons were deemed susceptible, and the remaining mice resistant. Susceptible miR-652$^{-/-}$ mice presented notably higher bacterial count in the liver and spleen at 4 days post-infection. Additionally, histology showed larger and more numerous lesions in the livers of susceptible mice. Bacterial counts in resistant miR-652$^{-/-}$ mice were not different from wild type mice. Significantly greater CD8^{+} CD44^{+} CD62L^{-} and CD4^{+} CD44^{+} CD62L^{-} effector T cells were present in the spleen of miR-652$^{-/-}$ mice 10 days post-infection.

The mechanism by which miR-652 ensures protection during *L. monocytogenes* infection is being elucidated, though the onset of mortality before the typical T cell peak response at 7 days post-infection suggests the effect is related to induction of a protective innate immune response.

250

The unique type-I Interferon-epsilon (IFNε) constitutively protects the female reproductive tract from Zika Virus infection.

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Zika virus (ZIKV) can be transmitted sexually with infection of the female reproductive tract (FRT) and the developing foetus *in utero*. Like many RNA viruses ZIKV abrogates type-I and type-III IFN antiviral responses that is mediated by viral non-structural (NS) proteins. However, we hypothesise that the constitutively expressed IFNε in the FRT can circumvent ZIKV mediated IFN evasion by constitutively protecting the FRT from infection

To explore the role of IFNε *in vivo* we intravaginally inoculated IFNε$^{-/-}$, type-I IFN receptor KO (IFNAR1$^{-/-}$) and WT mice with ZIKV (PRVABC59). IFNε$^{-/-}$ mice had increased viral burden in the uterus and ovary (qRT-PCR) and increased ZIKV in vaginal washes when compared to WT mice (plaque assay). We intravaginally administered 4 µg of recombinant IFNε to IFNε$^{-/-}$ mice that resulted in the restoration of antiviral activity compared to buffer treated controls. Furthermore, application of 100 µg IFNε neutralising antibody to WT mice increased their susceptibility to infection compared to isotype controls.

We demonstrated that in primary transformed FRT cell lines, IFNε induced the expression of hundreds of ISGs similar to the antiviral profile induced by IFNλ-III, not IFNα as expected (NextSeq550 V2.5, qRT-PCR). Interestingly, IFNε did not induce expression of IRF1 and displayed limited induction of pro-inflammatory cytokines compared to IFNα and IFNλ-III. Pre-treatment with IFNε in FRT cell lines inhibited ZIKV replication like IFNα and IFNλ-III (qRT-PCR, plaque assay). However, post-infection these antiviral responses were blocked via NS5 mediated STAT2 degradation. Conversely, the expression of IFNε was impervious to ZIKV NS4A, NS1 and NS5 mediated inhibition

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downstream of activated RIG-I that delayed the production of other type-I and III IFNs (qRT-PCR). Furthermore, we demonstrated by immunoblot that IFNε signals constitutively despite IFN induced receptor desensitisation that limits IFNα mediated signal transduction.

Collectively these findings highlight the unique biological role of constitutively expressed IFNε in prevention of ZIKV infections in the FRT. Importantly, this shifts our understanding of FRT innate immunity from reliance on reactionary responses to pre-emptive protection against viral infections. This knowledge will help to inform sexual health recommendations for ZIKV and other infections of the FRT.

251

PI3K gamma suppresses macrophage mediated inflammation in cystic fibrosis cells

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Monocytes and macrophages are equipped with Toll-like receptors (TLRs) and cytokine receptors for sensing cues in their microenvironments, to which they respond by differentiating into either pro-inflammatory, classically-activated (M1 polarized) killer macrophages or inflammation-resolving, alternatively-activated (M2 polarized) repair macrophages. Macrophage polarization is a key determinant of inflammation in homeostasis and disease. In patients with cystic fibrosis (CF), M1 macrophages constantly activated by recurrent infection are coupled with defective M2 polarization, resulting in exaggerated pulmonary inflammation. Recently, phosphatidylinositol 3-kinase gamma (PI3Kg) has emerged as a key molecular switch in M1 macrophages, biasing them towards M2 outputs to suppress inflammation. The role of PI3Kg in M2 macrophages is not well understood and its potential role in CF macrophages has not been investigated. In this study we assessed the roles of PI3Kg and the cystic fibrosis transmembrane conductance regulator (CFTR) in regulating macrophage polarization. Our data demonstrate that both PI3Kg and CFTR in macrophages contribute to constraining inflammation during M1 polarization and promoting inflammation-resolving M2 polarization. Healthy macrophages with pharmacologically inhibited CFTR and macrophages from patients with CF both show higher pro-inflammatory responses and reduced anti-inflammatory responses. Anti-inflammatory Akt signaling is also defective in these macrophages. Macropinosomes in macrophages, besides being a nonselective endocytic pathway for extracellular fluid uptake, also serve as a platform for PI3K/Akt signaling during macrophage activation. In CF, defective CFTR causes reduced macropinocytosis resulting in lower Akt signaling and dysregulated anti-inflammatory responses in both M1 and M2 macrophages. PI3Kg and CFTR are thus shown to modulate inflammatory responses at multiple levels that can contribute to non-resolving inflammation in CF.

252

Monophasic *Salmonella* Typhimurium strains exhibit increased replication capacity in human macrophages by modulating intracellular host responses.

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Australia has the highest reported incidence of salmonellosis in the developed world, with three times higher reported cases compared to the UK or US. Recently, there has been a rapid emergence of a monophasic variant of *Salmonella enterica* serovar Typhimurium, 4,[5],12:i:-. These monophasic strains are characterised by increased multi-drug resistances and deletions in the *FljAB* operon, preventing expression of the phase two flagellar antigen FljB. In the absence of this key antigenic factor, we hypothesised that monophasic *S.*Typhimurium variants would induce altered host intracellular responses to infection and replication. We infected human macrophages and colonic epithelial cells with a panel of monophasic *S.* Typhimurium isolates and compared their intracellular growth and effect on cell viability to control biphasic isolates (expressing both flagellar antigens). We observed limited infection of monophasic strains in epithelial cells with the exception of two strains which were highly attenuated. However, in macrophages we observed 5-10-fold higher intracellular replication of most monophasic strains compared to biphasic isolates, despite lower initial infection rates. Intriguingly, this did not adversely affect macrophage viability, which remained equal to or higher than biphasic-infected cells. We also saw decreased activation of caspases 1, 3 and 8 at early stages of replication, suggesting that the monophasic isolates were able to replicate to high levels within macrophages without activating the intracellular cell death pathways typically observed in *S.* Typhimurium infections. This was also associated with an early type I interferon and NF-κB activation signature, which we are currently investigating *in vitro* and *in vivo* to determine the mechanism by which monophasic *S.*Typhimurium strains can modulate host detection and immune responses.

253

Simultaneous Induction of Cardiac and Neurobehavioral Autoimmune Pathology Following Exposure to Streptococcal Antigens

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Streptococcal infections constitute more than 700 million cases annually. Repeated or/untreated streptococcal infections lead to post-streptococcal sequelae including Acute Rheumatic Fever, Rheumatic Heart Disease (ARF/RHD) and associated neurobehavioral disorder Sydenham's Chorea (SC). These autoimmune multisystem disorders are complex and require detailed mechanistic investigations. An animal model, which exhibit both characteristics of cardiac and neurobehavioral defects associated with ARF/RHD, is important for such

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studies. The objective of this study was to assess the mechanisms including functional and neurobehavioral defects following exposure to streptococcal antigens in a rodent model.

Following exposure to Group A streptococcus (GAS) recombinant M5 protein (rM5), whole killed GAS, and recombinant Group G streptococcus M protein (GGS-*stg*480) we assessed impairments in fine motor control (Food manipulation), gait and balance (beam walking) and obsessive-compulsive behaviour (Grooming and Marble burying). Cardiac tissue damage was assessed by electrocardiography and histology. Rats were euthanised 70 days post exposure; heart and brain were collected for immunopathological analysis and serum assessed for cross-reactivity with cardiac myosin, collagen, tropomycin, laminin, lysoganglioside$_{GM1,}$ dopamine receptors and tubulin.

Significant differences were observed in food manipulation (*p*=0.0086), beam walking (*p*=0.0101) marble burying (*p*=0.003) and grooming behaviours (*p*=<0.0001) in rats injected with streptococcal antigens. In contrast, there were no differences in all of the behavioral tests in control rats. Prolonged P-R intervals were observed in rats injected with streptococcal antigens compared to control rats (*p*=0.0007). Histological examination of heart sections from these rats demonstrated infiltration of mononuclear cells (carditis score *p*=<0.0001) and brain sections with deposition of antibodies whereas control rats showed little or no evidence of inflammation.

In conclusion, for the first time we have characterised a model that provides longitudinal stability of age-dependent behaviour, to simultaneously investigate both neurobehavioral and cardiac autoimmune abnormalities associated with post streptococcal sequelae.

254

Screening for *Chlamydia trachomatis* infection in tubal ectopic pregnancy patients in India: Is serology the answer

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Introduction:

Ectopic (tubal) pregnancy (EP) is of major concern worldwide as it is the main cause of maternal morbidity and mortality during the first trimester. Infection with *Chlamydia trachomatis* (CT) is considered as an important risk factor for EP. A high prevalence of CT has been reported from our country in various studies. However, studies on frequency of infection are still meagre in women with EP. It seems worthwhile to find a reliable estimate about CT infection in women with EP in a developing country like India prior to formulating guidelines for effective screening strategy and clinical management. Hence, the aim of the present study was to find the magnitude of CT infection by quantitating the anti-chlamydial IgG antibodies in women with tubal EP in a major tertiary hospital in north India.

Materials and Methods:

50 EP patients were enrolled from Department of Obstetrics and Gynecology, VMMC and Safdarjung hospital (SJH), New Delhi (India) for collection of 5.0 ml of non-heparinized blood. The study had the hospital ethical approval and prior informed written consent was further obtained from each patient. The presence of CT infection was studied by detecting IgG antibodies specific to CT by a commercial enzyme-linked immunosorbent assay (ELISA) kit *(Euroimmun, Germany)* as per manufacturer's guidelines.

Results:

27/ 50 (54%) tubal pregnancy patients were found to have IgG antibodies to CT. The cut-off value of absorbance (A) was calculated as 0.0577. Mean value of (A) in seropositive EP women was statistically significant (*P*<0.05) in comparison to the seronegative women (1.122 *versus* 0.178). Patients with (A) ranges from 0.0577 – 0.999 were considered as low positives with n=12 and (A) value >0.0001 were high positives (HP) with n=15. Maximum seroprevalence (55.5%; *P*<0.05) was found among those in the HP group of EP patients.

Conclusions:

Results indicate that quantitation of anti-CT IgG antibodies in HP patients can serve as an initial screening tool for detection of chlamydial infection in women with tubal EP. However, serology should be followed by more confirmatory testing, *viz.:* molecular diagnosis of infection for clinical management.

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255

Investigating the impact of helminth infection on young and aged T_{VM} cells

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Virtual memory T cells (T_{VM}) are antigen-naïve CD8 T cells that have undergone partial differentiation in response to common γ (γc) chain cytokine signalling. T_{VM} cells respond more rapidly than conventional naïve CD8 T cells to antigen stimulation, thereby providing the initial wave of effector CD8 T cells and early control of bacterial and viral infections. While T_{VM} cells comprise 20% of the naive $CD8^+$ T cell pool in young mice, they are selectively retained with age and comprise up to 50% of all antigenically naïve $CD8^+$ T cells in aged mice. In addition, young T_{VM} cells are highly proliferative but aged T_{VM} cells lose proliferative capacity and exhibit markers of senescence. Recently, it was shown that generation of the γc cytokine, IL-4, during helminth infection drove T_{VM} cell expansion and consequently improved pathogen clearance during subsequent infectious challenge. However, it is not known whether helminth infections similarly expand and improve responses of aged T_{VM} cells. To evaluate this, we investigated the expansion and functional changes in T_{VM} cells in aged helminth-infected mice. Acute infection of aged mice with helminths led to negligible expansion of the T_{VM} subset and no gain in proliferative capacity. Furthermore, there was reduced IFN-γ production by aged T_{VM} cells after infection. Surprisingly when transferred to a young host, aged T_{VM} cells appeared to retain proliferative capacity in response to helminth infection, although at a slightly reduced rate compared to young T_{VM} cells. Going forward we will investigate if the cytokine environment in aged mice is less supportive of T_{VM} proliferation. We will also explore if helminth infection in young mice has any long-term effect on T_{VM} number and function that influences the aged immune response. This study will provide information on how early life exposure to helminths may influence T cell subsets and functionality in advanced age, which will improve our understanding of aging-related immune dysfunction.

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256

Histone deacetylase 7 promotes innate immune defence against bacterial infection, as well as Pkm2-dependent production of the monocyte recruiting chemokine Ccl2

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We previously showed that the class IIa histone deacetylase (HDAC), HDAC7 drives production of key Toll-like receptor inducible inflammatory mediators, such as Il-1β and Ccl2, as well as systemic inflammation after lipopolysaccharide (LPS) challenge. It may therefore be a potential target for intervention in inflammation-linked diseases, thus necessitating assessment of its involvement in infectious disease processes. Here we report that, in a uropathogenic *E. coli* intraperitoneal challenge model, both pharmacological inhibition of class IIa HDACs with TMP195 and myeloid knockout of *Hdac7* increased bacterial loads in the liver and spleen. Systemic inflammation was also exacerbated, as evidenced by increased core body temperature and elevated sera levels of specific inflammatory mediators. Similarly, inhibition of class IIa HDACs with TMP195 in murine bone marrow-derived macrophages (BMM) and human macrophage-like PMA-differentiated THP-1 cells impaired their capacity to kill both non-pathogenic and uropathogenic *E. coli*. These data demonstrate that myeloid Hdac7 is required for effective host defence against bacterial infection. We previously reported that Hdac7 promotes glycolysis-associated inflammatory mediators, which is licensed by deacetylation of the glycolytic enzyme pyruvate kinase M isoform 2 (Pkm2) at lysine 433. However, the antimicrobial effect of Hdac7 in murine macrophage is independent of Pkm2, as overexpression of wild type and deacetylation mimics of Pkm2 in BMM not alter macrophage antibacterial responses. Instead, we show that Hdac7 is required for effective phagocytosis in macrophages. Furthermore, LPS-inducible Ccl2 production requires both Hdac7 enzyme activity and Pkm2, but occurs independently of Pkm2 deacetylation at K433. These findings thus demonstrate that Hdac7 acts through multiple mechanisms to drive both antimicrobial and inflammatory pathways in macrophages.

257

Epithelial lytic cell death promotes monolayer permeability, apical elimination and lamellipodia-driven mechanism of epithelia resealing

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The epithelium is an active and key innate immunity component acting as a biological, chemical and physical barrier in mucosal tissues. Epithelial cell infection triggers its pyroptotic cell death and elimination from the monolayer to restore epithelia barrier function and homeostasis. However, the morphological features and molecular mechanisms associated with lytic death of an epithelial cell, and the resultant response of the monolayer, remains unknown. Herein we addressed this topic using genetic, pharmacological and live cell imaging techniques in colonic epithelial monolayers expressing an activatable Caspase1 (Casp1) system. Casp1 activation leads to Casp1 and GSDMD cleavage and sequential membrane permeabilization; features of pyroptotic (lytic) cell death. In epithelial monolayers, lytic cell death is followed by apical/luminal elimination of the dead cell, due to rearrangement of the neighbouring cells. Strikingly, and distinct

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from epithelial cell apoptosis, epithelial cell lytic death leads to breaks in cell-cell junctions and a transient increase in monolayer permeability. Also, lytic cell death triggers neighbouring cells to produce lamellipodia and membrane ruffles but rarely actin rings. Pharmacological inhibition of these structures considerably delayed resealing of the epithelial monolayer. To address forces generated by these structures, we used Traction Force Microscopy. Curiously, epithelial pyroptosis induces force signatures in neighbours that resemble a lamellipodia-driven mechanism for monolayer resealing; this involves outward forces generated by cell crawling and inward forces generated by actin cables. Collectively our results show that lytic cell death in epithelial tissues induces a transient break in barrier function and compared to apoptosis, a distinct mechanism for eliminating the dead cell from the monolayer.

258

Therapeutic targeting of the myofibroblast NLRP3 inflammasome as a novel approach to treating fibrosis

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Background: The NLRP3 inflammasome is a key immunomodulatory platform induced by the immune system to produce the pro-inflammatory cytokines, interleukin(IL)-1β and IL-18 following tissue injury. However, the chronic activation of IL-1β and IL-18, by pro-fibrotic stimuli such as transforming growth factor (TGF)-β1 and toll-like receptor (TLR)-4 can drive fibrosis (tissue scarring) progression[1]. The anti-fibrotic hormone, relaxin, has been found to limit the pro-fibrotic effects of TGF-β1, TLR-4 and/or IL-1β on myofibroblast differentiation and excessive collagen deposition.

Aim: Hence, the extent to which the anti-fibrotic effects of recombinant human relaxin (RLX) targeted the myofibroblast NLRP3 inflammasome was determined.

Methods: Primary human cardiac (HCFs)[2] or dermal (HDFs)[3] fibroblasts ($1.5x10^5$ cells/well in 12 well-plates) were left untreated or stimulated with TGF-β1 (T; 2 ng/mL; to promote myofibroblast differentiation) + LPS (L; 100 ng/mL; to prime the NLRP3 inflammasome) + ATP (A; 5mM; to activate the NLRP3 inflammasome) for 8h or 72h; in the absence/presence of RLX (100ng/mL)[2,3]. HDFs were also subjected to lipofectamine-siRNA-induced knockdown of NLRP3 inflammasome priming components (NLRP3, ASC and caspase-1)[3], and treated with/without RLX (100ng/mL) for 72h (n=6-8) *in vitro*. 8-10 week-old-male 129/sv mice were subjected to isoprenaline (ISO, 25 mg/kg, s.c)-administration over 5 consecutive days, then left for 9 days for fibrotic healing to occur. Subgroups of ISO-injured mice were either left untreated or treated with RLX (0.5 mg/kg/day) or the NLRP3 inflammasome inhibitor, MCC950 (10 mg/kg/day) from days 7-14 post-injury (n=4-6/group) *in vivo*.

Results: T+L+A-stimulation of HCFs[2] or HDFs[3] *in vitro* significantly increased TLR-4, measures of NLRP3 inflammasome priming (NLRP3, ASC, caspase-1) and activity (IL-1β, IL-18), α-SMA (myofibroblast differentiation) and collagen-I (interstitial fibrosis) after 8h or 72h. Likewise, repeated ISO-administration promoted all these measures in the left ventricle of mice after 14-days of injury *in vivo*[2]. These T+L+A- or ISO-stimulated effects were significantly inhibited *in vitro* or ameliorated after 7 days *in vivo* by RLX. However, these NLRP3 inflammasome-inhibitory and anti-fibrotic effects of RLX were abrogated when caspase-1 was knocked-down.

Conclusion and Significance: Targeting inducers (TGF-β1, TLR-4) and/or components (caspase-1) of the myofibroblast NLRP3 inflammasome may represent a previously unrecognised approach for treating IL-1β- and IL-18-mediated fibrosis progression.

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259

Application of activity-based probes to interrogate the contribution of cathepsin X to dendritic cell function

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Cathepsin X/Z/P (Cat X) is a lysosomal cysteine protease that exhibits monocarboxypeptidase activity. Increased Cat X expression is associated with cancer and inflammation, although its roles in normal physiology are still poorly understood. It is expressed by antigen-presenting cells such as dendritic cells (DCs). DCs undergo functional changes upon agonism of pattern recognition receptors (*e.g.,* Toll-like receptors) by pathogen-associated molecular patterns such as CpG. Mature DCs can secrete cytokines, present antigens, etc. We hypothesise that Cat X contributes to DC function, but this has not been examined in detail until now.

We used activity-based probes, immunoblotting, and immunofluorescence imaging to measure active and total levels of Cat X in naïve DCs (DC1940) or those stimulated with TLR-1/2, 2/6, 3, 4, 7/8, and 9 agonists (Pam3, FSL-1, Poly I:C, LPS, R-848, and CpG, respectively). Lysosomal and secreted Cat X levels were significantly elevated in response to CpG treatment, and to a much lesser extent with the other agonists.

To examine the impact of Cat X on DC function, we generated Cat X-deficient DC1940 cells using CRISPR-Cas9. Expression of surface markers indicating DC maturation was unaffected by Cat X deficiency. By contrast, we observed a reduction in the secretion of several chemokines and cytokines (CCL3, CCL5, CD14, IL-12 and TNF-α) upon CpG stimulation in Cat X-deficient cells. Cat X-deficient cells exhibited altered processing of cathepsin L, while the activities of other lysosomal proteases were unaffected. We also found that Cat X-deficient cells had impaired antigen presentation.

Collectively, these data indicate that Cat X is strongly upregulated by TLR-9 agonism in DCs. While it may not be essential for DC maturation, Cat X may regulate cytokine secretion, processing of related endolysosomal proteases, and antigen presentation. Future studies will rescue Cat X expression in Cat X-deficient cells to validate the phenotypes discovered.

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260

MAIT cells expand in the absence of NKT and γδT cells

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Unconventional T cells, namely MAIT, NKT, and γδT cells, recognise non-peptide antigens presented by non-classical MHC-like molecules, and can rapidly mount robust cytokine responses after activation, leading to their implication in modulating the host immune response to disease. In particular, MAIT cells recognise riboflavin biosynthesis metabolites, such as 5-OP-RU, presented by MR1, and utilise a semi-invariant TCR comprising a *Trav1-Traj33*⁺ TCRα chain that pairs with a limited range of TCRβ chains. Despite their relative abundance, MAIT cell frequencies vary widely between individuals, ranging from 0.1-10% of total blood T cells in humans, the cause of which is understudied. Recent evidence has suggested that MAIT and other unconventional T cells may co-exist in the body within a shared developmental or homeostatic niche and are regulated by similar genetic and/or environmental factors.

We confirm earlier findings that CD1d-deficient mice, which lack NKT cells, have increased MAIT cells, and show that this is due to the loss of NKT cells rather than CD1d itself. Likewise, MAIT cells are also markedly increased in TCRδ-deficient mice, which lack γδT cells, and further expand in CD1d/TCRδ-doubly deficient mice. Expanded MAIT cells phenotypically and functionally resemble their WT counterparts. Accordingly, we hypothesise that MAIT cells may compete with NKT and γδT cells for similar factors and subsequently expand in their absence. As increased MAIT cells were also observed in the thymus, we sought to investigate *Trav1-Traj33* rearrangements and found that they were increased within developing thymocytes from TCRδ- and CD1d/TCRδ-doubly deficient mice. Consequently, we hypothesise that modification of the TCRδ locus to create TCRδ-deficient mice may have affected TCRα rearrangements during T cell development, in particular resulting in greater usage of distal *Trav* gene segments such as *Trav1.*

Thus, in addition to providing insight into factors that affect MAIT cell frequencies, our work also sheds light on previously unappreciated alterations in TCRα chain rearrangements in TCRδ-deficient mice, and cautions the use of CD1d- and TCRδ-deficient mice for studying the role of NKT and γδT cells in disease, respectively.

261

Live imaging of pyroptotic macrophages reveals novel GSDMD-dependent cytoskeletal remodelling

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The NLRP3 inflammasome assembles in response to a wide range of microbial and host stress signals that are indicative of infection or cell damage. It is well-characterised that upon activation, NLRP3 drives the redistribution of ASC into a large cytosolic cluster, known as the ‘speck’. Inactive caspase-1 undergoes proximity-induced dimerisation and self-cleavage at the speck to generate a transiently active protease. Active caspase-1 triggers a form of inflammatory cell death (pyroptosis) by cleaving full-length GSDMD, releasing a p30 fragment which can oligomerise to perforate the plasma membrane. Loss of plasma membrane integrity causes membrane rupture and release of cytosolic contents into the extracellular space. Cytoskeleton dynamics during NLRP3 inflammasome signalling are not well characterised, and our data show that cytoskeletal catastrophe is an underappreciated yet important feature of pyroptosis. We use high resolution live microscopy, including lattice light sheet microscopy, to characterise these cytoskeletal events in pyroptotic primary murine macrophages. Here, we show that macrophages assemble caspase-1-dependent stress-fibres upon treatment with NLRP3 activating-stimuli. Though much of this cellular polymerised actin (F-actin) is depolymerised and released upon pyroptotic cell lysis, we discovered that macrophages rapidly assemble filopodia at the plasma membrane upon GSDMD pore assembly. Extracellular F-actin is linked to a number of pathologies, including septic shock, and so F-actin production during NLRP3-dependent pyroptotic cell death may represent a novel pro-inflammatory pathway.

262

Activation of stimulator of interferon genes in dendritic cells induces interferon-lambda and subset- and species-specific dendritic cell death

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Stimulator of Interferon (IFN) Genes (STING) is a cytosolic DNA sensor that recognises cyclic dinucleotides (CDNs), such as the bacterial product cyclic-guanosine monophosphate-adenosine monophosphate (cGAMP). Other forms of cytosolic DNA from viruses or host cells can be converted into CDNs by an enzyme cGAMP synthase (cGAS). Activation of STING results in the production of Type I IFN through the phosphorylation of TANK-binding kinase 1 (TBK1) and IFN regulatory factor 3 (IRF3). As dendritic cells (DCs) are key antigen presenting cells that link the innate and adaptive immune systems, it is important to understand the role and effects STING activation have in DCs and how this subsequently mediates immunity against foreign or aberrant DNA insults. However, DCs interrogated in many

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studies investigating STING activation use *in vitro*-generated DCs instead of putative *ex vivo* DCs. We have previously shown that there is differential expression of STING among various DC subsets. However, the direct effects of STING activation on these DC subsets are incompletely understood. Here, we report that mouse and humanised mouse splenic DC subsets as well as human blood DCs are activated by CDN stimulation and all produce Type III IFN but only conventional DC2 (cDC2) and plasmacytoid DCs (pDCs) produce Type I IFN in response to CDN stimulation. However, only mouse pDCs aberrantly express extremely high levels of activation markers CD86 and CD80 and are ablated rapidly after STING activation. Some DC death was also observed in mouse and human cDC2s, but not in human pDCs. This ablation is STING-dependent and occurs via a cell-intrinsic mechanism involving intrinsic apoptosis. These observations demonstrate the differential effects STING activation has on DC subsets as well as highlight a discordance amongst mouse and human DCs during activation, which serve as an important consideration in future translational research and therapeutic design.

263

Viral Immune Evasion: There are more than two STATs

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Many viruses target signal transducer and activator of transcription (STAT) 1 and STAT2 to antagonise antiviral interferon signalling, but targeting of other STATs such as STAT3, a pleiotropic molecule that mediates signalling by diverse cytokines, is poorly understood. Here, using virus infection, quantitative live cell imaging, innate immune signalling and protein interaction assays, and complementation/depletion of STAT expression, we show that STAT3 antagonism is conserved among P-proteins of diverse pathogenic lyssaviruses and correlates with pathogenesis. We also find that another negative sense RNA virus, Ebola virus, targets STAT3. Importantly, targeting of STAT3 differs between viruses, such that some viruses use highly selective targeting of specific cytokine-activated dimers, variously using mechanisms such as direct interaction with specific STATs, antagonism of nuclear trafficking pathways, or combinations of both. RT-qPCR and reporter gene assays indicate that this results in specific modulation of cytokine pathways, effecting differential antagonism of target genes. These data provide novel insights into mechanisms by which viruses can modulate cellular function to support infection through discriminatory targeting of immune signalling complexes. The findings also highlight the potential application of selective interferon-antagonists as tools to delineate signalling by particular STAT complexes, significant not only to pathogen-host interactions but also cell physiology, development and cancer.

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264

IL-37 employs multiple strategies to suppress inflammasome-mediated IL-1β bioactivity and inflammation

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Background: The interleukin (IL)-1 family member IL-37 is one of the rare anti-inflammatory cytokines, its broad and powerful inflammation-inhibiting properties are well described. However, the mechanisms of action of IL-37 are poorly defined and especially its effects on the inflammasome, a multi-protein complex required for the maturation of the pro-inflammatory IL-1β and IL-18, are not well understood.

Methods: To study how IL-37 affects the expression and inflammasome-dependent maturation of IL-1β and IL-18, we performed gene expression analyses as well as inflammasome activation assays (ASC oligomerization/speck assays, caspase-1 assays) in bone marrow-derived macrophages (BMDM) of IL-37-transgenic and wild-type mice. Moreover, we investigated the effects of IL-37 on pyroptosis, an inflammatory form of cell death, by gene expression analyses and lactate dehydrogenase release assays in activated BMDM. Employing an endotoxemia model, we also investigated the role of IL-37 in vivo.

Results: IL-37 inhibited IL-1β production by NLRP3 and AIM2 inflammasomes, and IL-18 production by the NLRP3 inflammasome. This inhibition was partially attributable to effects on gene expression: whereas IL-37 did not affect LPS-induced mRNA expression of *Il18* or inflammasome components, IL-37-transgenic BMDM displayed an up to 83% inhibition of baseline and LPS-stimulated *Il1b* compared to their wild-type counterparts. Importantly, IL-37 suppressed nigericin- and silica-induced ASC oligomerization/speck formation (a step required for inflammasome activity) and subsequent caspase-1 activation. IL-37 also inhibited pyroptosis (50% reduction); and in mice subjected to endotoxemia, IL-37 reduced the abundance of plasma IL-1β (78% reduction compared to wild-type animals) and IL-18 (61% reduction).

Conclusion: Our study adds to the portfolio of anti-inflammatory pathways employed by IL-37. We demonstrate that IL-37 uses multiple strategies to suppress the bioactivity of inflammasomes and consequently of IL-1β and IL-18. Moreover, we identify IL-37 as an inhibitor of inflammation-amplifying pyroptosis. Importantly, our results highlight IL-37 as a potential tool for treating inflammasome-driven, hyper-inflammatory diseases.

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265

Bactericidal capacity of ascitic fluid from patients with decompensated cirrhosis

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Introduction: Patients with decompensated cirrhosis are highly susceptible to bacterial infections, associated with compromised liver and immune function, and outcomes following infection in these patients are poor. Increasing antibiotic-resistant infections have been reported worldwide leading to emerging interest in host-directed therapeutic strategies. Ascites, an accumulation of fluid in the peritoneal cavity, is the commonest complication in patients with decompensated cirrhosis, and is the most common site of infection (Spontaneous Bacterial Peritonitis). Knowledge of ascitic fluid (AF) innate immune mechanisms and potential strategies to enhance immune function to prevent or treat infections is limited. This study investigated the bactericidal capacity of AF for *E. coli,* the commonest cause of SBP, and the mechanisms enabling a uropathogenic *E. coli* (UPEC) strain to resist AF killing.

Methods: AF killing assays were conducted with the non-pathogenic *E. coli* strain (MG1655) and the multidrug resistant UPEC ST131 strain EC958. Bacteria were incubated in cell-free AF for 90 minutes to assess survival. Deposition of complement and immunoglobulin on the *E. coli* strains was assessed by flow cytometry. Transposon mutagenesis in combination with multiplexed transposon directed insertion-site sequencing (TraDIS) was used to identify genes required for EC958 survival in AF.

Results: *E. coli* MG1655 was susceptible to AF-mediated killing, but survived in heat-inactivated AF, suggesting an important contribution of complement. Consistent with this, robust deposition of complement C3 and the C5-9 membrane attack complex, but not C1q or IgG, on the surface of MG1655 was observed. In contrast, EC958 was resistant to AF-mediated killing and no complement deposition was observed. TraDIS analysis of an EC958 transposon mutant library identified 41 genes required for survival in AF. Similar to serum resistance mechanisms, many of these genes were related to capsule synthesis. Mutation of genes encoding the transcription factors RfaH, which controls capsule and lipopolysaccharide expression, and Fis, a nucleoid associated DNA-binding protein, rendered EC958 susceptible to AF killing, associated with restoration of complement C3 and C5-9 deposition.

Conclusion: AF has potent bactericidal capacity, mediated in part by the alternative complement pathway, however cell-surface components such as the capsule enable pathogenic *E. coli* to evade AF killing.

266

Exploring the MIF family of cytokines: Different roles for MIF and D-DT in inflammation

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Macrophage migration inhibitory factor (MIF) is an inflammatory cytokine with critical pathological roles in many disease, including rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, inflammatory bowel disease and certain malignancies. While has been reported to have myriad functions, its primary function and mechanism of action are still not well understood. Moreover, while MIF clearly acts as a secreted factor, it also has intracellular roles potentially independent of its cytokine activity, including a direct role in facilitating NLRP3 inflammasome activation. A second MIF family member, D-dopachrome tautomerase (D-DT) shares structural homology to MIF and has been reported to have similar functions in the context of inflammatory responses. However, we have reported significant differences in serum levels of MIF and D-DT in patients with systemic sclerosis (SSc, scleroderma) and SLE. To further investigate the role of D-DT in inflammation, we have created *Ddt*$^{-/-}$ and *Mif/Ddt*$^{-/-}$ mice. These mice do not display any significant phenotypic differences, compared to wilt type and *Mif*$^{-/-}$ mice. Moreover, in contrast to *Mif*$^{-/-}$ macrophages and dendritic cells, which show decreased NLRP3 activation, these responses are unaffected in *Ddt*$^{-/-}$ cells. Similarly, while multiple small molecule MIF inhibitors abrogate NLRP3 activation in macrophages, the D-DT inhibitor 4-CPPC had no effect. Other cytokines were also inhibited by MIF antagonists, including IL-1α, TNF and IL-10, while 4-CPPC affected only IL-1α release. Our data suggest that MIF and D-DT may have different roles to play in inflammation and immune cell responses to infectious and inflammatory stimuli. These data take us closer to understanding how MIF and D-DT influence pathology in inflammatory diseases, potentially informing future targeting strategies.

267

Tracking variability in the antimicrobial zinc toxicity response of human macrophages against *Escherichia coli*

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Uropathogenic *E. coli* (UPEC) are the primary cause of urinary tract infections, one of the most common bacterial infections in humans. Understanding effective host defence pathways could facilitate the development of new therapeutics to treat infections caused by UPEC, which are frequently antibiotic-resistant. During infection, innate immune cells, including macrophages, can restrict essential trace elements from pathogens in a phenomenon termed 'nutritional immunity'. Conversely, these cells can also toxify microorganisms with high concentrations of trace elements. Innate immune cells use both zinc starvation and zinc toxicity as antimicrobial strategies, but molecular mechanisms underlying the latter pathway are poorly understood. Here, we show differences between human monocyte-derived macrophages (HMDM) differentiated with CSF-1 versus GM-CSF in the deployment of these two antimicrobial responses against *E. coli.* Intramacrophage bacterial gene expression analyses as well as intracellular survival assays for specific *E. coli* mutants revealed that CSF-1-derived HMDM preferentially employ zinc toxicity, whereas GM-CSF-derived HMDM are more effective at zinc starvation. However, each population could still deploy both zinc toxicity and zinc starvation, suggesting that there may be variability in engagement of these responses at a single cell level. Using an *E. coli* strain that reports zinc stress, we demonstrate variation of zinc toxicity responses between individual cells within CSF-1-derived HMDM. To further delineate the heterogeneity of macrophage zinc toxicity responses, we are currently using single-cell RNA sequencing methods to determine both host and pathogen transcriptomes associated with the zinc toxicity response in macrophages. To this end, we have optimised reporter tools and protocols that will allow for single-cell bacterial and mammalian transcriptome sequencing. Together, our study shows the deployment of zinc toxicity is likely affected by heterogeneity between and within macrophage populations. Mapping macrophage transcriptional profiles that align with bacterial zinc stress at the single cell level should enable the identification of host genes and pathways that mediate the zinc toxicity response. Such knowledge may ultimately guide the development of host-derived therapies for the treatment of antibiotic-resistant bacterial infections.

268

WNT signatures are an integral part of macrophage responses to infection

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Actionable molecular targets for host-directed interventions in challenging bacterial infections are the subject of intense investigation. The WNT signalling network has emerged as an integral component of host responses to bacterial infection. With roles in shaping immune functions such as inflammatory cytokine responses and cellular antimicrobial defense, WNT signalling is being explored as a potential target for tailoring immune responses. As our understanding of immune-related WNT functions is evolving rapidly and some evidence suggests that pathogens may actively manipulate WNT-controlled cellular functions, it is now important to delineate factors that define the nature of the WNT response to infection. To address this, we profiled WNT responses in macrophages upon infection with phylogenetically diverse bacterial pathogens. We observed that a subset of the 19 mammalian WNT ligands was differentially expressed upon bacterial infection, with substantial similarities WNT responses induced by extracellular and intracellular Gram-positive and -negative bacteria. WNT responses induced by viable compared to killed bacteria were identical, indicating that active modulation of macrophage WNT expression is not a common feature of bacterial pathogens. In line with these observations, we identified TLR signalling as a major molecular driver of macrophage WNT responses. As we expand these studies, our findings to date indicate that macrophage WNT responses are controlled by classical innate immune recognition mechanisms, resulting in WNT responses that might be indicative of infection, without revealing distinct signatures for different groups of bacterial pathogens. This has important implications for the understanding of how WNT ligands contribute to the host response to bacterial infection and how this might be exploited for the design of WNT-targeting strategies.

269

Dengue Infection Causes Membrane Recruitment of FH Through Ligands other than Glycosaminoglycans

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Dengue virus (DENV) infection causes dengue, the most prevalent arboviral infection in the world and modelling suggests it infects 390 million people each year. Although, there is no specific treatment or safe vaccine. Dengue has hallmark signs of vascular leakage, fever, and thrombocytopenia, where vascular insufficiency can lead to hypovolemic shock and death.

Disease severity and patient outcome have been correlated to the changes in regulators and activators of the complement system through analysis of patient sera and GWAS. Specifically, reduced alternative pathway regulatory proteins, such as Factor H (FH), the most important complement alternative pathway regulator, are correlated with increased disease severity.

Previously our lab has demonstrated that DENV infection induces FH mRNA production in primary cells but no significant change in the concentration of secreted FH. Here we have demonstrated that HeLa can be used to reproduce this phenomenon.

Further, ELISA and Western blot of cell lysates demonstrates that FH protein is increased in DENV-infected cells but localised on or within the cell. To investigate the underlying mechanisms by which DENV facilitates FH retention by cells, the abundance of the two most prominent cell surface FH ligands, sialic acids (Sia) and heparan sulphate (HS) were quantitated using a novel fluorescence-based technique and UV spectroscopy respectively. No significant difference in the abundance of these FH ligands was found on the surface of DENV- compared to mock-infected cells. Membrane manipulation by enzymatic cleavage of HS and Sia from cell surfaces also yielded no difference in the amount of FH released from DENV infected cells compared to controls. Thus, DENV infection causes retention of FH by infected cells but not due to increased binding to Sia or HS. FH may be binding other known ligands such as oxidised fatty acids, C-reactive protein or C3 within the cell which is now being investigated.

Our results demonstrate dysregulation of FH ability to be secreted despite increased FH mRNA transcription due to cellular retention. Thus, restoring the regulatory capacity of FH by facilitating release and circulation from cells may be an effective therapeutic avenue to treat dengue.

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270

Ficolin-1 is an innate pattern recognition receptor that binds *Plasmodium falciparum*-infected red blood cells and may promote parasite clearance

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Malaria, due to *Plasmodium falciparum* infection, causes a significant burden of disease in vulnerable populations globally. A successful immune response is a strong determinant of clinical outcome. The ficolins are a family of proteins that act as secreted Pattern Recognition Receptors (PRRs) able to activate complement and facilitate clearance of bacterial pathogens such as *Staphylococcus aureus*. A role for ficolins in the response to and clearance of *P. falciparum* has not been previously investigated.

Here, we have investigated the role of ficolin-1 and ficolin-2 in *P. falciparum* infection and infected RBC (iRBC) clearance. We measured serum ficolin levels in a cohort of Malawian children with severe and uncomplicated malaria, as well as in uninfected, healthy children. Ficolin-1 levels were significantly increased in the serum of children with uncomplicated (ref 1.92 (1.27 to 2.90); p = 0.002) and severe malaria (1.71 (1.14 to 2.57); p = 0.011), when compared to the healthy children. In contrast ficolin-2 levels did not change with *P. falciparum* infection. We next sought to determine the role of ficolin-1 in *P. falciparum* infection. Using flow cytometry, we determined that ficolin-1 bound preferentially to iRBCs compared to uninfected RBCs (2.8-fold increase in MFI, p < 0.01). Using a recombinant mutant ficolin-1 (Y271F) (which doesn't bind sialic acid), the iRBC receptor was identified as sialic acid. To determine whether ficolin-1 could promote iRBC clearance, we set up an *in vitro* assay and demonstrated ficolin-1 promoted complement dependent lysis of iRBC but not of uninfected RBC (p < 0.01).

Together, our data suggest that ficolin-1 is an innate pattern recognition receptor that is increased with *P. falciparum* infection, binds to iRBCs and may contribute to parasite clearance.

271

Enteric viral infections prior to gluten intake exacerbate type I interferon response through an RNA methylation dependent mechanism

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Enteric viral infections have been related to the risk of developing different autoimmune disorders. Celiac disease (CeD) is an intestinal autoimmune disease in which dietary gluten is the main triggering agent, but enteroviral infections have been suggested as additional contributing environmental factors. The viral related type I interferon (IFN-I) pathway has been involved in CeD pathogenesis. Additionally, m6A RNA methylation is implicated in the control of the innate immune response to infections and has also been related to the development of autoimmunity.

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We hypothesized that gluten consumption can reactivate the IFN-I pathway in patients that have been previously infected by enteric virus, and that this is regulated by an m6A-dependent mechanism. We confirmed the alterations in the IFN-I pathway and the m6A machinery in intestinal biopsies of CeD patients. Additionally, patients presented higher serum titers of antibodies against intestinal reovirus. PIC treatment, used as a viral mimic, in combination with gluten, induced an IFN-I response and altered the expression of certain m6A machinery genes with an overall increase in m6A levels in intestinal cells. Interestingly, when PIC treatment was combined with gluten, the alterations in m6A eraser *ALKBH5*, m6A writer *METTL3* and *IRF7* were stronger than those observed with PIC or gluten alone. We confirmed the methylation of *IRF7* mRNA by meRIP-qPCR and observed that *ALKBH5* silencing and *METTL3* overexpression were able to increase *IRF7* and downstream gene expression levels, verifying the involvement of m6A machinery in the regulation of this pathway. Although *METTL3* overexpression decreases *IRF7* mRNA stability, higher protein amounts are observed suggesting that m6A regulates its transcription.

To sum up, our results suggest that in the context of viral infection, gluten consumption may lead to the development of an autoimmune response through an alteration of the m6A machinery and the induction of innate immune pathways.

272

Interferon-lambda: a key cytokine for dendritic cell development?

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In light of the current pandemic, COVID-19, our understanding of how the immune system combats viral infection has become more important than ever. Interferons (IFNs) are an integral component of the immune system's anti-viral response. While the functions of Type I Interferons have been extensively studied, the newest member of the Interferon family, Interferon-Lambda (IFN-λ), is yet to be fully investigated. Currently, it is believed that the canonical signalling pathway of the IFN-λ receptor (R, defined as IFN-λR1 and IL-10Rb) is shared with Type I IFN-αR signalling and leads to the transcription of Interferon Stimulated Genes (ISGs), key genes involved in antiviral immunity. However, there is a growing body of evidence that indicates that IFN-λR1 is involved in signalling pathways that differ to IFN-αR. Notably amongst this evidence is IFN-λR1's restricted expression to epithelial and some haematopoietic cells, as well as non-redundant roles being discovered at mucosal barriers(1). Building on novel preliminary data, this project seeks to differentiate IFN-λR1 signalling from this canonical pathway and determine whether other receptor chains are involved. Dendritic Cells (DCs), key mediators linking innate and adaptive immunity, are the focus of this project as they are major producers of IFN-λ and are also one of the few haematopoietic cells which express IFN-λR1. Using DCs, this project will investigate the signalling pathways regulated by IFN-λR1 and other potential pathways instigated by the interaction between IFN-λR1 and other receptors. Additionally, this work will investigate how IFN-λ signalling contributes to cellular function in the context of DC development, maturation and metabolism. Data shown in this presentation will be focussing on DC differentiation and development.

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273

The role of intracellular pattern recognition receptors in recognition and immunomodulation of dendritic cells in response to daptomycin resistant *Staphylococcus aureus*.

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Methicillin resistant *Staphylococcus aureus* (MRSA) infection is one of the leading causes of hospital-acquired infections and is more difficult to treat than other bacterial infections[1]. In recent years, resistance to last line anti-staphylococcal antibiotic daptomycin has been observed during treatment of MRSA bacteremia[2]. Protection against MRSA infection is challenging and requires both innate and adaptive immune effector mechanisms. Being at the interface between innate and adaptive immune responses, DCs are thus central to the immune protection against *S. aureus.* We have previously shown that distinct and paired clinical isolates of MRSA have differential capacity to induce DC activation, with MRSA strains resistant to the last-line antibiotic daptomycin inducing a compromised response [3]. However, this study explores the molecular mechanism by which MRSA strains impede DC activation, we explored the role of intracellular PRRs in the recognition and immunomodulation of these clinical isolates. We found that the cytosolic sensors cGAS and STING are implicated in the sensing of clinical isolates of MRSA, but their use is more prominent in response to Daptomycin sensitive (DapS) strains. We also determined that the TLR3 ligand poly(I:C) induced a highly synergistic increase in the activation of DC in response to both DapS and Daptomycin resistant (DapR) MRSA. Our work, thus provides important insights for the mechanism of differential recognition of clinical isolates of MRSA and immunomodulation through engagement of PRR pathways which are critical to achieve effective and comprehensive immune defense against this pathogen.

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274

Interferon epsilon alters peritoneal myeloid cell populations in a murine model of ovarian cancer

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The novel type I interferon, interferon epsilon (IFNε), is a unique cytokine which is constitutively expressed by epithelial cells under hormonal regulation in the female reproductive tract (FRT)[1]. Although IFNε is known to be protective against FRT infections [2], its role in the anti-cancer immune response remains unknown. Ovarian cancer is a common yet lethal FRT cancer, which metastasises to the peritoneal cavity in the majority of cases. Preliminary research has suggested that IFNε may protect against metastasis of ovarian cancer through action on both tumour and immune cells.

Here, we have investigated the activity of IFNε in an orthotopic mouse model of ovarian cancer, using the ID8 tumour cell model. IFNε treatment was found to significantly reduce tumour burden, ascites development and peritoneal hemorrhaging in challenged mice, compared to both IFNβ and vehicle control (PBS). This was accompanied by stark differences in peritoneal immune cell populations; IFNε treatment increased numbers of resident large peritoneal macrophages, and reduced infiltration of pro-inflammatory small peritoneal macrophages. Furthermore, while a population of myeloid derived suppressor cells (MDSC) was observed in tumour-bearing mice, these MDSC were absent in mice which received IFNε treatment. These results indicate that modulation of myeloid cell populations in the peritoneal cavity may contribute to the efficacy of IFNε in preventing peritoneal metastasis of ovarian cancer. Further investigation into the immunomodulatory activity of IFNε within the peritoneal cavity is required to elucidate the potential of IFNε to be used to treat ovarian cancer and other peritoneal pathologies.

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275

Development of a 3D cell culture model to study macrophage biology and infection with *Leishmania mexicana*

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Macrophages play a fundamental role in innate immunity by phagocytosing and eliminating invading pathogens upon their pro-inflammatory activation. Conversely, anti-inflammatory macrophages promote tissue regeneration and extracellular matrix (ECM) remodeling. While macrophage activation is a well-recognised modulator of tissue homeostasis, the influence of their local tissue microenvironment is not understood. In this study, we set out to examine the effects of ECM protein composition and tissue stiffness on macrophage activation. Bone marrow derived-macrophages (BMDM) or RAW264.7 murine macrophage cell lines were cultured in 3D by suspension within collagen gels. Macrophages cultured in 3D were compared to 2D controls (traditional plastic tissue-culture plates either with no ECM or a thin collagen monolayer), and a 2D cushion model (cells cultured as a monolayer on top of a pre-set collagen gel). Viability of BMDMs grown in 2D was ~80% after 6 days culture with media replacement every 2 days. By contrast, BMDM viability in the 2D cushion model was to ~75%, and only ~10% in the 3D model after 6 days, despite nutrient replenishment. By contrast, RAW264.7 macrophages exhibited increased viability in 3D compared to BMDM, with ~60% live cells after 6 days culture. Moreover, a human prostate cancer cell line (PC3*) exhibited normal growth and viability in 3D, suggesting BMDM are particularly sensitive to their culture environment. To determine whether 3D culture influenced the ability of macrophages to clear pathogens, we next infected BMDM with the protozoan parasite *Leishmania mexicana* prior to seeding within collagen gels. Strikingly, infected BMDM in 3D showed an increased viability to ~60% after 6 days of culture, compared to ~10% in uninfected 3D counterparts. All together, we show that culturing macrophages in 3D has an effect on their biology and may ultimately facilitate more accurate drug screening or the development of new therapeutics.

276

Unravelling mechanisms of TLR-inducible mitochondrial fission in macrophages

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Mitochondria have a multitude of functions ranging from energy generation to regulation of cellular processes. Mitochondrial dynamics (the balance between mitochondrial fission and fusion) has been implicated in regulating key macrophage functions, such as inflammatory and antimicrobial responses. This study focused on molecular mechanisms responsible for Toll-like Receptor (TLR)-inducible mitochondrial fission in macrophages. Here we reveal that lipopolysaccharide (LPS) stimulation of mouse bone marrow-derived macrophages (BMM) promotes toll-like receptor 4 (Tlr4)-dependent mitochondrial fission. Fission is triggered as early as 1 h post-stimulation, is sustained over a 24 h time course, and is dependent on the TLR adaptor protein MyD88. Drp1, a GTPase that is essential for mitochondrial fission, was also required for LPS-inducible fission; LPS-inducible fission was defective in both *Drp1*$^{-/-}$ mouse embryonic

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fibroblasts and *Drp1*-silenced BMM. Furthermore, LPS increased Drp1 recruitment to mitochondria, with acute LPS-induced Drp1 phosphorylation at serine 635 (S635) and delayed Drp1 dephosphorylation at serine 656 (S656) correlating with the early and late fission responses, respectively. Since fission has been linked to LPS-inducible glycolysis, the role of the glycolysis-promoting class IIa histone deacetylase (HDAC), Hdac7, in LPS-inducible fission was next investigated. Fission was abrogated in Hdac7-deficient macrophages and amplified in primary macrophages that overexpress Hdac7. Pre-treatment of BMM with the class IIa HDAC inhibitor TMP195 resulted in decreased LPS-inducible fission. However, both wild type Hdac7 and enzyme-dead Hdac7 could restore LPS-inducible fission in Hdac7-deficient BMM. This suggests that the deacetylase activity of Hdac7 is not required for Drp1-mediated fission in macrophages. Interestingly, LPS-mediated S656 dephosphorylation was abrogated in both MyD88- and Hdac7-deficient BMM, suggesting that this modification may be particularly important for TLR-inducible fission. In summary, this study has thus far delineated a pathway in which TLR4 activation promotes Drp1-mediated mitochondrial fission in a MyD88- and Hdac7-dependent pathway.

277

Characterizing a novel anti-inflammatory activity of Genistein

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INTRODUCTION: The cGAS-STING pathway plays a major role in aberrant immune responses following recognition of cytosolic DNA seen it many diseases such as Parkinson’s disease, Myocardial infarction or Systemic Lupus. Critically, this pathway can be amplified through formation of direct cell: cell interactions, known as gap junctions. Naturally occurring flavonoid compounds have been suggested to impact the activity of gap junction, but whether this can alter propagation of cGAS-STING signalling is not known.

METHODS: Here we used in vitro co-culture methods to assess the activity of a small set of flavonoid compounds on the propagation of cyclic GMP-AMP, the product of cGAS, between cells forming gap junctions. Since cGAMP directly binds to activate STING, we also studied the effect of flavonoids on STING downstream signalling, in human and murine cell models.

RESULTS: In co-cultures of cGAMP donor cells with STING expressing recipient cells, Genistein was the only flavonoid tested to decrease adjacent cell transactivation. This effect was concurrent with a decreased gap junction intercellular communication. Critically, Genistein pre-treatment of STING expressing recipient cells abolished cellular transactivation, indicating a direct effect on STING signalling. Accordingly, Genistein significantly decreased STING signalling upon activation by its agonists in human and murine cell models. Short pre-treatment with Genistein impacted STING phosphorylation, along with that of IRF3 and IKKe, demonstrating a direct effect on STING signalling. These findings establish a converging inhibitory activity of Genistein on cGAS-STING signalling, acting on preventing cGAMP transfer between adjacent cells, and preventing STING activation.

CONCLUSION: Collectively these findings indicate that select flavonoid compounds may present novel therapeutic opportunities to inhibit the cGAS-STING pathway involved in a growing number of auto-inflammatory diseases.

278

Differential modulation of innate immunity by *Bacteroides fragilis* bacteria and their secreted outer membrane vesicles

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Outer membrane vesicles (OMVs) are nanoparticles produced by bacteria as part of their normal growth. OMVs can package cargo from their parent bacterium including proteins, lipids and nucleic acids, which ultimately determine their function. OMVs produced by pathogenic bacteria have been extensively characterised as vehicles for the delivery of toxins and immunogenic molecules, thereby modulating host immune responses. By contrast, whilst commensal bacteria are recognised to modulate host immunity, the role of OMV secretion by the human microbiota remains poorly characterised. The commensal gut bacterium *Bacteroides fragilis* plays an integral role in maintaining gut homeostasis and modulating inflammation, in addition to their OMVs that can also mediate anti-inflammatory effects. In this study, we aimed to delineate the pathways by which *B. fragilis* and their OMVs mediate host innate immune responses.

To do this, *B. fragilis* OMVs were purified using density-gradient centrifugation and their size, concentration and contents were determined. OMVs ranged from 40-300 nm in size and contained immunogenic cargo including protein, DNA and RNA. Using HEK-Blue reporter cell lines, we determined that *B. fragilis* OMVs are detected by Toll-like receptor (TLR)-2, TLR4 and the cytoplasmic immune receptor NOD1. Preliminary results indicate that *B. fragilis* OMVs may also activate TLR7 and NOD2. By contrast, *B. fragilis* cells were only detected by TLR2. Using colonic epithelial cells, we are currently elucidating the ability of *B. fragilis* OMVs and their parent bacteria to modulate cytokine responses and to induce potential changes in epithelial cell barrier integrity.

Collectively, our findings indicate that the commensal bacterium *B. fragilis* produces OMVs which may exert anti-inflammatory effects via pathways independent of those activated by their parent bacterium. Furthermore, these findings highlight a role for OMVs produced by the human microbiota in shaping immune responses in the gut without the need for direct cell-to-cell interactions.

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279

Lactic acid produced by an optimal vaginal microbiota promotes cervicovaginal epithelial barrier integrity: implications for HIV transmission

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Women with a *Lactobacillus* spp.-dominated vaginal microbiota have a decreased risk of HIV acquisition compared to women colonized with 'non-optimal' vaginal microbiota, the latter being associated with decreased cervicovaginal epithelial barrier integrity. In an optimal vaginal environment, paracellular penetration of the epithelium by HIV is blocked by tight junctions. Lactic acid (LA) is a key metabolite of *Lactobacillus* spp. with antimicrobial and anti-inflammatory properties that is differentially produced by *Lactobacillus* spp. as L- and D-isoforms. However, the impact of LA in promoting epithelial barrier integrity through modulation of junctional molecules is unknown.

Cervicovaginal epithelial (Ect) cells were cultured in a transwell system and treated apically for 1 h with 0.3% L-LA or D-LA (pH 3.9), or acidity alone (pH 3.9, HCl adjusted). Transepithelial electrical resistance (TEER) across the cell monolayer was determined prior to and 24 h post-treatment to measure epithelial barrier integrity. Expression of junctional molecule mRNA after L or D-LA treatment was determined by RNASeq and qRT-PCR, and protein levels were determined by Western blot.

Treatment of Ect cells with L- or D-LA significantly increased TEER by 1.5-fold (n= 4; p<0.05), in contrast to the pH 3.9 (HCl) control treatment. RNASeq and gene ontology enrichment analysis were consistent with the TEER functional data demonstrating that L- and D-LA caused significant differential expression (FDR<0.05) compared to untreated cells of at least 11 genes associated with intracellular junctions and barrier function, including claudin-1 (CLDN1, L-LA Fold change [FC] 2.17 and D-LA 2.3-fold), claudin-4 (CLDN4, 2.8 and 3.1-fold) and occludin (OCLN, 1.4 and 1.5-fold), with no differential gene expression between isoforms. These findings were confirmed by qRT-PCR. In addition, tight junction protein levels were significantly increased by L-LA treatment (CLDN1 FC = 1.56, TJP2 FC = 1.42) but not with the pH control (n= 5; p<0.05).

LA significantly increases cervicovaginal epithelial barrier integrity by increasing the expression of junctional molecules, which has implications for the paracellular penetration of HIV through cervicovaginal tissue and subsequent HIV acquisition.

280

The gut microbiome: A source of antimicrobial resistance for pathogens

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The human gut microbiome is a community of microorganisms, including many species of bacteria, viruses and fungi that play a vital role in human health. These bacteria, particularly those within the gastrointestinal tract, have been linked to several diseases including inflammatory bowel disease and metabolic disease. However, what we now know is that these bacteria harbor antimicrobial resistance (AMR) genes that can be transferred between bacterial species, including pathogens. Currently, studies looking at this transfer of resistance have only been able to use sequencing data to infer that a transfer event has occurred, and they have not been able to culture many of the bacterial species involved. These studies have also relied upon the previous annotations of AMR genes and so have not been able to identify any novel modes of resistance.

This project is assessing the mechanism and frequency of AMR transfer from the commensals in the gut microbiota to gut enteropathogens. Initially, commensals from the gut microbiome were screened for resistance to three antibiotics (Tetracycline, Amoxicillin-clavulanic acid, and Vancomycin), and resistant strains were collected to form a panel of 95 AMR strains. This panel of AMR commensals was then mixed with either *Enterococcus feacium* or *Klebsiella pneumoniae*. The transfer of resistance has been phenotypically determined and the genes which have been transferred have been identified through whole genome sequencing of transconjugants. This work has identified AMR genes and mobile genetic elements capable of transfer between gut commensal bacteria and enteropathogens. Analysis has demonstrated shared phenotypic activity and phylogenetic barriers for transfer. These results will allow for more tailored treatments for patients with bacterial infections to improve treatment outcomes and reduce spread of AMR, and aid potential development of novel antimicrobials.

281

Identification of novel bacterial species associated with paediatric inflammatory bowel disease through direct mucosal sampling

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Inflammatory bowel disease (IBD) is a chronic, incurable condition, comprised of Crohn's Disease and Ulcerative Colitis. Over 20% of IBD is diagnosed in childhood; however, there are no universally effective treatments and clinical management is complicated by a plethora of side-effects. Until recently, investigation of the microbiota has been limited by an inability to culture the majority of intestinal bacteria. This has led to reliance on microbial sequencing to understand bacterial community structure; however, sequencing is limited by an inability to unambiguously identify causative bacteria.

Applying recently developed culturing techniques[1], we investigated the microbiota in Paediatric Inflammatory Bowel Disease (PIBD) patients presenting for colonoscopy at Monash Children's Hospital. Mucosal biopsy samples were collected across three intestinal regions (terminal Ileum, caecum and rectum) in order to characterise microbial signatures among a PIBD cohort. Concurrent bacterial culturing, shotgun metagenomic sequencing and molecular profiling was performed on 242 samples from this paediatric cohort. Bacterial culturing allowed 8347 purified isolates to be archived and identified using 16S rRNA sequencing, with whole genome sequencing performed on 192 of these isolates. Microbial analysis was complemented with examination of the molecular responses initiated at sample sites. RNA-sequencing, combined with qPCR validation of 12 genes of interest (IL6, IL8, IL12, IL17A, IL17F, IL23, CXCL10, TNF-α, STAT3, TREM1, EPCAM, IFN-γ), provided a detailed understanding of the transcriptional responses initiated.

Reference based metagenomic analysis[2] allowed for correlation of the bacterial isolates present with disease states and molecular inflammatory profiles. The therapeutic candidates identified through the unification of these multi-omics approaches were functionally validated in-vitro via Caco2 cell culture models, to begin testing their causative potential. This work develops and applies a novel workflow for the identification, classification and functional validation of microbiome-based disease biomarkers and may be significant for a huge multitude of diseases, unrestricted to the gastrointestinal microbiome.

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2 Forster, S. C. *et al.* A human gut bacterial genome and culture collection for improved metagenomic analyses. **37**, 186 (2019).

282

Characterisation of host-microbiome interactions using microfluidic organ-on-a-chip models

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The human gut microbiome plays an integral role in the development, maintenance, and function of the host immune system. Together, these interactions shape the balance between health and disease states, however, when disrupted can drive the development of infectious and inflammatory diseases. Advances in culturing and sequencing tools have paved way for comprehensive analysis of the intestinal ecosystem and microbial composition in the gut to functionally validate the microbiome. Despite numerous efforts to identify novel keystone species in health and disease, there is an unmet need to dissect the specific functions of host-microbiome interactions through experimental validation. Although extensive research has established various host-microbial factors that contribute to chronic immune disorders of the gut such as inflammatory bowel disease (IBD), further work is essential to fully understand the complex pathophysiology of IBD and determine causation of the microbiome. Through culturing and sequencing approaches, we have isolated novel bacteria from IBD patients that are associated with non-inflamed and inflamed intestinal states. However, the distinct functions of these bacterial isolates have yet to be characterised. Advancements in bioengineering has allowed the emergence of microfluidic organ-on-a-chip systems, which has revolutionised the ability to model organ physiology and function at a cell and tissue interface. Here, we describe the ability to functionally validate and characterise host-microbiome interactions based on the organ-on-a-chip system that mimics the intestinal niche and model diseases such as IBD. Intestinal epithelial cells, endothelial and immune cells are cultured in distinct nutrient-filled compartments on a multichannel, polymeric chip and can be co-cultured with bacteria or molecules of interest. This system will advance our current understanding of microbiome function and host responses, and will ultimately facilitate the development of high-throughput screening platforms and personalised precision medicine.

283

Functional Characterisation of Gastrointestinal Microbes

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The human gastrointestinal tract harbors a diverse ecosystem of commensal microbes that play an integral role in human health. Alterations to microbial composition, including those driven through diet or medication, are associated with a plethora of diseases, including inflammatory bowel disease, diabetes and obesity. However, limited knowledge of the functional requirements of individual bacterial

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species has limited the transition from associative taxonomic profiling to causative validation. This has also limited our ability to study microbial interactions and the formation of an optimal microbiome community structure, to inform therapeutic development.

Applying culturing techniques, we aimed to improve functional microbiome analysis methods to enhance the mechanistic understandings of the processes determining microbiome community structure. Initial phenotypic and whole genome analysis of 89 gastrointestinal isolates was performed to assess the varying nutrient requirements of common gastrointestinal isolates, highlighting isolate level growth variation and carbohydrate dependencies. This analysis demonstrates a diverse array of nutrient requirements and highlights the need for detailed, strain level investigation of therapeutic candidates. Future application of these studies at the community level with multi-omic analysis of temporal datasets will be required to assess the role of nutrient availability in community establishment.

284

Identification of suitable host conditions for the hatching of *t muris t suis* and *t trichiura* in the mucosal microbiota of pigs humans and a humanised mice model to understand the molecular basis of hatching in trichuris species

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Trichuriasis is a disease caused by infection with *Trichuris trichiura*, the human whipworm; affecting over 700 million people. Infection occurs upon ingestion of whipworm eggs that hatch and establish in the cecum and proximal colon. Trichuriasis has been studied in murine and porcine models, *T. muris* and *T. suis* respectively; revealing the role of the host intestinal microbiota in inducing hatching of whipworm eggs. *Escherichia coli* surface molecules play a role in the hatching of *T. muris*. We hypothesise that the same or similar molecules found in specific conformations on members of the host gut microbiota– specifically those closely associated with the intestinal mucosa, trigger hatching across other *Trichuris* species, through physical interaction and enzymatic activity. To understand the molecular basis of hatching across species and identify human gut microbiota capable of hatching *T. trichiura* we probed the interactions between *E. coli* and *T. muris*; and have identified classes proteins and proteases important to the hatching process by observing reductions in *T. muris* hatching. We investigated the effect on hatching of these proteases *in vitro* using lumenal and mucosal microbiota samples from a range of hosts including pigs, humans, and a humanised mice model. We hope to build a picture of hatching across the *Trichuris* species by using metagenomics, transcriptomics, and proteomics to analyse these samples which include human intestinal biopsies, porcine and humanised murine intestinal mucosal samples, purified gut microbiota, and faecal samples to search for bacteria expressing molecules key to the hatching process. Additionally, we are using these techniques to build a high throughput hatching screening platform to test and validate our candidate molecules and human gut microbiota, and can be applied to other large-scale investigations.

285

MicroRNA-16: Potential role in *Chlamydia trachomatis*-induced recurrent spontaneous abortion

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INTRODUCTION

Infection with the microorganism, *Chlamydia trachomatis* (Ct) is a frequent cause of sexually transmitted diseases globally including India. Ct is responsible for causing genital diseases chiefly in women including adverse obstetric outcome such as recurrent spontaneous abortion (RSA). Ct-induced RSA largely remains unreported/ underdiagnosed due to the multifactorial etiology of RSA. Micro-RNAs (miRNAs) play pivotal roles in regulating pathological/ physiological processes in diseased conditions. There is a lacunae regarding their role in infection (Ct)-associated RSA. Since miRNA-16 has been reported to be involved in placental angiogenesis, therefore the aim of the present work was to study the quantitative expression of urine miRNA-16 in Ct-positive women with RSA.

MATERIALS AND METHODS

This cross-sectional study was carried out in 25 non-pregnant women with history of first trimester RSA (after ruling out other risk factors associated with RSA) attending OPD, Department of Obstetrics and Gynecology, Safdarjung hospital, New Delhi (India) subsequent to ethical approval. Freshly-voided urine was collected after obtaining informed written consent from each patient and PCR was done for detection of Ct major outer membrane protein (537bp). Quantitation of chlamydial load was carried out by SYBR green-based chemistry real-time PCR using commercial Amplirun Ct DNA (*Vircell, Grenada, Spain*). Real-time PCR assay was also used for quantitating miRNA-16 expression by SYBR green-based chemistry using *C. elegans* miR-39_1 as an endogenous control (*Qiagen, MD, USA*). Graphpad prism software version 9.0 was utilized for statistical evaluation.

RESULTS

05 patients were diagnosed as positive for Ct (4-8 copies) infection. The expression of miRNA-16 was significantly upregulated ($p < 0.001$) in Ct-positive RSA *versus* uninfected RSA women (controls; n=20), with fold-change of 4.3.

CONCLUSIONS

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Overall data suggests the potential of miRNA-16 to serve as diagnostic biomarker that can be used during screening of Ct-positive RSA patients in a non-invasive sample such as urine. However, validation of findings by miRNA profiling is required in larger cohort for clinical management/ intervention.

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286

Is there potential clinical significance of miR-323-3p in *Chlamydia trachomatis*-associated tubal ectopic pregnancy?

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Introduction:

Ectopic pregnancy (EP) is associated with maternal morbidity/mortality. Diagnostic methods involving ultrasonography/hCG have poor clinical utility. There is no reliable diagnostic method till date. *Chlamydia trachomatis* (Ct) is an important risk factor for tubal EP. MicroRNAs (miRs) are present in body fluid and have been reported to be involved in pregnancy regulation. miR, *viz.*: miR-323-3p is biomarker in immune/inflammatory responses; however, its role in EP is unclear and remains to be established in Ct-positive tubal EP. It was hypothesized that expression level of circulating miR-323-3p might provide non-invasive method for early diagnosis of Ct-associated tubal EP. Hence, aim of study was to quantify expression of pregnancy-associated miR, *viz.:* hsa-miR-323-3p in serum of tubal EP patients(Ct-infected *versus* non-infected controls).

Materials and Methods:

This was a cross-sectional study wherein, 30 tubal EP patients (first trimester; age 22-36 years), undergoing surgical treatment at Department of Obstetrics and Gynecology, VMMC and Safdarjung hospital, New Delhi, India were enrolled with ethics approval from hospital's Ethical Committee. Fallopian tube (2x6 mm) and non-heparinized blood (serum) were collected from each enrolled patient. Ct detection was performed in FT by PCR usingprimers for MOMP (537 bp) and plasmid (200 bp) genes. Quantitative expression of serum hsa-miR-323-3p was done by real-time PCR by using commercial kit, miRNeasy serum/ plasma kit *(Qiagen, USA)* as per manufacturer's guidelines. Statistical analysis was performed using Graphpad Prism software version 9.

Results:

Ct MOMP and plasmid were found in 4/30(13%) and 5/30(16%) respectively among tubal EP women. Expression of hsa-miR-323-3p was found to be altered and significantly upregulated ('p'<0.05) in Ct-positive tubal EP women *versus* controls with fold-change of 3.1.

Conclusions:

Our study showed that hsa-miR-323-3p has potential to serve as possible clinical marker for screening such patients. However, detailed study with large number of cases is further required for use in clinical management.

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287

Disulphide bond dependent bacterial pathogenesis

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The disulphide bond catalyst DsbA, a key component of the disulphide bond (Dsb) forming machinery, is a central mediator of bacterial pathogenesis, as it promotes the assembly of a wide range of virulence factors required at different stages of the infection process. Even though extensive research has been focused in deciphering the Dsb systems in pathogens, to date, little is known about the way Dsb proteins interact with and fold virulence substrates. Interestingly, the main causative agent of urinary tract infections, Uropathogenic *E. coli* (UPEC), possesses two DsbA enzymes, namely DsbA and DsbL, for virulence factor folding. Through a combination of molecular biology, structural biology and biophysics, this work is focussed on understanding the structural and molecular determinants behind the substrate interaction and specificity of these two distinct DsbA homologues. Outcomes of this research will elucidate how bacterial pathogens use the Dsb system to generate virulence proteins involved in the establishment of infection. This knowledge will allow us to understand how bacteria cause disease and will provide important information to current campaigns aiming at developing DsbA inhibitors as potential anti-virulence agents.

288

Investigating molecular markers of Plasmodium falciparum artemisinin resistance in Papua New Guinea

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Mutations in the *P. falciparum kelch 13* gene (*Pfk13*) are associated with artemisinin resistance and emerged in Cambodia in 2008. While there is growing evidence of widespread *Pfk13* mutations throughout South East Asia and recent reports of a C580Y mutant parasite isolated in Wewak, a port town in Papua New Guinea (PNG), insufficient evidence exists to determine the extent of the local spread of mutant parasites. We investigated the prevalence of *Pfk13* mutations in geographically distinct regions of PNG from samples collected in 2015 – 2018 (n=1332). Isolates were screened by a standard *Pfk13* PCR assay and sequenced. Sequencing showed 663 high quality *Pfk13* sequences with five NS mutations; C580Y and 4 novel mutations R471C, N499K, K586E and Y635C. All mutations were found in a single isolate indicating that *Pfk13* mutations are rare in the surveyed areas, with no C580Y mutation in the previously identified area. The single C580Y isolate in our study, was found in another port town, in the Morobe Province, indicating a potential entry site for the importation of drug resistant infections into PNG. Whilst these results show that mutants are rare, the identification of a C580Y mutation has increased ongoing surveillance of antimalarial resistance mutations nationwide.

290

Bacterial membrane vesicles: a potential reservoir for antimicrobial resistance genes within bacterial communities

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The release of bacterial membrane vesicles (BMVs) is a conserved mechanism common to all bacteria, which contributes to the dissemination of bacterial components including proteins, DNA, and RNA. Recently, BMVs have been described as a novel mechanism of horizontal gene transfer (HGT), as DNA-containing BMVs can transfer antimicrobial resistance genes (ARGs) to recipient bacteria, however this has only been described for a limited number of bacterial species. In this study, we examined BMVs produced by the mucosal opportunistic pathogen, *P. aeruginosa*, and by a mixed gut microbiota culture, to determine if they contain DNA encoding for ARGs which can be transferred to other bacteria.

We found that *P. aeruginosa* grown using planktonic conditions released BMVs containing plasmid-encoded ARGs. These BMVs could mediate HGT, resulting in antibiotic-resistant transformants at a significantly higher rate than plasmid DNA alone. As biofilms have been shown to enhance HGT, we next investigated the ability of BMVs produced by *P. aeruginosa* grown as a biofilm to facilitate HGT. BMVs produced by *P. aeruginosa* biofilms contained an increased level of plasmid ARGs compared to planktonic-derived BMVs, and we are currently comparing their HGT efficiencies in the transfer of DNA to planktonic and biofilm *P. aeruginosa*.

We next wanted to examine the ability of BMVs to contribute to HGT in a mixed microbial setting. As the human gut microbiome is considered a reservoir for ARGs, we purified BMVs produced by a mixed culture containing 95 microbiota bacteria to determine if they

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contain ARGs. Currently, we are sequencing the DNA contained within microbiota-derived BMVs to identify ARGs contained within them and whether they can facilitate HGT.

Collectively, these studies will advance our limited knowledge regarding the contribution of BMVs to the transfer of ARGs during physiological settings, such as within biofilms and the human gut microbiota.

291

Molecular mechanisms of autotransporters

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Introduction:

Autotransporters are the largest and most widespread group of bacterial surface and secreted proteins. They are used to promote important pathogenic functions such as colonisation, biofilm formation, invasion and tissue destruction. However, very little is known with regard to the structures and functions of these proteins. Understanding the molecular details of how these proteins function is key to better understanding bacterial infections from diverse pathogens and will provide critical information that can be used to target these proteins for the development of new antimicrobials.

Aims:

We sought to uncover the structures, mode of action, regulation and roles in bacterial pathogenesis of autotransporters, and then to use this information to develop new inhibitors and other medical tools.

Methodology:

We combined a multidisciplinary approach of X-ray crystallography with biophysical, biochemical, cellular and microbiology methods.

Results:

Our findings show that the crystal structures of autotransporters reveal that these proteins form long β-helices that incorporate different features to allow binding and action on their target proteins. This common β-helix based domain allows for (i) direct binding to host epithelia to promote bacterial colonisation[1] (ii) the formation of self-associations to create protective biofilms[2] (iii) switching of binding modes to mediate bacterial invasion (iv) intracellular degradation of host tissue (v) and the ability to interact with both host and bacterial proteins to modulate infection and immune processes.

Conclusions:

We are now finally uncovering for the first time the roles, mechanisms and structures of this large and uncharacterised group of bacterial proteins. We can now appreciate in molecular detail how these autotransporters promote various types of bacterial infection processes. We are now using these findings to develop new types of antimicrobials and other medical tools[3].

292

Genomic characteristics of *exoU* and *exoS* ocular *Pseudomonas aeruginosa* isolates

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Twenty-five ocular *P. aeruginosa* isolates, 12 *exoU*+ (cytotoxic) and 13 were *exoS*+ (invasive), were analysed for their genotypic and phenotypic characteristics. Among the *exoU* isolates, four were multidrug resistant. *ExoU*+ isolates had a large pan genome (average 6553 genes) whereas *exoS*+ had a smaller pan genome (average 6401 genes). *ExoU*+isolates carried a median of 1403 (963-1894) whereas *exoS*+ strains have 1137 (909-4033) accessory genes. Out of a total of 32 acquired resistance genes, 29 were present in the *exoU*+ and 11 in the *exoS*+ strains. Five clones (ST233, ST491, ST316 and ST308) were present in the isolates. ST308 and ST316 clones were multi drug resistant and acquired most of the resistance genes. Among *exoS* strains, none of the clonal isolates were multidrug resistant. *ExoU* isolates were resistant to seven of the eight tested antibiotics, except polymyxin B. In conclusion, the two different type III secretion system genotypes of ocular *P. aeruginosa* were diverse and possessed distinct genotypic and phenotypic characteristics.

293

Identification of dengue virus (DENV) NS1 protein residues that are critical to its secretion

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Dengue virus (DENV) is a Flavivirus of the Flaviridae 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 others, multiple individual mutations in the ß-ladder domain of NS1 (including V220D, A247V, T283A, C313S and R336S) impaired its secretion by >90%. The impact of these mutations on viral RNA replication and infectious virus production are

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currently under investigation. Together, we suggest that residues that are critical for NS1 secretion may be targeted in future antiviral drug and attenuated vaccine development.

294

Galectin-1 Plays a Significant Role in Preterm Birth in Women Infected with *Ureaplasma urealyticum*

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Introduction

Preterm Birth (PTB) is associated with 75% perinatal mortality and >50% long-term morbidity, such as neuro-developmental problems/ pulmonary dysfunction/ visual impairment. Infections and infection-driven inflammatory responses are considered as leading cause of PTB. Intrauterine infections can induce various stimulatory factors such as galectins/ proinflammatory cytokines/ chemokines/ prostaglandins which can trigger the early onset of labour and lead to PTB. Galectins are endogenous β-galactoside binding lectins, emerging as potential inflammatory mediator. Galectin-1 expresses at the feto-maternal interface and plays key role in maintenance of pregnancy by regulating the maternal immune response against placental alloantigens. Present study aimed to study *in situ* expression of galectin-1 in placenta of PTB patients infected with the pathogenic bacteria, *Ureaplasma urealyticum* (UU).

Materials and Methods

After obtaining hospital ethics committee permission, 35 patients (age = 19 - 31 years) undergoing premature delivery at Department of Obstetrics and Gynaecology, Safdarjung hospital, New Delhi, India were enrolled for collection of placental tissue (5cm x 5cm). Polymerase chain reaction assay was performed for UU detection by targeting MBA gene (364 bp) and for ruling out other sexually transmitted diseases (STD) pathogens. Real-time PCR was done for quantification of galectin-1 mRNA expression level by using commercial kit (*Vivantis Technologies, Malaysia*) as per manufacturer's guidelines. Data was statistically analyzed by GraphPad Prism version 9.0.

Results

Placental UU was detected in 7 /35 (20%) of PTB patients. Galectin-1 expression was altered and found to be significantly ($p < 0.05$) upregulated in UU-positive PTB patients *versus* uninfected PTB patients ($n = 28$) with a fold change of 2.4.

Conclusions

Results suggest significant association of galectin-1 with PTB and its potential as a determinant for pathological pregnancies. Future studies targeting galectins in larger number of patients undergoing premature delivery are needed.

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295

Investigating the molecular evolution of carbapenem-resistance in *Klebsiella quasipneumoniae*

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The evolution of multidrug resistance in Klebsiella species in response to selective pressure and the extent to which this may be reversible is not fully understood. Since the spread of carbapenem-resistant Enterobacteriaceae (CRE) is considered a public health threat by the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC), more studies are needed to understand the evolution of antibiotic resistance mechanisms to determine if it is possible to reverse this trend.

A clinical Klebsiella quasipneumoniae isolate called FK688, which caused a bloodstream infection in a hospital patient, is resistant to multiple antibiotics, including third-generation cephalosporins and carbapenems. Analysis of the complete FK688 genome using short-read and long-read sequence data provided the first view of the gene synteny surrounding each of the outer-membrane porin genes in this species. Loss of functional porins is a mechanism by which bacterial cells can develop drug resistance due to reduced permeability of antibiotics into the cell. Genome sequencing showed that *ompK35* has an insertion sequence in the promoter region and *ompK36* contains a 48-bp deletion in the open-reading frame, resulting in the expression of a truncated non-functional outer membrane porin.

The genome data for FK688 also revealed the presence of a megaplasmid carrying a $bla_{\text{DHA-1}}$ gene that encodes a plasmid-mediated class C-type (pAmpC) β-lactamase. A machine learning predictor, DeepBL, was used to confirm that FK688 does not encode any known

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carbapenemases. Gene conversion experiments were performed to demonstrate that it is the presence of *bla*$_{DHA-1}$ together with non-functional porins that serve as a primary determinant of the CRE phenotype in *K. quasipneumoniae* FK688 and DHA-1 is contributing to a decreased susceptibility to carbapenems even in an OmpK36 expressing strain.

296

Evolutionary Glycomics: A Comprehensive Study of Vertebrate Host Serum/Plasma Glycome Using Orthogonal Glycomics Techniques

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Eukaryotic Vertebrate host glycosylation machinery has been subject to immense evolutionary pressure. Besides, being crucial for host sustenance, glyco-epitopes are also key-regulators of inter-species pathogen transmission. Significant evolutionary events such as loss of N-glycolyl neuraminic acid (Neu5Gc) or alpha Galactose (α-Gal) epitopes in species such as humans have been relevant for species development and have significantly impacted the ongoing arms race between pathogen and its host. Comprehensive knowledge on Vertebrate species-specific glycosylation is still scattered, thereby leaving a substantial gap in our understanding of their glycome evolution.

Serum/plasma N- and O-glycomes of 35 different Vertebrate species (mammals, marsupials, birds, reptiles and fish) were analysed by two orthogonal glycomics methods Porous Graphitised Carbon nano- Liquid Chromatography coupled to Electrospray Ionisation Tandem Mass Spectrometry (PGC nano-LC ESI-MS/MS) and Matrix Associated Laser Desorption Ionisation Time-of-flight Mass Spectrometry (MALDI TOF MS).

Vertebrate serum/plasma glycome predominantly contained complex di-, mono sialylated glycans. Core fucosylation of these N-glycans was strongly species-dependent and so were the non-reducing end modifications. Most species showed a preference for the incorporation of either N-acetyl neuraminic acid (NeuAc) or NeuGc, with exceptions such as rats that use both. Anseriformes (Goose, Duck) N-glycans carried GalNAc-GlcNAc (LacDiNAc) epitopes that, however, were absent in Galliformes (Chicken, Turkey). Reptilians (e.g. Green sea turtles) had high levels of α-Gal epitopes, and Saltwater Crocodile uniquely showed high amounts of oligomannose N-glycans in serum. This largest, vertebrate species-specific serum/plasma glycan and MS/MS spectral data is currently in the process of being made available open access through our collaboration with glycan focussed database such as Glyconnect and UniCarbDB.

297

Bioinformatics analysis of chemotaxis related genes in gram negative bacterial colonisers of the gastrointestinal tract

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Colonisation of the gastrointestinal tract (GIT) is a key factor in the ability of pathogenic bacteria to cause disease in humans, where the GIT's mucous layers form a barrier against infection. The chemotaxis properties of gut colonising bacteria, like *Escherichia coli* and *Campylobacter jejuni* have been well studied. Motility and attraction of *C. jejuni* to components of the GIT mucous layer have been regarded as facilitators for penetration of the gut epithelial cells. However, chemotaxis genes of hydrogen-requiring *Campylobacter* species, known to colonise the GIT, have not been thoroughly investigated.

This study compared the presence and location of chemotaxis related genes in the genomes of *C. concisus*, *C. curvus*, *C. mucosalis* and *C. showae*. The 3-D predicted protein structure and ligand binding domains were analysed to assess their colonisation capabilities. Whole genome sequences for *E. coli* (K-12, MG1655), *C. jejuni* (NCTC 11168) and *C. concisus* (ATCC 33237), *C. curvus* (525.92), *C. showae* (B91 SC) and *C. mucosalis* (ATCC 43265) were downloaded from the NCBI website and annotated using DFAST. Protein identity was established by multiple sequence alignment using Clustal-omega. The predicted chemotaxis-related genes and their encoded protein structures were compared to those previously identified in *E. coli.*

Several chemotaxis-related genes similar to those previously identified in the *E. coli* model were found, in some form, in all of the analysed *Campylobacter* spp genome sequences. Two groups of these gene clusters were close enough in their location to possibly be in operons. Furthermore, the high similarity between the size and the predicted 3-D protein structures of *Campylobacter* spp and *E. coli* suggests that chemotaxis in these GIT colonisers, operates in a similar manner, and show the conservation of chemotaxis genes in these selected enteric bacterial species. However, differences in the binding sites of chemotaxis receptor proteins suggests variation in the sensed substances, and may indicate their colonisation potential and pathogenic properties, which is worth of further investigation.

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298

Understanding Plasmodium vivax antigen diversity and identifying targets of immune selection

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There has been limited attention on Plasmodium vivax infection and its impact on the global malaria control and elimination campaign. One of the major gaps in vivax research is the lack of information on the diversity of surface antigens, which could provide relevant details on the parasite population structure and vaccine development. Population genetic data can not only assess transmission intensity but also provide insights in detecting genes under immune selection. We aimed to determine the global diversity of P. vivax vaccine candidates and serosurveillance antigens and identify gene regions under positive balancing selection. Global diversity and evidence of immune selection were measured from gene sequences extracted from whole genome sequences. Haplotype network diagrams were constructed to visualize the genetic relatedness of the analyzed sequences. Moreover, the protein sequence of each antigen was mapped to predict B cell epitopes to identify immunodominant domains. Initial results showed varying levels of diversity in some of the major P. vivax antigens across different populations. The antigens' RAMA, $MSP1_{19}$, FAM-a-1, MSP8, and S16 demonstrated remarkably conserved sequences while RBP2a, RBP2b, MSP3-a, MSP5 and DBP exhibited extensive genetic diversity. The haplotype network plot also showed distantly related sequences among highly diverse antigens reflecting their known extensive variation. Lastly, scanning the full-length antigen sequences revealed protein regions that are likely to be under immune selection and could be used as a guide in selecting domains to be included in a candidate vaccine. The data from this study will guide developers in designing widely effective vaccines and serological tools against P. vivax parasites.

299

Single cell transcriptional profiles of immune cell subsets from children and adults with malaria.

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In 2018, there were 228 million cases of malaria, leading to more than 400,000 deaths, of which ~70% were among children under the age of five. In areas of high malaria incidence, naturally acquired immunity to malaria increases with repeated infection. However, age seems to be a factor in immune acquisition, for example adults gain protective immunity more rapidly than children in families moving from malaria naïve to malaria endemic settings. To investigate the impact of age on immune responses during malaria (*Plasmodium falciparum*) infection, we applied single cell RNA sequencing (scRNA-Seq) to profile peripheral blood mononuclear cells (PBMCs) of 3 children and 3 adult patients during acute infection, 7 days post-treatment and at 28 days post treatment during convalescence. We found that the composition of circulating immune cells varied significantly between malaria infection and convalescence, with significant changes to monocytes and B-cells, whereas the proportions of CD8 and CD4 T cells remained relatively consistent. Multiple transcriptional changes were identified which were both unique to specific cells types, and shared globally across multiple cell subsets. By comparing transcriptional changes during infection between children and adults, we identified multiple age dependent differences which may influence acquisition of protective immunity. Together data generated are a unique resource to investigate malaria induced and age specific changes to immune responses.

300

Nanoparticle-mediated pulmonary delivery of the peptide hormone relaxin abrogates chronic allergic airways disease

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Chronic allergic airways disease (AAD) is a hallmark T Helper type 2 (T_H2) disease characterised by airway inflammation (AI), airway remodeling (AWR) and airway hyperresponsiveness (AHR). Current treatments of AAD mainly focus on targeting AI and its contribution AHR, with the use of corticosteroids. However, there are no therapies for the direct treatment of AWR, that can contribute to AHR independently of AI, and which contributes to corticosteroid resistance. The acute heart failure drug, serelaxin (recombinant human gene-2 relaxin/RLX), has been shown to ameliorate lung fibrosis and related AHR/lung dysfunction in various pre-clinical disease models[1,2], but requires continuous systemic delivery or daily intranasal administration[3]. We developed serelaxin-conjugated biodegradable nanoparticles (NP-RLX) and demonstrated for the first time the use of these particles as translational therapies for the treatment of AAD. Intranasally-delivered NP-RLX was primarily taken up by alveolar macrophages. Characterisation of inflammatory myeloid cell influx in the lung demonstrated the necessity of CD206 (mannose receptor) and CD68 (a pan-macrophage/dendritic cell) markers for uptake of NP-RLX in mitigating these effects. Furthermore, intranasally-delivered NP-RLX was found to abrogate AI by reducing the levels of pro-inflammatory cytokines (specifically IL-1β) and the influx of pro-inflammatory cells such as alveolar macrophages and eosinophils. NP-RLX also reduced

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several features of AWR by reducing the TGF-β1/IL-1β axis at the level of phosphorylated Smad2, as well as AHR. These findings demonstrate the therapeutic efficacy of nanoparticle-conjugated RLX in the airways/lung and represent a clinically translatable and effective strategy for the treatment of chronic AAD, which present with several features of human asthma.

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301

Rapid profiling of polymyxin B susceptibility in gram-negative bacteria with a novel polymyxin-derived fluorescent probe

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Polymyxins are considered as the last-resort antibiotics for the treatment of bacterial infections, however, the prompting concern of polymyxin-resistant bacteria demands faster antimicrobial susceptibility testing method to guide reasonably therapy in clinic. Traditional antimicrobial susceptibility testing methods are usually time-consuming. Here, we have designed and synthesized a fluorescent polymyxin-fluorophore derivative, Polymyxin-NBD, which has been successfully applied to develop a flow cytometry assay that can rapidly determine polymyxin B susceptibility of various Gram-negative species including *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The short time frame, easy data analysis, and application to multiple species for this qualitative test make it a promising antimicrobial susceptibility test method.

302

Development of a Novel Antimalarial Class with a Slow Onset of Parasite Activity

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Malaria is one of the most significant parasitic diseases in human history with approximately half of the world's population at risk of infection.[1] Infected individuals are estimated to total 219 million annually with 435,000 succumbing to the disease in 2016.[2] Parasite resistance has developed against all available classes of antimalarials, including the current first-line treatment Artemisinin combination therapy (ACT).[3] Therefore, an urgent need has arisen towards the development of antimalarials with novel mechanisms of action.

In collaboration with Janssen Pharmaceuticals and Medicines for Malaria Venture, we have undertaken a high-throughput screen of a large drug-like library against the asexual blood stage of Plasmodium falciparum and identified a number of hit chemical series. One of these series is the focus of the present studies and is mediated by an unknown mechanism of action with an interesting delayed parasite killing profile. Medicinal chemistry techniques have been used to identify a tight SAR and have generated a potent nanomolar inhibitor. These optimised hits are now being used for further mechanistic studies towards the identification of novel P. falciparum cellular targets.

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303

Genetic characterisation of zoonotic rotavirus strains identified in children and adults with acute rotavirus gastroenteritis in Australia

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Background

Rotavirus strains are classified into G and P genotypes based on the two outer capsid proteins. To date, 36 G types and 51 P types have been characterised from humans and animal species. Whole genome sequencing allows for the classification of all 11 rotavirus genes to describe genome constellations and reassortment events. Rotavirus is capable of infecting human and animal hosts, and zoonotic transmission occurs. Zoonotic transmissions have the potential to result in highly competitive viruses that circulate widely in the human population; antigenically distinct to human strains. The Australian Rotavirus Surveillance Program conducts an ongoing investigation of the rotavirus strains causing disease in Australia. The aim of this project was to investigate the genetic diversity and origins of unusual rotavirus strains.

Methods

Unusual rotavirus strains were selected between 2009 to 2019, from adult and paediatric patients with acute gastroenteritis. Following random amplification of viral RNA, whole genome sequencing was performed on an Illumina MiSeq platform. Sequence assemblies were performed using Geneious Prime. Maximum likelihood and Bayesian phylogenetic analysis was performed.

Results

In this study, G3P[3], G3P[8] and G3P[9] samples were identified as canine/feline-like and were derived via independent reassortment events between human and animal strains. These strains were detected over a number of years in multiple regions highlighting sustained human-to-human transmission. Several strains with bovine origins were detected including G6P[14], G8P[14], G10P[14], G8P[1], G8P[4] and G10P[25]. These strains exhibited a highly conserved genome constellation. This suggests a highly conserved bovine-like strain has undergone several independent reassortment events and been introduced into the human population multiple times with widespread human-to-human transmission. A number of strains also had genes that may have been derived from viruses circulating in bats, camels and other animal species.

Conclusions

This study highlights the diversity of rotavirus strains circulating in Australia and the wide potential for zoonotic transmission. The continued detection of unusual strains is of great importance in the era of routine rotavirus vaccination. While zoonotic transmissions often represent sporadic cases, the sustained circulation of antigenically novel strains has the potential to challenge vaccine efficacy.

304

Keep holding on: the role of antibody avidity in immunity to malaria

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A major hurdle in the development of a highly efficacious and long-lasting malaria vaccine is the absence of clear correlates of protective immunity. Antibodies are critical to mediate immunity to malaria but concentration alone is not sufficient to guarantee clinical protection. Therefore, antibody properties and functional activity must be more closely investigated to identify highly protective antibodies. Antibody binding strength, or avidity, is frequently described as the quality of an antibody response and has proven a valuable marker of infection and immunity in other diseases. However, although avidity in malaria is often associated with pathogen exposure, previous studies have demonstrated an inconsistent association with protection from parasitemia or clinical disease. Elucidating the role of high avidity interactions in the immune response to malaria will identify beneficial antibody properties which should be targeted in future vaccine development. In this work, IgG avidity against key vaccine targets was assessed in separate malaria vaccine clinical trials, comprising malaria-naïve and naturally-exposed individuals, and considered in relation to antibody maintenance and functional mechanisms. In a Phase II trial of the leading malaria vaccine candidate, RTS,S, high avidity binding did not improve recruitment of complement proteins or Fcγ-receptor binding but was associated with improved maintenance of IgG over time. This effect was also observed when investigating the antibody response to the major B cell epitope of the RTS,S target protein. The association between high avidity and IgG longevity, but not function, was supported in a cohort of Australian adults enrolled in a Phase I trial of an alternative vaccine candidate. We further investigated the presence of high avidity, well-maintained antibody populations in vaccine-induced responses in mice. Defined markers of long-term immunity are crucial for the design and testing of improved malaria vaccines. To eliminate this global health burden, the development of a vaccine capable of inducing sustained protection is essential.

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305

Investigation of the optimal strategy to deliver a B cell immunogen for a HCV vaccine.

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Approximately 71 million people are infected with hepatitis C virus causing substantial morbidity and mortality worldwide. Efforts to eliminate HCV have been improved with the development of safe and highly effective direct-acting antivirals, but for elimination to be achieved, a vaccine must be developed to reduce the number of new infections and prevent reinfection. To date, no pathogen has been eliminated without the development of a prophylactic vaccine. Chimpanzee adenovirus (ChAd) is a leading vaccine platform for COVID-19 aimed at generating neutralizing antibodies to the SARS-CoV-2 spike protein. It is a viral vector platform developed at Oxford University from a genetically modified attenuated rare serotype (ChAdOx1), therefore there is low seroprevalence in the human population which will circumvent pre-existing immunity. We investigated the use of this platform with a leading HCV vaccine candidate aimed at generating humoral immunity. We have previously reported that by removing the hypervariable regions from glycoprotein E2 a soluble recombinant protein (D123) can be produced and was shown to elicit cross neutralising antibodies in guinea pigs when administered as an adjuvanted soluble protein. In this study, we delivered the D123 vaccine candidate in ChAdOx1 and investigated the optimal prime-boost strategy. Animals were administered three doses, three weeks apart of either ChAdOx1D123, ChAdOx1D123 and boosted with soluble adjuvanted D123, or three doses of soluble adjuvanted D123 protein. All strategies elicited E2 specific antibodies in mice, however, titres were significantly higher in groups that received D123 protein, either as a boost with ChAdOx1D123 prime or three doses of D123. Enhanced antibody mediated E2-CD81 inhibition was observed to be statistically highest using ChAdOx1D123 prime/D123 protein boost, with this heterologous combination also eliciting both type specific and cross neutralising antibodies. Type specific neutralising antibodies were targeted to 3 major neutralisation epitopes located at residues 412-428, 429-448 and 523-549 which includes the CD81 binding loop. The results of this study show that addition of a protein boost after a ChAdOx1 prime using D123 is the optimal strategy for delivering a B cell immunogen for an HCV vaccine and suggests that this strategy could be explored for other pathogens including COVID-19.

306

Intrapulmonary vaccination with delta-inulin adjuvant stimulates non-polarised chemotactic signalling and diverse cellular interaction

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There is an urgent need for novel vaccination strategies to combat respiratory pathogens. Mucosal vaccine delivery is an attractive option as it directly targets the site of infection; however, preclinical development has been hindered by a lack of suitable mucosal adjuvants and a limited understanding of their immune effects in the lung environment. Herein, we define the early immune events following the intrapulmonary delivery of a vaccine incorporating the adjuvant delta-inulin. Analysis of the early inflammatory response showed vaccine-induced innate cell recruitment to lungs and local lymph nodes (LN) was transient and non-polarised, correlating with an increase in pulmonary chemotactic factors. Use of fluorescently labelled adjuvant revealed widespread tissue dissemination of adjuvant particles, coupled with broad cellular uptake and transit to the lung draining LN by a range of innate immune cells. Mass cytometric analysis revealed extensive phenotypic changes in innate and adaptive cell subsets induced by vaccination; this included identification of unconventional lymphocytes such as gd-T cells and MAIT cells that increased following vaccination and displayed an activated phenotype. This study details a comprehensive view of the immune response to intrapulmonary adjuvant administration and provide pre-clinical evidence to support delta-inulin as a suitable adjuvant for pulmonary vaccines.

307

Synchrotron-based X-ray fluorescence microscopy reveals accumulation of polymyxins in single human alveolar epithelial cells

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Intravenous administration of the last-line polymyxins results in poor drug exposure in the lungs and potential nephrotoxicity; while inhalation therapy offers better pharmacokinetics/pharmacodynamics for pulmonary infections by delivering the antibiotic to the infection site directly. However, polymyxin inhalation therapy has not been optimized and adverse effects can occur. This study aimed to quantitatively determine the intracellular accumulation and distribution of polymyxins in single human alveolar epithelial cells. A549 cells were treated with an iodine-labeled polymyxin probe FADDI-096 (5.0 and 10.0 μM) for 1, 4, and 24 h. Concentrations of FADDI-096 in single A549 cells were determined by synchrotron-based X-ray fluorescence microscopy. Concentration- and time-dependent accumulation of FADDI-096 within A549 cells was observed. The intracellular concentrations (mean ± SEM, n≥189) of FADDI-096 were 1.58 ± 0.11, 2.25 ± 0.10, and 2.46 ± 0.07 mM following 1, 4 and 24 h of treatment at 10 μM, respectively. The corresponding concentrations following the treatment at 5 μM were 0.05 ± 0.01, 0.24 ± 0.04, and 0.25 ± 0.02 mM (n≥189). FADDI-096 was mainly localized throughout the cytoplasm and nuclear region over 24 h. The intracellular zinc concentration increased in a concentration- and time-dependent manner. This is the first study to quantitatively map the accumulation of polymyxins in human alveolar epithelial cells and provides crucial insights for deciphering the mechanisms of their pulmonary toxicity. Importantly, our results may shed light on the optimization of inhaled polymyxins in patients and the development of new-generation safer polymyxins.

308

Identification of estrogen receptor modulators as inhibitors of flavivirus infection

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Flaviviruses such as Zika virus (ZIKV), dengue virus (DENV) and West Nile virus (WNV) are major global pathogens for which safe and effective antiviral therapies are not currently available. To identify antiviral small molecules with well-characterized safety and bioavailability profiles we screened a library of 2,907 approved drugs and pharmacologically active compounds for inhibitors of ZIKV infection using a high-throughput cell-based immunofluorescence assay. Interestingly, estrogen receptor modulators raloxifene hydrochloride and quinestrol were amongst 15 compounds that significantly inhibited ZIKV infection in repeat screens. Subsequent validation studies revealed that these drugs effectively inhibit ZIKV, DENV and WNV (Kunjin strain) infection at low micromolar concentrations with minimal cytotoxicity in Huh-7.5 hepatoma cells and HTR-8 placental trophoblast cells. Since these cells lack detectable expression of estrogen receptors-α and -β (ER-α and ER-β) and similar antiviral effects were observed in the context of subgenomic DENV and ZIKV replicons, these compounds appear to inhibit viral RNA replication in a manner that is independent of their known effects on estrogen receptor signaling. Taken together, quinestrol, raloxifene hydrochloride and structurally related analogues warrant further investigation as potential therapeutics for treatment of flavivirus infections.

1. Identification of Estrogen Receptor Modulators as Inhibitors of Flavivirus Infection Eyre, N. S., Kirby, E. N., Anfiteatro, D. R., Bracho, G., Russo, A. G., White, P. A., Aloia, A. L. & Beard, M. R. (2020) Antimicrobial Agents and Chemotherapy. 64, 8, 20 p., e00289.

309

Fortrexo: New approach to prevent and treat viral infectious diseases

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Viruses rely on their surface proteins to engage and enter their host target cells. By grafting those protein ligands onto the extracellular vesicles (EV) surface viral tropism is transferred to the EV, allowing the engineered EVs to interact preferentially with the same cells that are targeted by the virus. The concept of transferring viral tropism, in a process called pseudotyping, is an established approach in virus research and viral-based therapies.

Exopharm's proprietary EV engineering technologies enable predictable surface ligand modification (EVPSÔ) and ability to enhance specific nucleic acid cargo loading (LOADÔ) into EVs. LOADÔ and EVPSÔ form the basis of Exopharm's FortrexoÔ platform, the first iteration of which has allowed the rapid development of EV-based drugs for the prevention or treatment of viral diseases. The first product to be developed is Fortrexo CoVÔ, to address the current COVID-19 pandemic.

An advantage of FortrexoÔ is that it does not require prior knowledge of the target cell receptor that facilitates viral entry into the host cells. It is also not affected by mutations in the coat protein which might render an established vaccine ineffective, as the mutated virus will likely target the same receptor.

The RNAi cargo be designed to be completely specific for viral pathogens' target RNA sequences. The Fortrexo platform can be applied to any virus (or other RNA sequence) by differential and appropriate siRNA design.

Viral sequences quickly become accessible when new viruses emerge, therefore coat proteins can be readily incorporated into the next iteration of Fortrexo. Similarly, the genome sequence can be used to identify targets for RNAi to be used as cargo.

Fortrexo has been conceived to bridge the gap between the identification of new potentially pandemic viral strains and the successful development of effective prophylaxes.

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310

Taming the beast: disarming pathogenic bacteria with novel anti-virulence agents

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The emergence and spread of antibiotic resistance has jeopardized the effectiveness of current treatments that fight bacterial infections and motivated an active search for novel antibacterial targets and strategies. Bacteria rely on the biosynthesis of virulence factors to establish an infection in a host and cause disease. Disarming bacteria by anti-virulence agents has emerged as a promising approach to contain bacterial infections. This approach may have advantages over traditional antibiotics as it specifically targets virulence factors required for pathogenesis and do not inhibit bacterial viability or growth, potentially reducing selection pressure for resistance development. Many of these virulence factors, such as secreted toxins, adhesins, components of secretion systems and motility organelles, require folding into their native state by the thiol-disulfide oxidoreductase enzyme DsbA.[1] Bacteria lacking a functional DsbA displays reduced virulence, increased sensitivity to antibiotics and diminished capacity to cause infection in many Gram-negative pathogens.[1] DsbA thus has been identified as a viable target to sabotage bacterial virulence.

In our drug discovery program, we carried out multiple compound screening campaigns against DsbA and identified several classes of small molecule inhibitors binding to two proximal sites adjacent to the catalytic site of DsbA.[2-4] We examined simultaneous targeting of both DsbA binding sites by separate inhibitors and evaluated their impact on DsbA function *in vitro* and *in vivo*. Using X-ray crystallography, molecular modelling along with other biochemical assays, we demonstrated that combining these two inhibition modes enhanced DsbA inhibitory activity *in vitro*. More importantly, a synergistic effect of these compounds was also observed *in vivo*, where co-treatment of the pathogen *Salmonella enterica* serovar Typhimurium with two types of inhibitors resulted in increased attenuation of virulence. Overall, this work provides a new avenue for designing more potent DsbA inhibitors with enhanced anti-virulence activity and could lead to a new generation of antibacterial agents to treat drug-resistant bacterial infections.

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311

Molecular surveillance over 14 years confirms reduction of *Plasmodium vivax* and *falciparum* transmission after implementation of Artemisinin-based combination therapy in Papua, Indonesia

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Genetic epidemiology can provide important insights into parasite transmission that can inform public health interventions. The current study compared long-term changes in the genetic diversity and structure of co-endemic *Plasmodium falciparum* and *P. vivax* populations. The study was conducted in Papua Indonesia, where high-grade chloroquine resistance in *P. falciparum* and *P. vivax* led to a universal policy of Artemisinin-based Combination Therapy (ACT) in 2006. Microsatellite typing and population genetic analyses were undertaken on available isolates collected between 2004 and 2017 from patients with uncomplicated malaria (n=666 *P. falciparum* and n=615 *P. vivax*). The proportion of polyclonal *P. falciparum* infections fell from 28% (38/135) before policy change (2004-2006) to 18% (22/125) at the end of the study (2015-2017); p<0.001. Over the same period, polyclonal *P. vivax* infections fell from 67% (80/119) to 35% (33/93); *p*<0.001. *P. falciparum* strains persisted for up to 9 years compared to 3 months for *P. vivax*, reflecting higher rates of outbreeding in the latter. Sub-structure was observed in the *P. falciparum* population, but not in *P. vivax*, confirming different patterns of outbreeding. The *P. falciparum* population exhibited 4 subpopulations that changed in frequency over time. Notably, a sharp rise was observed in the frequency of a minor subpopulation (K2) in the late post-ACT period, accounting for 100% of infections in late 2016-2017. The results confirm epidemiological evidence of reduced *P. falciparum* and *P. vivax* transmission over time. The smaller change in *P. vivax* population structure is consistent with greater outbreeding associated with relapsing infections and highlights the need for radical cure to reduce recurrent infections. The study emphasizes the challenge in disrupting *P. vivax* transmission and demonstrates the potential of molecular data to inform on the impact of public health interventions.

The conference acknowledges CSL

Human Amniotic Epithelial Stem Cells attenuate Crescentic Glomerulonephritis

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Background

Crescentic glomerulonephritis (GN) is a disease which causes severe inflammation and damage of the kidney. The current treatments are non-specific, toxic and cause many serious side effects. Therefore, safer and effective treatments are needed. In this study, we tested if human amniotic epithelial stem cells (hAECs), obtained from the human placenta after birth, can attenuate crescentic GN using a mouse model.

Methods

GN was induced by injection of sheep anti-mouse glomerular basement membrane globulin on day 0. Then, saline or hAECs were given on day 10, when injury is already present. On day 21, we assessed GN development and immunity against sheep globulin. hAECs were also cultured *in vitro* and their expression of pro- and anti-inflammatory mediators analysed by flow cytometry.

Results

hAECs significantly attenuated the development of GN. This was shown by decreased proteinuria and crescent formation in glomeruli. Furthermore, they significantly reduced leukocyte infiltration in the kidney. hAECs did not affect the levels of mouse anti-sheep globulin antibody in serum. *In-vitro* studies showed that hAECs expressed anti-inflammatory cytokines, TGFβ and IL-10, but did not express pro-inflammatory molecules such as MHC-II, CD40 or CD86.

Conclusion

hAECs inhibit the development of crescentic GN in mice. Therefore, hAECs may be a safer, effective therapy for this disease.

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INDEX OF ABSTRACT AUTHORS

Guerra-Assunção, J 35
Liffner, B 236
McMillan, C.L 207
Moreno, P 303
Abeysekera, W 28
Acharya, D 206
Adams, F.G 224
Adderley, J 237
Afroz, S 276
Afzal, M 247
Afzal, R 40
Ahmed, S 210
Ailin, L 210
Ainslie, C 210
Aitken, E 270
Aktepe, T 214
Al Mushafi, A 312
Alagesan, K 296
Alam, M 108
Alexander, W.S 245
Alhammad, Y 102
Alisoltani, A 17
Allali, I 17
Aloia, A.L 308
Alwan, W 310
Amado, M 301
Amanat, F 3
Ambrose, R 252,32
Ammit, A 242
Anders, R.F 304
Andersen, S.B 267
Anderson, D 14
Anderson, G.P 241
Andrew, D.W 110,220,299
Andronicos, N.M 253
Anfiteatro, D.R 308
Ang, C 108
Angel, P.W 117
Annett, S 40
Anstey, N.M 220,299,311
Aponte, J.J 304
Appukuttan, B 269
Arora, R 254,285,286,294
Arpaia, N 16
Arulraj, T 205
Asari, K 309
Ashayeripanah, M 53
Ashdown, G 218
Ashhurst, T.M 3,306
Assmann, N 40
Atack, J.M 224
Athan, E 232
Auburn, S 311
Audsley, J 3
Azad, M.A 307
Bachem, A 101,114,15
Bafit, M 272
Bailey, B.L 302
Baker, M.G 33
Baker, M.L 6
Baldwin, C 309
Balka, K 262
Balle, C 17
Bannister, S 4
Barber, B.E 299
Bareng, P 298
Barnabas, S 17
Barnes, E 305
Barr, J.J 282
Barry, A 232,288,298
Barugahare, A 203
Baschuk, N 32
Bauer, M.J 22
Beard, M 250
Beard, M.R 293,308
Beatson, S.A 22
Beattie, L 104
Bedoui, S 101,114,15,214,32
Beeson, J 233,25,26,52
Beeson, J.G 304
Beilharz, T 36
Bekker, L 17
Belinda, S 210
Bell, L 17
Bell , P 215
Belz, G 115,49
Bennett, T 203

The conference acknowledges CSL

Bentley, M 297
Berger, P.J 264
Berriman, M 284
Bhat, P 14
Bhati, T 294
Bielefeldt-Ohmann, H 239,244
Bilbao, J 271
Binder, S.C 205
Bines, J.E 303
Binger, K 15
Binger, K.J 275
Bitto, N 278
Blackburn, J.M 17
Blaskovich, M.A 22
Blaskovich, M.A 21,301
Blumenthal, A 268,276
Bohlmann, L 22
Bolisetti, R 22
Bolton, R 268
Bonfim-Melo, A 257
Bonnici, R 303
Boo, I 102
Bottomley, A 217
Bouchery, T 255
Boyle, M.J 110,220,299,304
Bracho, G 308
Brewster, J 25
Britton, W 19
Britton, W.J 306
Brouwer, S 22
Brown, J.S 35
Browning, G.F 234
Bryant, C 113
Bullen, H 238
Burdam, F 311
Burgner, D 4
Calder, B 17
Caminschi, I 104
Campbell, N.K 274
Cao, W 115
Carlton, L 33
Carmo, O 230
Carr, J.M 269
Carr, P 33
Castellanos-Rubio, A 271
Catton, M 3
Center, R 102,14
Center, R.J 305
Cervantes-Silva, M.P 40
Chabikwa, T.G 299
Chakraborty, A 300
Chalk, J 26
Chambers, D.C 215
Chan, A 257
Chan, J 233,304,52
Chappell, K 48
Chen, Y 207
Cheng, A.C 3
Chew, K 208
Chin, W 282
Chinnakannan, S 305
Chishimba, S 233
Chitnis, C 25
Choi, J 117
Chong, M 54
Chong, M.M 106
Chonwerawong, M 281,282
Chua, B.Y 3
Chua, S 235
Chung, A.W 3
Church, W 207
Clark, A 242
Clark, M.P 229
Clemens, B.E 3
Clifford, V 4
Coldbeck-Shackley, R 250
Coley, A 309
Colledge, D 243
Condon, N.D 267
Conroy, T 22
Cook, G.M 22
Cook, L 225
Cooper, L 103
Cooper, M.A 21
Coppo, M.J 234
Cork, A.J 22
Costello, S 283
Coulibaly, F 102
Counihan, N 238
Counoupas, C 12,306
Coussens, A 218

The conference acknowledges CSL

Cowan, L 47
Cowman, A 2,302
Cox, A 245
Cozsijinsen, A 104
Crabb, B 238
Crameri, G 6
Crawford, N 4
Cristofaro, M 266
Cryle, M.J 31
Cunningham, A.C 221
Currie, A 107,246
Curson, J.E 112
Curson, J 42
Curtis, A 40
Curtis, N 50
Cutter, S.W 275
Cutts, J 233
D'Adamo, G.L 55
Da Costa, R 221
Dabee, S 17
DAdamo, G 282
DAdamo, G.L 281
Dankers, W 266
Daraj, G 262
Das Gupta, K 256,276,42
Daunt, C 255
Davey, G 40
Davies, M.R 22
Davis, G 40
Davis, T 233
Day, C 210
Day, N 26
de Bie, B 225
de Geus, E 113
de Geus, E.D 55
de Jonge, M.D 307
de Koning-Ward, T 20,238
De los Santos, K 16
De Oliveira, D.M 22
de Ribeiro, A 207
de Souza, D 15
De Weerd, N 113
de Weerd, N.A 55
Dearnley, M 6
Delgado Diaz, D 279
Demosthenous, E 303
Denardo, D 262
Deng, Y 117
Denholm, J 3
Dent, A 304
Deplazes, E 24
Derseh, H.B 234
DeSanti, C 40
Deshpande, C 207
Desvaux, M 227
Dhorda, M 26
Dhouib, R 219,310
Di Fazio, A 207
Dickerman, B 238
Dimri, A 254,286
Divithotawela, C 215
Dixon, M.W 230
Doak, B 310
Dobano, C 304
Doerflinger, M 114,229,32
Doerig, C 237
Donato, C.M 303
Dondorp, A 26
Donnison, T 305
Donovan, M.L 268
Doolan, D.L 3
Dooley, N.L 110,220
Dowling, J.K 264,40
Drew, D 52
Drummer, H 102,14
Drummer, H.E 305,6
du Plessis, M 17
Dubowsky, J.G 269
Duggin, I 217
Duque-Correa, M.A 284
Ebert, G 32
Edgington-Mitchell, L.E 259
Edwards, D.N 3
Eijkelkamp, B.A 224
El-Deeb, I.M 22
Ellett, F 31
Elliott, A.G 21
Elliott, A.G 301
Ellis, D 216
Ellisdon, A.M 264
Enders, M.H 104
Eng, V 32

The conference acknowledges CSL

Engel, J	27
Engel, S	114
Engelberg, D	235
Eriksson, E.M	28
Essilfie, A	212,219
Evelyn, C	2
Everest-Dass, A	22
Eyre, N.S	293,308
Fahimi, F	108
Fairlie, D	256,42
Fan, F	210
Fantino, E	219,251
Feng, G	233,304
Ferguson, P	245
Fernandez Ruiz, D	104
Ferrell, K.C	306
Ferrington, N	270
Ffrench, R	14
Finlay, D.K	40
Finlayson, K.A	296
Fitzgerald, K	43
Fleischmann, M	241
Fletcher, A	212
Forde, B.M	22
Forster, S	281,283,290
Forster, S.C	280,282,55
Forsyth, W	33
Fowkes, F.J	26
Freyne, B	50
Frolich, S	236
Fu, J	307
Fulcher, A.J	307,31
Gabriela, M	238
Gaggar, A	243
Gamieldien, H	17
Gan, J	222
Ganio, K	24
Gantier, M	277
Garnish, S	245
Gaspari, T.A	258
Gearing, J	250,281
Gearing, L.J	118,40,55
Gebhardt, T	15
Geoghegan, N	218
Geoghegan, N.D	2
Germano, S	50
Gibney, K	283
Giles, E	113,281
Giles, E.M	55
Gillies, J.K	225
Gilmore, W.J	278
Gilson, P	238
Gilson, P.R	233
Giogha, C	222,23
Goddard-Borger, E	23
Godfrey, D	260
Godfrey, D.I	3
Godzik, A	17
Gomes Moriel, D	201
Good, M	210
Good-Jacobson, K	103,240
Goodman, C.D	302
Gorrell, M.D	207
Gough, D.J	40
Gould, J	280,281,283
Gould, J.A	55
Gout, E	270
Grant, E	8
Grant, E.J	202
Grant, M	215
Gras, S	13,202,8
Gray, D	54
Grencis, R.K	284
Grigg, M.J	299
Gu, J	102,117
Gugasyan, R	279
Gulliver, E	283,290
Gulliver, E.L	280
Gupta, S	257
Gwillim, J	266
Habel, J.A	3
Hakkinen, E	225
Hamlin, A.S	253
Hammond, R	243
Hancock, S	265
Handayuni, I	311
Hansen, D.S	28
Hansford, K.A	21,22
Hanssen, E	52
Haque, A	204,27
Harawa, V	270
Harbers, M	25

The conference acknowledges CSL™

Harper, M 23
Harris, J 266
Harris, N 255
Harry, E 217
Hartland, E 222,32
Hartland, E.L 23,241
Hasang, W 270
Hawas, S 204
Hayes, C.A 296
Hayward, J 279
Hayward, J.A 6
Healer, J 2,25
Hearps, A 279
Heath, W.R 104
Helbig, K.J 7
Henderson, I.R 221
Henningham, A 219
Hensen, L 3
Heras, B 116,213,227,287,291,000
Herold, M 108,114,32
Hertzog, P 113,250,272,281
Hertzog, P.J 118,40,55
Hertzog, P.J 274
Hess, C 40
Hetzel, M 288
Heyderman, R.S 35
Hibbert, J 246
Hickson, S 223
Hildebrand, J.M 245
Hill, D.L 51
Hille, G 6
Ho, U 111
Hochrein, H 262
Hoehensteiger, J 223
Hogarth, M.P 233
Hogarth, P 52
Holden, K 309
Holdsworth, S 312
Holley, C.L 261
Holly, S 210
Holmes, E.C 6
Holmes, N.E 3
Hopkins, P.M 215
Hor, L 213,227,287,291
Horton, J.L 304
Hosmer, J 212,219
Howard, D.L 307
Huang, L 309
Huang, Q 225
Huang, S 113
Hughes, C.M 3
Hung, Y 251
Hussain, T 203,255
Hussell, T 56
Idris, A 206
Imwong, M 26
Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR) Group, M 50
Ingle, D 252
Ioannidis, L.J 28
Iranzadeh, A 17
Irima, D 31
Irvine, K 265
Irving, A 5
Isaacs, A 207
Istivan, T 297
Iwasaki, A 1,44
Iyer, A 42
Jackson, D.C 3
Jackson, P 302
Jackson, S 237
Jahan, N 273
James, A 111
James, K 27
James, S.A 307
James, W 210
Jameson, V 117
Jarnicki, A.G 241
Jaspan, H.B 17
Jayasinghe, D 8
Jen, F.E 22
Jennings, M 210
Jennings, M.P 22
Jenzelewski, V 52
Jesaveluk, B 279,6
Jia, X 3

The conference acknowledges CSL

Jiang, J	273
John, G	210
Johnson, A	279,6
Johnston, E.L	290
Jonsdottir, T	238
Joubert, I	107
Juno, J	9
Juno, J.A	3
Kaiser, K	16
Kanci Condello, A	234
Kaparakis Liaskos, M	290,30
Kapetanovic, R	267,276,42
Kappler, U	212,216,219
Karahalios, E	270
Karlsson, N.G	296
Karunakaran, D	256,42
Kauppi, M	245
Kazura, J	25
Kazura, J.W	304
KC, S	301
Kearney, E	26
Kedzierska, K	3
Kedzierski, L	3
Keller, N	22
Kenangalem, E	311
Kent, S.J	3
Ketheesan, N	253
Khalouf, H	266
Khan, M	292
Kho, S	311
Khoury, D	27
Kidd, T	265
Kidd, T.J	22
Kile, B	262
Kim, J	239,244
Kim, Y	307
King, N.J	3
Kirby, E.N	308
Kirkwood, C.D	303
Klein, S	14
Klenerman, D	113
Klock, E	14
Koay , F	260
Kobe, B	24
Koch, B	36
Koh, Y	112
Kohl, C	6
Kolarich, D	296
Koleala, T	288
Kontos, D	6
Kostoulias, X	31
Kotsimbos, T.C	3
Kouskousis, B	238,52
Koutsakos, M	3
Krammer, F	3
Kueh, A	108
Kulappu Arachchige, S.N	234
Kumari, S	41
Kurowska-Stolarska, M	117
Kurtovic, L	304,52
La Gruta, N	203,255
Labzin, L.I	208
Lacerda, M	25
Lacy, J.A	22
Laetitia, H	210
Lai, H	111
Lalaoui, N	32
Laman, M	233,288
Langer, C	6
Laslett, A.L	117
Latz, E	118
Lau, H	202
Laura, D	264
Lautu, D	288
Lawlor, K	228,262
Laws, J	7
Lay, C	102
Le, A	312
Le, G	242
Lê Cao, K	117
Ledger, E	215
Lee, A	101,107
Lee, H	27
Lee, S.J	26
Legarda, M	271
Legione, A.R	234
Levings, M.K	225
Lewin, S.R	3
Li, B	113
Li, F	14
Li, J	203,22,307, 307

The conference acknowledges CSL

Li Wai Suen, C.S	28
Liaskos, M	226,278
Lim, L	235
Lim, R	312
Lim, S	250
Lisacek, F	296
Lithgow, T	295
Liu, L	112
Liu, X	106,54
Liu, Z	245,25
Ljungberg, J.K	268
Lo, A	291
Locarnini, S	243
Longley, R	25
Lonnberg, T	27
Lorne, T	210
Loseff-Silver, J	309
Loughland, J.R	110,220,299
Low, J.K	207
Lowes, K.N	302
Lucas, R.M	112
Luo, L	112,251
Luo, Z	24
Ly, A	28
M Pacheco, C	117
Mackay, C	108
Mackay, J.P	207
Mackenzie, J	214
Macri, C	262
Maizels, R.M	225
Mak, J	210
Makita, L	288
Manabe, Y	14
Mandala, W	270
Mangan, N	113
Mangan, N.E	55
Manhanzva, M.T	17
Mann, E	18
Manning, L	233
Mansell, A	264
Marcelino , V	281,283
Marcelino, V.R	55
Marfurt, J	311
Mariethoz, J	296
Marks, Z.R	274
Marsh, G.A	6
Marsh-Wakefield, F	3
Martelotto, L	103
Martin, J.L	310
Mass, E	104
Masson, L	17
Masterman, K	262
Maude, R.J	26
McCarthy, J.S	110,304
McCarthy, K.L	223
McCaughan, G.W	207
McConville, M	15
McConville, M.J	275
McCoy, C	40
McDevitt, C	24
McDevitt, C.A	22
McDonald, M	37
McDonald, Z	17
McEwan, A	212,219
McEwan, A.G	22
McEwen, S	288
McFadden, G.I	104
McGregor, J	305
McGregor, R	33
McMahon, J	14,3
McMillan, D.J	253
McMillan, N	206
McMillan, P.J	109
McQuilten, Z	10
Mehra, S	298
Menassa, J	46
Mendoza, L	271
Mengist , A	270
Messina, N.L	50
Meyer-Hermann, M	205
Meyer, W	45
Michael, J	210
Milling, S	117
Mills, J	210
Minigo, G	220
Mintern, J.D	259,53
Mkandawire, T.T	284
Modak, J	238
Modhiran, N	239,244
Mojtahedinyazdi, Y	296
Monson, E.A	7
Monteiro, W	25

The conference acknowledges CSL

Monteith, J	223
Monteleone, M	261
Moore, R.J	297
Morand, E	266
Mordant, F.L	3
Moreland, N.J	201,33
Morris , K	206
Moseley, G	263
Mueller, I	233,25,288
Mulder, N	17
Murphy, J.M	245
Murthy, A	42
Murugan, A.V	296
Nadvi, N	207
Nasreen, M	212,219
Nate, E	288
Naung, M	298
Nedeva, C	46
Neeland, M	4
Neild, A	282
Nejsum, P.L	284
Newson, J	222
Ng, G	222
Ng, G.Z	241
Ngo, A	302
Nguitragool, W	25
Nguon, C	26
Nguyen, A	202,203,8
Nguyen, N	265
Nguyen, T.H	3
Nguyen , W	302
Nie, S	7
Nitsche, A	6
Njie , M	270
Nold, M.F	264
Nold-Petry, C.A	264
Noordstra, I	257
Noormohammadi, A.H	234
Nothaft, H	296
Noursadeghi, M	35
Noviyanti, R	28,311
Nutt, S	115
O'Brien, D	232
O'Flaherty, K	26
O'Keeffe, M	262,272
O'mara, M	116
Odobasic, D	312
Oehlers, S.H	34
Oh, S	106,54
okeeffe, M	273
Olazagoitia-Garmendia, A	271
Oliveira, T	296
Omotainse, O.S	234
Opi, D	25,26
Osborne, B	207
Oyong, D	220
Palchaudhuri, R	14
Palmer, C	52
Pandey, M	210
Pang, E	262,272
Panjikar, S	291
Pant, S	254,286
Parish, I	103
Park, H	16
Parvez, S	285,286,294
Pasparakis, M	41
Passmore, J.S	17
Pasternak, M	2
Patel, K	207
Paterson, D.L	22
Pathirana, R	278
Pava, Z	311
Paxman, J	213,287,291
Paxman, J.J	227
Paxman, J.J	310
Payne, J.A	31
Pearson, J	222,228,252
Pearson, J.S	23,32
Pederick, V	24
Peleg, A.Y	31
Peleg, A	273
Pellegrini, M	218,229,32
Pellicci, D	260
Persson, K	304
Peters, K.M	267
Peterson, E	217
Peto, T.J	26
Petrovski, S	290
Petrovsky, N	306
Pham, A	215
Phan, M	22,265,267
Phetsang, W	301

The conference acknowledges CSL

Philpott, D 29
Phyo, A 26
Piera, K.A 299
Pietrzak, H.M 28
Pilapitiya, A 213,291
Pilapitiya, A.U 227
Pinar, A 300
Pinar, A.A 258
Piontek, M 52
Pliego, A 239,244
Poespoprodjo, J.R 311
Pollara, G 35
Pollock, G 222,23
Poole, J 224
Potgieter, M 17
Poumbourios, P 305,6
Poumbourious, P 102
Price, D 26
Price, R.N 311
Proft, T 201
Puspitasari, A.M 311
Puthalakath, H 46
Putri, G.H 3
Qin, J 116
Quinn, K 203,255
Radford, K 262
Radzey, N 17
Rafeek, R.A 253
Raisuddin, S 254
Rajab, N 117
Ralph, S.A 109
Ramnath, D 256,276,42
Ramos-Sevillano, E 35
Randall, L.M 270
Rankin, L.C 16
Rastogi, S 254,285,286,294
Ratnasekera, I.U 265
Ray, A 285
Ray, R 206
Raynes, J.M 33
Razook, Z 288
Read, M.V 3
Reid, D 216
Reid, K 225
Reid, R.C 256
Reid, R 42
Reiling, L 233,304,52
Revill, P 243
Reynolds, N.N 275
Rice, S 292
Richardson, M 232
Richardson, S 296
Rivera-Hernandez, T 22
Robert, R 108
Roberts, J.A 22
Roberts, K.D 307
Roberts, T 111
Robertson, S 250
Robinson, L 25,288
Robson, T 40
Rocker, A 295
Roczo-Farkas, S 303
Roe, M 26
Rogers, K 218
Rogers, K.L 2
Rogerson, S 233,270
Rollo, R.F 268
Rooke, J.L 221
Roquilly, A 53
Rosas Bastidas, N 295
Rosli, S 250
Ross, E 17
Rowntree, L.C 3
Royce, S 300
Rudloff, I 264
Rumaseb, A 311
Russ, B 15,203
Russo, A.G 308
Rutar, M 117
Rutten, E 281,283
Rutten, E.L 280
Sacarlal, J 304
Saffery, R 4
Salvamoser, R 309
Samuel, C 300
Samuel, C.S 258
Sánchez, C.A 6
Sanders, P 238
Santos Martin, C 287
Sara, M.M 248
Sarkar, S 116

The conference acknowledges CSL

Sattabongkot Prachumsri, J 25
Saunders, B 242,249
Scanlon, M.J 310
Schembri, M.A 22,227,267
Schembri, M 265,291,42
Schirra, H 219
Schlotthauer, F 102
Schmidt, F 214
Schroder, K 208,257,261
Scott, N 222
Scott, N.E 23
Scott, T 206
Sebastian-delaCruz, M 271
Selomulya, C 300
Seydel, K 270
Shakespear, M 42
Sharareh, E 210
Shaw, T 243
SheelaNair, A 220
Shibu, M.A 230
Shil, P.K 234
Short, K 208
Shreenivas, M.M 105
Silke, J 245,32
Simmer, K 246
Simpson, J 26
Siraj, F 254,286
Skwarczynski, M 210
Slade, D.J 221
Sleebs, B 302
Sloane, H 8
Sly, P 219,251
Smith, I 6
Smyth, D.J 225
Smyth, G 28
Solymosi, P.D 6
Soon, M 27
Soppe, S 243
Sriprakash, K.S 253
Stacey, K 244
Stacey, K.J 239
Stanisic, D 210
Stapleton, F 247,292
Steer, A 4
Stevens, M 249
Stewart, E.L 306
Stocks, C 42
Stocks, C.J 208,267
Stow, J 251
Stow, J.L 112
Strasser, A 114,32
Straub, S 118
Straube, J 27
Strugnell, R 115
Strugnell, R.A 105,221
Strunk, T 107,246
Studniberg, S.I 28
Subbarao, K 3
Subedi, P 287
Suckow, M 52
Sudhakar, S 238
Supramaniam, A 206
Svensson, V 27
Sweet, M.J 112,267,39
Sweet, M.J 256
Sweet, M 265,276,42
Szeto, C 202,8
Szymanski, C.M 296
Tabb, D.L 17
Tachedjian, G 279,6
Tachedjian, M 6
Tailhades, J 31
Takashima, E 25
Tan, A 22,24
Tan, B.E 293
Tan, H 235
Tan, M 215
Tanaka, S 225
Tapia-Caceres, F 258
Tarique, A 251
Tate, M 250
Tay, M 33
Taylor, T 270
Teichmann, S 27
Tham, W 25
Thevarajan, I 3
Thiele, D 203
Thielens, N 270
Thomas, S 303
Thomason, T 281,282,283
Thompson, A 243

The conference acknowledges CSL

Thompson, J.K 2
Thygesen, S 239
Tilley, L 109,230
Tipping, P.E 220
Tirta, Y.K 311
Tong, S.Y 3
Torres, V.L 223
Tosif, S 4
Toth, I 210
Totsika, M 116,204,287,310
Tram, G 224
Traven, A 36
Trenerry, A 214
Trianty, L 28,311
Triccas, J.A 306
Triccas, J 12
Trimarsanto, H 311
Tripura, R 26
Trussart, M 28
Tsuboi, T 25
Tuladhar, N 251
Turner, S 15,203
Tutau, C 271
Tutor, M.V 109
Typas, N 38
Tyssen, D 279
Ubillos, I 304
Udupa, V 203
Uhlemann, A 16
ullah, t.M 277
Ung, H.K 264
Utami, R.A 311
Utami, T 28
Van, H 14
van de Merwe, J.P 296
van de Sandt, C.E 3
Van der Hoek, K 250
van Driel, I 222
van Driel, I.R 241
van Gils, M 57
Vankadari, N 211
Vaz, P.K 234
Velkov, T 307
Verderosa, A.D 310
victoria, O 210
Vijay, A 247
Villadangos, J.A 53
Vincent, F 266
Volaric, J 113
Volaric, J.S 55
von Itzstein, M 22
von Pein, J.B 267
Von Pien, J 42
von Seidlein, L 26
Wachsmuth, L 41
Walker, M.J 22
Walsh, R 243
Wang, G 310
Wang, L 6
Wang, N 105,115
Wang, T 287,291
Wang, X 207
Wang, Y 106,256,42
Warner, N 243
Watterson, D 239
Wawegama, N.K 234
Weerasinghe, H 36
Wei, H 14
Weight, C.M 35
Wells, C.A 117
Wells, T.J 215,223,268
Wemyss, M 228
Werner, A 6
Wetzel, D 52
Wheatley, A.K 3
Whelan, D.R 7
Whisstock, J.C 264
Whitchurch, C 226
Whitcombe, A.L 201
White, M 225,25
White, P.A 308
Whitehead, L 2
Wibawa, R 222
Wijegunasekara, S 232
Wilksch, J 295
Willcox, M 247,292
Willcox, M.P 248
William, T 299
Williams, B.R 40
Williamson, D 14,252
Williamson, T 237

The conference acknowledges CSL

Willingham, S 108
Wilson, D 236
Wilson, K 116,14
Wines, B.D 233
Wines, B 52
Wirjanata, G 311
Wong, C 3
Wong, D 243
Wong, M.Q 225
Wong Fok Lung, T 222,23
Wright, M 262,272
Xi, C.R 207
Xiang, M.S 207
Xie, L 108
Xu, B 259
Xu, C 260
Xu, H 243
Yap, A.S 257
Yau, K 36
Yerkovich, S 215
Young, N.D 234
Young, P 239
Young, R 283
Yu, H.H 307
Yuferov, A 258
Zaini, A 240
Zaph, C 240
Zareie, P 203
Zavan, L 226
Zeigler, S.F 225
Zhan, Y 262
Zhang, B 301
Zhang, H 207
Zhang, S 307
Zhang, W 3
Zheng, D 270
Zheng, S 14
Zhong, Q 212
Zhou, Q.T 307
Zuegg, J 21
Zupan, M 24

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