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WELCOME FROM THE CONFERENCE CO-CONVENORS

Dear Colleagues and Friends,

On behalf of the organising committee, we welcome you to our 12th Lorne Infection & Immunity conference and first hybrid meeting. In the current climate, we are hopeful that in 2022 the I&I community will gather together again in person, and based on the success of the virtual meeting in 2021, we will also offer this format for those unable to visit Lorne. We are delighted by 12 years of support this meeting has received from all of you - delegates, presenters, our invited guests, conference committees, support teams, sponsors and exhibitors. You have all been crucial in establishing this conference as a fixture in the scientific calendar, joining other Lorne Conferences, some of which have been running 40+ years.

Immunologists and microbiologists here in Australia and globally have had another year of intensive work, leading public health responses and development programs for new diagnostics, therapeutics and vaccines for the COVID-19 pandemic underpinned by essential fundamental research. This excellent science has continued, sometimes under the toughest of funding conditions, across the broad diversity of host- and pathogen-related research fields represented by our community's expertise. We look forward to hearing about many examples of this outstanding work at the meeting. We will continue to support these efforts and ask that delegates read our COVID-19 guidelines on the conference website.

The past year has seen a re-structure of the conference's organising committee, aimed at improving the efficiency of our operations and at providing opportunities to committee members for leadership and greater input to the direction and activities of the conference. We now have three subcommittees, detailed on the following pages of this program. The Program Committee, chaired by Prof Ana Traven and Deputy Chair, Prof Begoña Heras, have led the development of the conference's program, selection of invited speakers, presentations from abstracts, and the meeting's overall structure. The Development Committee takes responsibility for its strategic direction, budget and sponsorship and registration targets. The Student and ECR Committee, chaired by A/Prof Justine Mintern and Deputy Chair, Dr Andrew Currie, have developed a new program of activities for the conference's students and ECRs. Thanks go to Dr Laura Cook and Julie Hibbert, the co-chairs of the student and ECR sub-committee, and all members of this team, who have organised the student / ECR pizza night, networking lunch and other activities. All students and ECRs should plan to join in these events. Each of these committees continues to be supported by our Program Manager, Dr Rebecca Smith and ASN's Gemma-Ann Taylor, Jim Fawcett, Nitesh Patel and co. Thanks to you all.

We are also indebted to our sponsors and exhibitors whose support for this field of science enables us to hold this meeting each year. In this difficult economic climate, we are especially appreciative of your investment in this conference. This enables us to keep prices down, to heavily subsidise student registrations and offer Career Development awards to ECRs. Please reciprocate the support of our sponsors by visiting their exhibition booths – in-person or on our virtual platform, attending technical symposia and by buying their quality products and services for your research. We especially thank our major sponsor CSL Ltd, who have loyally supported this conference for all 12 years. We especially acknowledge new committee member, Dr Eugene Maraskovsky from CSL, who has brought a valuable perspective to our decision-making.

The aim of this conference has always been to bring together basic, clinical and translational researchers who examine microbes and their impact on innate or adaptive immunity, who study the mechanisms that regulate immune responses determining the fate of infection, and who apply this knowledge to preventing and treating infections and inflammatory diseases. We have endeavoured to do this while being responsive to emerging trends in our field. The Lorne Infection and Immunity Conference has been a celebration of the highest-quality Australian and international research and we are particularly pleased to continue fostering new collaborations and promoting emerging scientists in our discipline. Thank you all for supporting the Lorne Infection and Immunity conferences of the past decade. It has been a pleasure and honour to get to know so many of you at Lorne.

With best wishes,

Paul Hertzog
Co-Convenor

Heidi Drummer
Co-Convenor

WELCOME FROM THE PROGRAM CHAIR

Dear Colleagues and Friends,

As the inaugural Program Chair for the Lorne Infection and Immunity conference, I am delighted to add my welcome to you to our 12th meeting. On behalf of the Deputy Program Chair, Prof Begoña Heras and the Program Committee, I hope you will enjoy this year's conference.

I would like to begin by acknowledging all the committee members who have worked so hard to develop the program. It has been a pleasure to work with my colleagues Begoña Heras, Cameron Stewart and Michelle Boyle, as well as welcome new members in Matt Johnansen (Centenary Institute), Kimberly Kline (Nanyang Technological University), Si Ming Man (Australian National University), Eugene Maraskovsky (CSL Ltd), Wai-Hong Tam (Walter and Eliza Hall Institute), Makrina Totsika (Queensland University of Technology) and Daniel Utzschneider (Peter Doherty Institute) to the committee. Thanks so much to all of them for the great speaker suggestions and insights that helped us to create an exciting and diverse program.

In developing the program for 2022 we built on the tradition of the Lorne Infection and Immunity conference in bringing together researchers working at the interface of microbiology and immunology, to understand the molecular mechanisms that regulate host-pathogen interactions in infections. We have included a session on clinical challenges to promote discussions between fundamental and clinical scientists, and incorporated some of the major topics that have been driving research in our field, such as antimicrobial resistance, metabolism, vaccines, microbiomes and the new developments in the research of SARS-CoV-2 and COVID-19. We hope that the program will inspire new collaborations and a cross-fertilisation of ideas coming from related but distinct research areas.

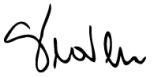
We extend a special welcome to our international speakers and thank them for contributing to the high calibre of this conference. We hope you will have the opportunity to visit Lorne and Australia in person in the future. We encourage all attendees to engage with our international speakers virtually in each session. Students and ECRs – come and meet our invited speakers at Thursday's networking lunch. This is your opportunity to interact with some of the best Infection and Immunity researchers in Australia and internationally.

The Program Committee has also developed a new code of conduct for the conference, as well as guidelines for diversity and inclusion, and social media use. We thank our colleagues from the Lorne Protein meeting for sharing their policies and letting us reuse them and adapt them to our conference. Please take a moment to read these statements on the conference website. The Program Committee specifically, and organising committee generally, will continue to work to make this meeting diverse, accessible, relevant and enjoyable for all participants. We welcome your feedback on any element of the meeting.

It is with mixed feelings that I make the following special announcement. Prof Paul Hertzog has been the co-founder and co-convenor of 12 Lorne Infection and Immunity Conferences and the Victorian Infection and Immunity Network since 2009. At the conclusion of this meeting, Paul will be stepping down from these roles. I think that I can safely say that we all sincerely thank Paul for the enthusiasm, high standards and diligent oversight with which he has led the meeting and network for over a decade. To recognise his foundational role and contribution to the network and conference in perpetuity, I am delighted to announce the establishment of the Hertzog Oration. Each year, the Hertzog Oration will be delivered by an international invited speaker. In 2022, it is a pleasure to host our inaugural Hertzog Orator, Prof Caetano Reis e Sousa. It is our intention that the Hertzog Oration supports ongoing recognition of Paul's contribution to Infection and Immunity, and the development and promotion of the field of innate immunity internationally and in Australia.

It has been a pleasure to chair the Program committee this year and contribute to what I hope will be another great Lorne Infection and Immunity conference. Begoña and I now pass on the baton to Michelle Boyle and Cameron Stewart to chair the Program committee for the 2023 conference and stand ready to help them in any way we can.

Kind Regards,



Ana Traven,
Program Chair

ORGANISING COMMITTEE 2022

CO-CONVENORS

Paul Hertzog Hudson Institute of Medical Research	**Heidi Drummer** Burnet Institute

PROGRAM COMMITTEE

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

STUDENT & ECR COMMITTEE

CHAIR **Justine Mintern,** University of Melbourne **DEPUTY CHAIR** **Andrew Currie,** Murdoch University	**COMMITTEE MEMBERS** **Michael Beard,** University of Adelaide **Nicole Moreland,** The University of Auckland **Greg Moseley,** Monash University **ECR COMMITTEE MEMBER** **Laura Cook,** University of Melbourne **STUDENT COMMITTEE MEMBER** **Julie Hibbert,** University of Western Australia **SUB-COMMITTEE OF STUDENTS AND ECRS** **Laura Cook,** University of Melbourne (Co-Chair) **Julie Hibbert,** University of Western Australia (Co-Chair) **Ebony Monson,** La Trobe University **Rachael Zemak,** Telethon Kids **Eva Hesping,** Walter and Eliza Hall Institute **Kirsty McCann,** Deakin University **Catherine Jia-Yun Tsai,** University of Auckland **Rubaiyea Farrukee,** Peter Doherty Institute

BUSINESS DEVELOPMENT COMMITTEE

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

INVITED SPEAKERS

INTERNATIONAL INVITED SPEAKERS

DR RICHARD ALM, Boston University, *United States of America*
Richard joined CARB-X following 24 years in industry. He spent almost 20 years in multiple infection-based therapeutic R&D teams at AstraZeneca followed by 4 years at a small antibacterial biotech company. He has supported the progression of small molecule compounds from discovery through to late-stage clinical development. He obtained his PhD in molecular microbiology from the University of Adelaide, and prior to joining industry he had two post-doctoral positions in the antimicrobial resistance area, one at the University of Queensland in Australia and one at the University of Victoria in Canada.

A/PROF SANJAY CHOTIRMALL, Lee Kong Chian School of Medicine, *Singapore*
Assoc Prof Chotirmall graduated from the Royal College of Surgeons in Ireland (RCSI) and trained as a clinician-scientist in Respiratory and Critical Care Medicine through a 'Molecular Medicine Ireland Clinician Scientist Fellowship' (MMI-CSF) before establishing a translational respiratory research group at the Lee Kong Chian School of Medicine at NTU Singapore. To date, he has performed key work on endo-phenotyping pulmonary infection, including use of next generation sequencing approaches, in the context of chronic inflammatory respiratory diseases that have led to >130 publications including those in Nature Medicine, the New England Journal of Medicine (NEJM), the Lancet Respiratory Medicine and the American Journal of Respiratory and Critical Care Medicine (AJRCCM). He has been appointed Provost's Chair in Molecular Medicine at NTU Singapore since 2019, is the Assistant Dean (Faculty Affairs) since 2021 and leads "The Academic Respiratory Initiative for Pulmonary Health (TARIPH)", an interdisciplinary national academic initiative that aligns strategic academic expertise across Singapore to benefit Singaporeans with lung disease through research.

PROF ASHRAF IBRAHIM, The Lundquist Institute,
Dr. Ibrahim is a Professor of Medicine at the David Geffen School of Medicine at UCLA, and is based in the Division of Infectious Diseases at Harbor-UCLA Medical Center. He received his B. Sc. in Biochemistry-Microbiology in 1986 from Kuwait University, Kuwait with honors. He then attended Post graduate school at Loughborough University of Technology, UK where he obtained a Ph.D. degree in Microbial Physiology in 1991 under the mentorship of Professors R.J. Stretton and M. A. Ghannoum. Dr. Ibrahim completed his Post doctoral training in Infectious Diseases in the laboratory of Dr. John Edwards, Jr. at Harbor-UCLA Medical Center. Dr. Ibrahim's research focuses on molecular pathogenesis, host-pathogen interactions, immunotherapies, and models of infections to advance the understanding of the pathogenic mechanisms and virulence factors of fungal and bacterial infections and translate this knowledge into novel therapeutic strategies to combat Infectious Diseases. His research is focused on the following areas: 1) The host-pathogen interactions in mucormycosis and the development of rapid diagnostics and novel antibody-based therapy; 2) Unnatural immunity for the development of vaccine strategies that target multidrug resistant organisms including MDR Candida species, and Gram-negative bacteria; 3) Mechanisms of microbial sepsis for development of novel immunotherapies; and 4) Antifungal drug discovery.

A/PROF ILLIYAN ILIEV, Weill Cornell Medical College, Cornell University,
Iliyan Iliev is an Immunologist and an Associate Professor at the Department of Medicine and the Jill Roberts Institute for Research in IBD at Weill Cornell Medical College, Cornell University, New York. He earned his PhD from the European School of Molecular Medicine and the University of Milan and was previously associated with the Tohoku University in Japan, the European Molecular Biology Organization, LB Bulgaricum Plc., Meiji Co., Ltd and the Cedars Sinai Medical Center.

Iliev' s research defined a role of intestinal fungi (the gut mycobiota) in gut mucosal immunity, intestinal and lung inflammation. The laboratory develops and uses computational and experimental approaches to study the role of mycobiota early and later in life, upon therapeutic interventions and during conditions, such as inflammatory bowel disease, allergy, and immunodeficiencies, where fungi contribute to pathologies. Dr. Iliev's was a recipient of the NIH Pathway to Independence K99 Award, CTSI Publication Award, Malaniak Award for Excellence in Research, Irma T. Hirschl Scholar Award and Kenneth Rainin Foundation Innovator Award and was recently named a Burroughs Welcome Fund PATH Investigator.

ASSISTANT PROF CAROLYN KING, University of Basel, Siwtzerland
Carolyn King received her PhD in immunology from the University of Pennsylvania where she studied the role of TRAF6 in T cell tolerance. With support from the HFSP, she then carried out a postdoctoral fellowship in Basel Switzerland where she identified asymmetric cell division as an important mechanism linking T cell receptor signal strength with pathogenic T cell differentiation. In 2016, she became an assistant professor at the Department of Biomedicine, University of Basel. The King lab focuses on understanding the molecular underpinnings of CD4 T cell responses during infection with a focus on T cells that support antibody production by B cells. More recently this work has expanded to investigate how how tissue resident immune cells regulate host responses during pulmonary infection. Through the integration of proteomic, spatial transcriptomic and high dimensional flow cytometry data the lab is investigating how T cells, antibodies and macrophage remodeling intersect to modulate mucosal immunity to tuberculosis.

A/PROF SUZANNE NOBLE, University of California San Francisco, *United States of America*
Suzanne Noble is an Associate Professor of Microbiology & Immunology and Medicine at the University of California San Francisco (UCSF). Following her undergraduate education in Molecular Biophysics and Biochemistry at Yale University, she earned MD and PhD degrees at UCSF, followed by a residency in internal medicine at the Beth Israel Deaconess Hospital, Boston (Harvard Medical School). She returned to UCSF for subspecialty training in infectious diseases and postdoctoral training in medical mycology before joining the faculty there.

The Noble laboratory investigates fungal commensalism and virulence in mammalian hosts. Candida albicans is a yeast component of normal mammalian gut microbiota that is also the source of life-threatening infections in vulnerable hosts. The Noble lab utilizes a forward genetics approach to identify key fungal effectors and regulators of these interactions. C. albicans mutants exhibiting defects in a specific animal model of commensalism or disease are used as tools uncover the molecular mechanisms of these fungal-host interactions, using techniques from biochemistry, molecular biology, cell biology, genetics, systems biology, and other disciplines. This approach has yield surprising insights including the existence of a C. albicans cell type that is specialized for gut commensalism, reciprocal regulation of virulence and commensalism by an iron homeostasis regulatory circuit, and unexpected roles for the major regulators of C. albicans mating and virulence in control of commensal fitness. Dr. Noble's contributions to the field have been recognized with

her election to the American Society for Clinical Investigation (ASCI) and American Society for Microbiology (ASM).

PROF ERIKA PEARCE, Johns Hopkins Bloomberg School of Public Health, *United States of America*

Dr. Pearce obtained her Ph.D. in Cell and Molecular Biology in 2005 at the University of Pennsylvania in Philadelphia, where she studied the regulation of T cell responses during infection. During her postdoctoral studies, also at the University of Pennsylvania, she began her research into how cellular metabolic processes govern immune responses to infection and cancer. She launched her independent career in 2009, holding faculty positions at the Trudeau Institute in NY and then Washington University School of Medicine in St. Louis. She moved her research group to Europe in 2015 to become a Director at the Max Planck Institute for Immunobiology and Epigenetics in Freiburg, Germany. In 2018 she was awarded the Gottfreid Wilhelm Leibniz Prize from the DFG for her work on immunometabolism. In 2021 she became a Bloomberg Distinguished Professor at the Johns Hopkins University in Baltimore. Her work continues to investigate the connection between metabolism and cell function.

MARIA-GRAZIA PIZZA, GSK*, Italy*

Mariagrazia Pizza received her degree in Chemistry and Pharmaceutical Technologies at the University of Naples, Italy. Following a period at the EMBO laboratories in Heidelberg, Germany, Mariagrazia moved to Siena, Italy where she has been ever since, leading many bacterial projects. She has contributed to the discovery of a pertussis vaccine based on a genetically detoxified toxin, able to protect children from disease and to the discovery of new vaccine antigens by genome mining (reverse vaccinology), which are the basis of a new MenB vaccine now licensed in more than 40 countries worldwide. Mariagrazia is currently Senior Scientific Director for Bacterial Vaccines at GSK, and Preclinical Head at GVGH, the GSK Vaccine Institute for Global Health. She has received many scientific awards and is elected member of EMBO, of the European Academy of Microbiology and Academia Europaea, Fellow of the American Academy of Microbiology and Vice President of IUMS (International Union of Microbiology Societies). Mariagrazia is also Honorary Visiting Professor at the Imperial College (UK). She has over 200 publications and is co-inventor of many patents.

PROF CAETANO REIS E SOUSA, Francis Crick Insitute, *United Kingdom*

Caetano Reis e Sousa obtained a BSc Hons in Biology in 1989 from Imperial College London, and a DPhil in Immunology in 1992 from the University of Oxford. He subsequently trained as a postdoctoral fellow at the National Institutes of Health, USA, with Ron Germain. In 1998 he joined the Imperial Cancer Research Fund, later re-named Cancer Research UK London Research Institute, where he headed the Immunobiology Laboratory. His laboratory became part of the Francis Crick Institute in 2015. He is currently a senior group leader and assistant research director at the Crick and head of the Immunobiology Laboratory. He is also Professor of Immunology in the Department of Medicine at Imperial College London and holds honorary professorships at both University College London (UCL) and King's College London. Caetano's contributions have been widely recognised and he is included in the list of Highly Cited Researchers (Thomson Reuters) and has won the BD Biosciences Prize of the European Macrophage and Dendritic Cell Society (2002), the Liliane Bettencourt for Life Sciences Award (2008), the Award for Excellence in Basic/Translational Research from the European Society for Clinical Investigation (2011), the Louis-Jeantet Prize for Medicine (2017) and the Bial Award in Biomedicine (2019).

He is a fellow of the Royal Society (elected 2019), fellow of The Academy of Medical Sciences (elected 2006), a member of the European Molecular Biology Organisation (EMBO; elected 2006) and was made an Officer of the Order of Sant'Iago da Espada by the government of Portugal, his home country, in 2009.

NATIONAL INVITED SPEAKERS

DR JEREMY BARR, Monash University, School of Biological Sciences, *VIC*
Jeremy completed his PhD in microbiology at The University of Queensland in 2011. He then moved to San Diego, USA to complete a postdoctoral position with Prof. Forest Rohwer at San Diego State University. While there he studied the interactions of bacteriophage with mucosal surfaces and was involved in a word-first phage therapy case treating a patient with disseminated, multidrug-resistant infection. In 2016, he joined Monash University's School of Biological Sciences where he is currently a Senior Lecturer and Group Leader. His research group studies bacteriophages and their tripartite interactions with their bacterial hosts and the human body. In 2020, he joined the Centre to Impact AMR where he leads translational phage therapy work.

DR JUSTIN BEARDSLEY, University of Sydney, *NSW*
Dr Beardsley is an infectious disease specialist and clinical researcher, based at the University of Sydney's Institute for Infectious Diseases since 2017. He spent six years in Vietnam researching fungal infections, where his multi-national randomised clinical trial into adjunctive steroid therapy for HIV-associated cryptococcal meningitis (Beardlsey et al, NEJM 2016) revealed that steroids were harmful. He is active in researching the epidemiology of fungal infections and trends in antifungal drug resistance, taking a One Health approach. He is a co-author on the Lancet Global Burden of Disease series in his capacity as GBD specialist on Vietnam and HIV and a contributor to international guidelines on treatment of fungal infections. He is currently leading a WHO multi-criteria decision analysis project to determine a fungal priority pathogen list for fungi.

A/PROF LEA-ANN KIRKHAM, Telethon Kids Institute, *WA*
A/Prof. Lea-Ann Kirkham is an infectious disease microbiologist and Co-Director of the Wesfarmers Centre of Vaccines and Infectious Diseases at the Telethon Kids Institute in Perth, Western Australia. She leads the Bacterial Respiratory Infectious Disease Group, with a mission to develop improved therapies to significantly reduce the global burden of childhood ear and lung diseases. A/Prof. Kirkham is leading an NHMRC-funded first-in-human trial to evaluate the safety and tolerability of a novel bacterial therapy that she is developing to prevent ear (and possibly) lung infections. She is also an investigator on a clinical trial to prevent repeat grommet surgery and leads Industry-funded vaccine studies to develop the best approach to protect high-risk infants from respiratory tract infections.

A/PROF KATE QUINLAN, University Of New South Wales, *NSW*
Kate Quinlan is a Scientia Associate Professor at the School of Biotechnology and Biomolecular Sciences, UNSW Sydney. She received her PhD from the University of Sydney in 2006 and, following postdoctoral appointments at the Children's Hospital at Westmead and the University of Cambridge, established her research group at UNSW Sydney in 2014. Having spent her research career studying gene regulation and metabolism, she has combined these interests in her current program of research. Along with a dedicated team of PhD students and honours students, Kate is exploring how adipose tissue resident immune cells, in particular

eosinophils, play a role in the regulation of thermogenic beige fat. She hypothesises that signalling between immune cells and adipocytes may be able to be manipulated to drive adipose tissue beiging and weight loss.

DR KYLIE QUINN, RMIT*, VIC*

Kylie Quinn is a Vice-Chancellor's Research Fellow at RMIT University, where she heads the Ageing and Immunotherapies Group. After a PhD in New Zealand, Dr Quinn undertook post-doctoral roles at the Vaccine Research Center/NIH, the University of Melbourne and Monash University, building her expertise in T cell biology, vaccine design and ageing. With a move to RMIT in 2019, she established a research program exploring the impact of ageing on T cell immunity, with the aim of improving immune-based therapies for older patients. One such therapy is chimeric antigen receptor T cell therapy (CTT) for haematological cancers. It can achieve remarkable remission rates, but it can be more variable for older patients, as T cell population composition and quality shifts, leading to deficits in activation and expansion. Her work aims to identify the basis of these deficits and to reduce these barriers, to tailor CTT for older individuals.

PROF SUDHA RAO, QIMR Berghofer, Gene Regulation And Translational Medicine Laboratory, *QLD*

Professor Sudha Rao has extensive experience in transcriptional biology and genomic technologies that spans both pharmaceutical and academic settings. The primary focus of Sudha's research group has been to unravel complex epigenetic-signatures in the immune system, as well as to understand the deregulatory mechanisms operating in cancer settings as well as the role of epigenetic regulation in viral infection. She has obtained her BSC (Hons.) degree at Keele University, UK. PhD from the University of London, Kings College in 2000. During this period, she joined a team of scientists at Rhone Poulenc/Sanofi Pharma, both in UK and France. During this time, she was part of one of the first groups world-wide to establish the clinical genomics platform for therapeutics in the UK. She has developed close partnerships with global technology companies and established novel liquid biopsy clinical platforms, first of its kind in Asia, for non-invasive tracking of blood samples from cancer patients. She has attracted highly competitive NHMRC, ARC and commercial funding to advance her cancer work. Sudha's work has yielded national and international patents for both novel diagnostics and therapeutics in the emerging arena of immune-oncology as well as novel therapeutics and diagnostics for viral infections. Rao has a 20-year track record in translational epigenetics in both academia and industry, where she has led drug development and biomarker discovery projects to take novel therapeutics from bench to clinic. Her work has led to translational outcomes, an international patent portfolio, and EpiAxis Therapeutics, of which she is founder, director, and CSO (until commencing at QIMRb, 2020), with a successful completed clinical trial.

PROF LEANN TILLEY, University Of Melbourne, *VIC*

Leann Tilley is Professor of Biochemistry and Pharmacology, at the Bio21 Institute, University of Melbourne. Leann was awarded the Georgina Sweet Australian Laureate Fellowship from the Australian Research Council (2015-2020), to measure and model malaria parasites. Leann's laboratory applies techniques, such as structural cryoEM, X-ray crystallography and chemical biology, to identify the malaria parasite's vulnerabilities. She is interested in the action of and resistance to antimalarial drugs; and is working with industry partners to design better antimalarials. She believes that the development of drugs for diseases that affect patients who can't afford expensive treatments, requires radical new approaches, involving Academic/ Private/ Public partnerships. She would like to be part of the exciting developments in these areas.

A/PROF CHRIS TONKIN, WEHI, *VIC*

Chris Tonkin is an Associate Professor and Laboratory Head at The Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia. Chris has worked on Plasmodium falciparum, Toxoplasma gondii and has recently branched out into Cryptosporidium. His lab's interests lie in understanding host-parasite interactions and how parasites sense environmental cues to activate stage transition and lifecycle progression.

PROF DAVID TSCHARKE, The Australian National University, *ACT*

David Tscharke is a virologist who moonlights as an immunologist, or vice versa (depending on your point of view). He has diverse interests from antigen presentation to CD8+ T cells, to herpes simplex virus (HSV) latency and application of CRISPR/Cas9 to engineering DNA viruses. He got to this point of confusion via a PhD in from University of Adelaide on HSV pathogenesis and CD8+ T cell immunity and postdoctoral experience at the University of Oxford (where he swapped herpes for pox), the US National Institutes of Health, and QIMR in Brisbane. Since 2006 he has been at The Australian National University (ANU) and has had several positions with varying responsibility for research and teaching. Throughout his career he has had the benefit of working with excellent mentors, generous collaborators and brilliant students. With their help he has held a succession of fellowships and is currently Head of the Division of Immunity, Inflammation and Infection at the John Curtin School of Medical Research, ANU.

HERTZOG ORATION

Professor Paul Hertzog has been the co-founder and co-convenor of the Victorian Infection and Immunity Network since 2009 and the Lorne Infection and Immunity Conference co-convenor since 2011, overseeing all 12 of the Lorne meetings.

After over a decade steering VIIN and the Lorne Infection and Immunity Conferences, Paul will be stepping down as co-convenor of both initiatives at the conclusion of the 2022 meeting.

To acknowledge Paul's foundational role and contribution to the network and conference in perpetuity, we have established the Hertzog Oration at the Lorne I&I conference from 2022 onwards. The Hertzog Oration will be delivered by the international invited speaker whose research is mostly closely aligned to Paul's, to support ongoing recognition of his contribution to I&I and to help develop and promote the field of innate immunity internationally and in Australia.

We are delighted that the inaugural Hertzog Orator is Prof Caetano Reis e Sousa, Francis Crick Institute, UK.

More information: https://www.viin.org.au/news/the-hertzog-oration

The Hertzog Oration is one of two prestigious orations at the Lorne Infection and Immunity Conference, with the Hartland Oration recognising the other founding co-convenor of VIIN, Prof Elizabeth Hartland.

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THE ORGANISERS – ASN EVENTS

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WHAT YOUR REGISTRATION INCLUDES

All Delegate and Student registrations include:

- Access to the the virtual platform, Pheedloop
- Access to presentations and conference content for 12 months post conference
- Live chat function enabling interaction with speakers, delegates and exhibitors on Pheedloop
- Virtual Social Functions for Students

Those who will be in Lorne have additional access to:

- All sessions with face-to-face speakers
- Exhibition Hall
- Morning and afternoon teas and lunch on Thursday
- Drinks at the Poster Sessions
- Evening Social Functions (at an additional cost)

COVID-19 Guidelines

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

Please complete your pre-arrival and post-arrival declarations and take a moment to read all guidelines here.

Complete the **pre-arrival declaration** in the 24 hours prior to arriving in Lorne:
https://forms.gle/7cofMxZF6nWoFtPK8

Complete the **post-arrival declaration** within 12 hours of arriving in Lorne:
https://forms.gle/ATCrfSEG8VKew5y7A

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

Science Bite Presentations

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

Face-to-face Presentations

Presenters for the first poster session (Wednesday, 16th February at 8:00pm) will display their poster at the start of the conference and will remove them after morning tea on Thursday.

Presenters for the second poster session (Thursday, 17th February at 8:00pm) will display their poster from lunch on Thursday until morning tea on Friday.

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

Virtual Presentations

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.

SOCIAL FUNCTIONS

Student Dinner – Pizza Night

The Heriatage Room or Seagrass Lawn (weather permitting), Mantra Lorne
SpatialChat for virtual participation - https://spatial.chat/s/lorneii2022
Wednesday, 16th February, 6:20pm – 8:00pm

General Delegate Dinner

Lorne Central, 54-56 Mountjoy Pde, Lorne
Wednesday, 16th February, 6:20pm – 8:00pm

Student and ECR Networking Lunch

The Heriatage Room or Seagrass Lawn (weather permitting), Mantra Lorne
Thursday 17th February, 11:50AM - 1:00PM

Student and ECR Virtual Networking Function

SpatialChat - https://spatial.chat/s/lorneii2022
Thursday 17th February, 11:50AM - 1:00PM

Conference Dinner
Seagrass Lawn, Mantra Lorne
Thursday 17th February, 5:50pm – 8:00pm

Morning and afternoon teas, the general delegate lunch on Thursday and Poster Sessions will be held in the Exhibition Centre.

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

- Abcam - One free antibody or kit of your choice, to the value of $750 AUD
- Bio Strategy - $100 Westfield voucher and one of our branded brink bottles that has a Bluetooth speaker
- BMG LABTECH - $100 from the Bush gift voucher
- Cytek Biosciences - $50 voucher
- Genesearch - $50 gift card
- Miltenyi Biotec – Miltenyi prize pack (including some of our promotional gear and a $100 Amazon gift card)
- Mimotopes - $100 voucher for their online store
- MP Biomedicals – $50 gift card
- New England Biolabs – Bluetooth Speaker
- Transnetyx - $50 gift card
- TrendBio - $50 Coles/Myer Voucher

VIRTUAL EXHIBITOR PRIZE DRAW
Virtual delegates have the chance of winning a $100 gift card. The winner will be the virtual delegate who maximises their interaction with the virtual booths and company representatives. Visit all of the booths, access the resources, and chat to staff to increase your chance of winning. The winner will be announced in the last session on Friday, 18th February.

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Wednesday 16th February 2022

Session 1: Plenary 1 and Welcome Address

1:00PM - 2:00PM Heritage Ballroom

Chair: Ana Traven *(Monash University)*

Leann Tilley *(University of Melbourne, Melbourne)*
Hijacking proteostasis: new strategies for antimalarial drug development abs# 1

Session 2: Host-Pathogen interactions: Microbial evasion

2:00PM - 3:35PM Heritage Ballroom

Chairs: Antje Blumenthal *(University of Queensland)* & Thomas Naderer *(Monash University)*

Suzanne Noble *(University of California San Francisco)*
Deep tissue infection by an invasive human fungal pathogen requires novel lipid-based suppression of the IL-17 response abs# 2

Robson Kriiger Loterio *(Department of Microbiology and Immunology)*
Coxiella burnetii effector MceF employs the host protein GPX4 to protect mitochondria against oxidative stress-induced cell death pathways abs# 3

Natalie A Borg *(RMIT University)*
TRIM25 and DEAD-box RNA helicase DDX3X cooperate to regulate RIG-I-mediated antiviral immunity abs# 4

Ulrike Kappler *(School of Chemistry & Molecular Biosciences, The University of Queensland)*
Bacterial self defence – *Haemophilus influenzae* S-oxide reductases as determinants of successful host-pathogen interactions abs# 5

Chris Tonkin *(Walter & Eliza Hall Institute of Medical Research)*
Mechanisms of Survival of Latent Toxoplasma abs# 6

Afternoon Tea

3:35PM - 4:15PM Convention Centre

The conference acknowledges the support of:

Session 3: Science Bites 1

4:15PM - 4:45PM Heritage Ballroom

Chairs: Thomas Naderer *(Monash University)* & Makrina Totsika *(Queensland University of Technology)*

Thomas Ashhurst *(University of Sydney)*
Comprehensive mapping of innate and adaptive immune response dynamics across the blood and respiratory tract in COVID-19 abs# 51

Katherine R Balka *(Monash Biomedicine Discovery Institute, Monash University)*
Illuminating intracellular STING trafficking abs# 52

Ali Delbaz *(Griffith University)*
Chlamydia pneumoniae infects the brain via olfactory and trigeminal nerves and triggers

Alzheimer's disease pathologies abs# 53
Tirta (Mario) Djajawi *(Hudson Institute of Medical Research)*
A1 limits pathogen-induced macrophage and monocyte cell death and inflammatory responses abs# 54
William Gilmore *(La Trobe University)*
Differential immune detection of *Bacteroides fragilis* bacteria and their secreted outer membrane vesicles abs# 55
Gemma E Hartley *(Monash University)*
Durability of B-cell memory to SARS-COV-2 infection and vaccination abs# 56
Julie Hibbert *(University of Western Australia)*
Proteomics biomarker discovery for the early diagnosis of neonatal sepsis abs# 57
Patrick Hien-Neng PHN Kao *(Nayang Technological University)*
Polymicrobial infection interferes pathogen-specific neutrophil responses and impairs bacterial clearance. abs# 58
Emma McHugh *(University of Melbourne)*
Nonsense-mediated decay is highly divergent in the malaria parasite *Plasmodium falciparum* abs# 59
Khayriyyah Mohd Hanafiah *(Macfarlane Burnet Institute)*
Dimeric IgA as a novel biomarker of acute measles infection abs# 60

Session 4: Host-Pathogen Interactions: Immune attack

4:45PM - 6:20PM Heritage Ballroom
Chairs: Justine Mintern *(University of Melbourne)* & Meredith O'Keeffe *(Monash University)*

David Tscharke *(Australian National University)*
A human turn of the TAP to more faithfully model herpes simplex virus infection abs# 7
Sophie A Valkenburg *(University of Hong Kong)*
Antibody immunity to SARS-CoV-2 by infection and vaccination abs# 8
Demetra SM Chatzileontiadou *(La Trobe University)*
Unravelling the features of T cell Response to a dominant HLA B57-Restricted Gag epitope in HIV+ Controllers and non-Controllers abs# 9
Daniel HD Gray *(Walter & Eliza Hall Institute of Medical Research)*
Caspase-8 has dual roles in regulatory T cell homeostasis that balance immunity to infection and inflammatory pathology. abs# 10
Caetano Reis e Sousa *(The Francis Crick Institute)*
Regulation of immunity to infection by emergency cDCpoiesis abs# 11

Student Dinner

6:20PM - 8:00PM Heritage Room

General Delegate Dinner

6:20PM - 8:00PM Lorne Central

Poster Session 1 and Exhibition Launch

8:00PM - 9:30PM Convention Centre

Science Bite presenters from Session 3 and odd numbered poster presenters will be available at the Mantra and online for discussions during this session.

Thursday 17th February 2022

Session 5: Plenary 2 and Hartland Oration

8:30AM - 9:45AM Heritage Ballroom

Chair: Paul Hertzog *(Hudson Institute of Medical Research)* & Kate Lawlor *(Hudson Institute of Medical Research)*

Ebony A Monson *(La Trobe University)*
Analysis of the Dynamics and Composition of Lipid Droplets During Viral Infection abs# 12
Michael F Duffy *(University of Melbourne)*
Bromodomain proteins are essential regulators of gene expression and chromatin in *Plasmodium falciparum* abs# 13
Erika Pearce *(Johns Hopkins Bloomberg School of Public Health)*
Mitochondrial Shape Shifting in the T Cell Response abs# 14

Morning Tea

9:45AM - 10:15AM Convention Centre

Session 6: The big picture: metabolism and systems biology in Infection & Immunity - Concurrent Session I

10:15AM - 11:50AM Heritage Ballroom

Chairs: Ana Traven *(Monash University)* & Kate Lawlor *(Hudson Institute of Medical Research)*

Kate Quinlan *(University of New South Wales)*
Eosinophils in Adipose Tissue Thermogenesis abs# 15
Kylie Quinn *(RMIT)*
Metabolic adaptations in ageing T cells abs# 16
Katrina J Binger *(La Trobe University)*
Development of a 3D cell culture model to study macrophage activation and metabolism abs# 17
Katherine Martin *(Walter & Eliza Hall Institute of Medical Research)*
CD98 controls the metabolic flexibility of low-density neutrophils mobilized by G-CSF abs# 18
Jack Adderley *(RMIT)*
Deconvoluting host cell phosphorylation pathways during infection with a network-based modelling approach abs# 19

Session 7: Clinical challenges, translational solutions - Concurrent Session II

10:15AM - 11:50AM Heritage Room

Chairs: Richard Ferrero *(Hudson Institute of Medical Research)* & Claudia Nold *(Hudson Institute of Medical Research)*

Lea-Ann Kirkham *(Telethon Kid's Institute)*
Targeting host-microbial interactions to develop therapies to prevent and treat otitis media abs# 20
Johanna K Ljungberg *(University of Queensland)*
WNT signalling shapes inflammatory responses in patients with sepsis abs# 21
Richard M Lucas *(University of Queensland)*
The transmembrane TLR adaptor SCIMP is a spatiotemporal Erk1/2 scaffold to drive pro-inflammatory responses in macrophages abs# 23
Isaac M Barber-Axthelm *(University of Melbourne)*
Vδ2+Vγ9+ T-cells downregulate CCR6 following phosphoantigen-driven in vivo expansion in pigtail macaques (Macaca nemestrina) abs# 22

Justin Beardsley *(University of Sydney)*
Antifungal resistance – shaping the research agenda abs# 24

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

The conference acknowledges the support of: CSL

Student and ECR Networking Lunch
11:50AM - 1:00PM Heritage Room

The conference acknowledges the support of: QIMR Berghofer Medical Research Institute

Student and ECR Virtual Networking Function
11:50AM - 1:00PM SpatialChat

The conference acknowledges the support of: QIMR Berghofer Medical Research Institute

Session 8: COVID-19: What is new?
1:00PM - 2:45PM Heritage Ballroom
Chairs: Cameron Stewart *(CSIRO)* & Stephanie Gras *(La Trobe University)*

Ashraf Ibrahim *(The Lundquist Institute)*
Mucormycosis in the COVID-19 pandemic era abs# 25
Sudha Rao *(QIMR Berghofer Medical Research Institute)*
Unravelling novel epigenetic mechanisms underpinning SARS-CoV-2 infection and implications for novel epi-therapies abs# 26
Louise Rowntree *(Peter Doherty Institute for Infection and Immunity, University of Melbourne)*
SARS-CoV-2 infection in children does not necessitate establishment of adaptive SARS-CoV-2-specific immunological memory abs# 27
Aaron M Brice *(CSIRO)*
ILRUN downregulates ACE2 expression and blocks infection of human cells by SARS-CoV-2 abs# 28
Daniel Harari *(The Weizmann Institute of Science)*
SARS-CoV-2 suppresses IFNβ production mediated by NSP1, 5, 6, 15, ORF6 and ORF7b but does not suppress the effects of added interferon abs# 29

Session 9: Science Bites 2
2:45PM - 3:15PM Heritage Ballroom
Chair: Justine Mintern *(University of Melbourne)*

Paris Papagianis *(RMIT)*
Stromal cell heterogeneity in the lungs supports epithelial cell regeneration abs# 61
Matthew D Prokop *(University of New South Wales)*
A new in vitro colon simulating model to examine *Candida albicans* in the presence of human GI microbiota. abs# 62

Rachel F Rollo *(University of Queensland)*
Wollamide cyclic hexapeptides synergise with tuberculosis antibiotics to inhibit growth of *Mycobacterium tuberculosis* abs# 63
Vinod Sundaramoorthy *(CSIRO)*
Investigating the trans-synaptic transfer mechanism of rabies virus. abs# 65
Yulin Wang *(Griffith University)*
STING is a critical sensor in CD4+ T cells during malaria that stimulates type 1 IFN production, that in turn, promotes the development of IL-10-producing Th1 (Tr1) cells. abs# 67
Scott Williams *(La Trobe University)*
Identification and characterisation of the pH-dependent antifungal saltwater crocodile defensin CpoBD13 abs# 68
Remy B Young *(Hudson Institute of Medical Research)*
Characterising the functional diversity of the gastrointestinal microbiota abs# 69
Ming Z. M. Zheng *(Peter Doherty Institute)*
Single-cycle influenza virus vaccine generates lung CD8+ Trm with a diverse TCR repertoire that preclude the emergence of virus escape mutants abs# 70

Afternoon Tea

3:15PM - 4:15PM Convention Centre

Session 10: Immunity and vaccines

4:15PM - 5:50PM Heritage Ballroom
Chairs: Eugene Maraskovsky *(CSL)* & Nicole Moreland (*University of Auckland)*

This conference acknowledges the support of:

Joanna R Groom *(Walter & Eliza Hall Institute of Medical Research)*
Interplay of type I and II IFN for the generation of CD8+ Stem-like memory T cells abs# 30
Gautham R Balaji *(Monash University)*
Recognition of host Clr-b by the inhibitory NKR-P1B receptor provides a basis for missing-self recognition abs# 31
Nicole K Campbell *(Hudson Institute)*
Interferon epsilon limits ovarian cancer metastasis via tumour-extrinsic mechanisms abs# 32
Carolyn King *(University of Basel)*
CD4 T cells Sans Frontières: Division of labor in the lung abs# 33
Mariagrazia Pizza *(GSK Vaccines)*
Synergistic activity of antibodies in multivalent vaccines abs# 34

Conference Dinner

5:50PM - 8:00PM Seagrass Lawn

Poster Session 2

8:00PM - 9:30PM Convention Centre

Science Bite presenters from Session 9 and even numbered poster presenters will be available at the Mantra and online for discussions during this session.

Friday 18th February 2022

Session 11: Antimicrobial Resistance: mechanisms and therapies

8:30AM - 10:15AM Heritage Ballroom

Chairs: Megan Lenardon *(University of New South Wales)* & Erin Price *(University of the Sunshine Coast)*

Richard A Alm *(CARB-X)*
CARB-X after 5 years – lessons learned and future visions abs# 35
Tim Barnett *(Telethon Kids Institute)*
Host-dependent resistance of Group A Streptococcus to antifolate antibiotics abs# 36
Jilong Qin *(Queensland University of Technology)*
Dismantling blaKPC carbapenemase resistance: inhibiting the DsbA protein restores carbapenem susceptibility in KPC carbapenem-resistant bacteria. abs# 37
Coralie Boulet *(Burnet Institute)*
Investigating the mode of action of three compounds that block egress and invasion of malaria parasites abs# 38
Jeremy Barr *(Monash University)*
Phage-antibiotic combination is a superior treatment against multi-drug resistant *Acinetobacter baumannii* abs# 39

Morning Tea

10:15AM - 10:45AM Convention Centre

Session 12: Microbiomes, microbes and other hot topics

10:45AM - 12:30PM Heritage Ballroom

Chair: Philip Hansbro *(Centenary Institute)* & Matt Johansen *(Centenary Institute)*

Sanjay H Chotirmall *(Nanyang Technological University)*
Translating lung "microbiomics" into clinical application for sgronic respiratory desease abs# 40
Iliyan Iliev *(Cornell University)*
Immunity, IBD and the gut-brain axis through the prism of functional mycobiota exploration abs# 41
Sonja Frolich *(University of Adelaide)*
PfCERLI1, a novel rhoptry associated protein essential for rhoptry discharge and Plasmodium falciparum merozoite invasion of erythrocytes abs# 42
Tinashe G Chabikwa *(QIMR Berghofer Medical Research Institute)*
Single cell transcriptomics provides insights into innate and adaptive immune responses to malaria abs# 43
Tiana Koukoulis *(University of Melbourne)*
Microbiota-derived outer membrane vesicles and their proinflammatory role in Parkinson's disease progression abs# 44
Christopher McDevitt *(University of Melbourne)*
Breaking antibiotic resistance in *Streptococcus pneumoniae* abs# 45

Closing Remarks, Prizes, Awards and Photos

12:30PM - 1:00PM Heritage Ballroom

POSTER LISTING

Science Bite presenters from Session 3 and odd numbered poster presenters will be available at the Mantra and online for discussions during the poster session on Wednesday.

Science Bite presenters from Session 9 and even numbered poster presenters will be available at the Mantra and online for discussions during the poster session on Thursday.

Those posters with an * will be viewable on the virtual platform.

Posters are also available for viewing on the virtual platform for 12 months.

***Jacinta R Agius**
Investigating the immune control of herpesvirus infection in marine molluscs abs# 101

***Sultan Alanazi**
Tryptase regulates the epigenetic modification of core histones in mast cell leukemia cells abs# 102

***Marina R Alexander**
Restricted translation of antiviral cytokines and transcription factors by SARS-CoV-2. abs# 103

Jeremy Anderson
Preterm infants exhibit a reduced pro-inflammatory immune response to RSV abs# 104

***Dionne C. Argyropoulos**
Molecular surveillance of asymptomatic *Plasmodium falciparum* malaria in high-transmission regions in the context of interventions abs# 105

***Daniel Arsovski**
Cytomegalovirus infection drives an atypical human Vδ2+ T cell compartment abs# 106

***Stefanie M Bader**
Dissecting the role of programmed cell death and inflammation in SARS-CoV-2 infection in vivo abs# 108

***Miguel A Berrocal-Rubio**
Programmable macrophages understanding the role of nrg1 and macrophage plasticity through synthetic biology abs# 110

***Sneha Biniwale**
New insights into the immunological roles of macrophages adjacent to the rete testis and tunica albuginea abs# 111

Rob Bischof
Pathophysiological changes in a large animal model of COPD abs# 112

***Natalie Bitto**
Methods of vesicle production and quantification can influence immunological outcomes. abs# 113

***Rhiannon A Bolton**
Tissue-specific contributions of WNT production and signalling to cytokine and chemokine responses during *E. coli* infection abs# 114

Michelle Boyle
Targeting T follicular helper cells to improve immunity to malaria abs# 115

***Michaela Bulloch**
Apicoplast derived metabolites are essential for the biosynthesis of glycophosphatidylinositol anchors and egress of asexual stage *Plasmodium falciparum.* abs# 116

***Lauren H. Carlton**
Charting elimination in the pandemic: a SARS-CoV-2 serosurvey of blood donors in New Zealand abs# 117

***Jessie J-Y Chang**
Long-read RNA sequencing identifies polyadenylation elongation and differential transcript usage of host transcripts during SARS-CoV-2 in vitro infection abs# 118

***Priyanka PC Chevour**
Inflammatory bowel disease remodels circulating and tissue-resident populations of human Vd1+ T cells. abs# 119

***Julia G Chitty**
Inhaled pirfenidone reduces transforming growth factor-beta-enhanced lung viral infection and inflammation as effectively as oral pirfenidone abs# 120

***Jarny Choi**
Benchmarking transcriptional profiles of immune cells online at Stemformatics abs# 121

***Michelle Chonwerawong**
Specific gut microbiota-epithelial cell interactions induces the unfolded protein response and ER stress pathways abs# 122

***Sharon L Clark**
Australian Aboriginal children with otitis media produce high avidity serum IgG to potential non-typeable *Haemophilus influenzae* vaccine antigens at lower titres when compared to non-Aboriginal children abs# 123

***Allison Clatch**
Whole-body analysis of tissue-resident immune cells abs# 124

***Bliss A Cunningham**
Developing antimicrobial zinc ion embedded polymers for One Health applications abs# 125

***Destiny Dalseno**
Novel fluorescent TNF reporter systems for characterisation of TNF expression abs# 126

***Madeline Dans**
The sulfonylpiperazine MMV020291 prevents red blood cell invasion through interference with actin-1/profilin dynamics in the malaria parasite *Plasmodium falciparum* abs# 127

Sophia Davidson
PKR is a sensor of proteotoxic stress via accumulation of cytoplasmic IL-24 abs# 128

***Samantha Davis**
The good, the bad and the ugly: The functional IgA response in convalescent COVID-19 patients. abs# 129

Caleb A Dawson
Uncovering resident macrophages in the mammary gland by 3D and intravital imaging abs# 130

***Eveline D de Geus**
Interferon ε as a novel regulator of intestinal homeostasis abs# 131

***Habtamu B Derseh**
The efficacy and safety of pinocembrin in a sheep model of bleomycin-induced pulmonary fibrosis abs# 132

***Desmarini Desmarini**
Developing inhibitors against fungal inositol polyphosphate kinases (IPK) as a novel class of antifungal drug abs# 133

***Bethany Bowring**
The calcineurin and TOR signaling pathways are critical for mitigating stress caused by phosphate overload in invasive fungal pathogens abs# 134

***Marcel Doerflinger**
Functional overlap of different cell death pathways ensures host protection against intracellular bacterial pathogens abs# 135

***Leslie C Domínguez Cadena**
Rab6b localises to the Golgi complex in murine macrophages and promotes TNF release in response to mycobacterial infection abs# 136

***Nicholas Dooley**
Activation of cytotoxic Vγ9Vδ2 T cells during primary *Plasmodium falciparum* infection abs# 137

***Mariam Doualeh**
Rapid multiplex PCR to detect and characterise polymicrobial sepsis abs# 138

***Yianni Droungas**
Targeted HBeAg seroclearance in a chronic-like HBV mouse model using chimeric bionanoparticles. abs# 139

***Salimeh Ebrahimnezhaddarzi**
HCV infection induces prolonged inflammation and elevated chronic disease risk biomarkers even after curative direct-acting antiviral treatment abs# 140

***Mahtab ME Eivazitork**
Screening of a panel of FDA-approved drugs to identify CCL17-inhibiting candidates abs# 141

***Zahra Elahi**
An integrated transcriptional atlas of human dendritic cells abs# 142

***Vik Ven Eng**
RIPK1/3 regulates T cell responses in mucosal defence against bacterial gut infection abs# 143

***Sven Engel**
Molecular mechanisms of apoptosis induction during *Salmonella* Typhimurium infection abs# 144

***Ryan Farr**
Circulating microRNA biomarkers enable accurate identification of COVID-19 patients abs# 146

***Ryan Farr**
Rabies against the machine: using artificial intelligence to find cellular and exosomal microRNA signatures of lyssavirus infection in a human stem-cell derived neural culture abs# 147

***Rubaiyea Farrukee**
Exploring TRIM proteins as targets for broad spectrum antivirals abs# 148

Kirsty R Field
Programming Vγ9Vδ2 T cells for HIV immunotherapies abs# 149

***Cheng Xiang Foo**
The GPR183/oxysterol axis controls macrophage infiltration and inflammation in the lung during influenza infection and SARS-CoV-2 infection abs# 150

***Jiyao Gan**
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ORAL ABSTRACTS

1

Hijacking proteostasis: new strategies for antimalarial drug development

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As an organism that undergoes rapid growth and cell division, the malaria parasite is very reliant on two major cellular homeostasis pathways: protein translation and proteasomal degradation.

The *Plasmodium falciparum* proteasome is a potential antimalarial drug target for compounds with activity against multiple life cycle stages. Starting from inhibitors developed to treat cancer, new derivatives were designed and synthesized with the aim of increasing potency against the *Plasmodium* proteasome and decreasing activity against the human enzyme. Biochemical and cellular assays identified compounds that exhibit selectivity and potency at different stages of the parasite's lifecycle. We demonstrated curative oral efficacy in the *P. falciparum* SCID mouse model. Cryo-electron microscopy revealed the new inhibitors bind in a hydrophobic pocket that is structurally different in the human proteasome – underpinning their selectivity.

We have also identified a series of nucleoside sulfamates from phenotypic screening that exhibit high potency against *P. falciparum* blood stage cultures, high selectivity against mammalian cell lines and long half-lives in blood. An exemplar compound demonstrates multi-stage activity and oral efficacy in the *P. falciparum* SCID mouse model. Using *in vitro* evolution of resistance, we have identified the target as *P. falciparum* tyrosyl-tRNA synthetase (*Pf*Tyr-RS). *Pf*Tyr-RS catalyses the formation of a highly stable inhibitory sulfamate conjugate, via a mechanism we call reaction-hijacking. Enzyme kinetics and X-ray crystallographic studies of plasmodium and human Tyr-RS reveal that differential flexibility of a loop over the catalytic site determines differential susceptibility to inhibition by nucleoside sulfamates. The work points to the potential for the design of bespoke nucleoside sulfamates, with tuneable specificity for applications in a broad range of infectious diseases.

2

Deep tissue infection by an invasive human fungal pathogen requires novel lipid-based suppression of the IL-17 response

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Candida albicans is the most common cause of human fungal infection, but the mechanisms of invasive pathogenesis remain poorly defined. Here we identify an unexpected mechanism: lipid-mediated immunosuppression. Through forward genetics, we found that *C. albicans* secretes a lipase, Lip2, that is critical for invasive disease. Murine infection with *C. albicans* strains that lack Lip2 display an exaggerated host IL-17 response that leads to fungal clearance from solid organs and host survival. IL-17 signaling is required for Lip2 action. The lipase activity of Lip2 inhibits IL-17 production indirectly through suppression of IL-23 production by tissue resident dendritic cells. We conclude that *C. albicans* suppresses antifungal IL-17 defense in solid organs by altering the tissue lipid milieu.

3

Coxiella burnetii effector MceF employs the host protein GPX4 to protect mitochondria against oxidative stress-induced cell death pathways

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Coxiella burnetii is the causative agent of Q fever in humans. The bacterium is highly adapted to infect alveolar macrophages and subvert their functions. Its virulence relies on the translocation of effector proteins into the host cytoplasm through the Dot/Icm type 4 secretion system (T4SS). Collectively, these effectors facilitate the formation of a spacious vacuole that supports bacterial replication. Similarly, *Legionella pneumophila* also relies on a Dot/Icm T4SS, and previous studies have demonstrated that *L. pneumophila* can translocate *C. burnetii* effectors through this T4SS. Therefore, we have used *L. pneumophila* to individually express *C. burnetii* effectors and monitor their impact on pathways normally activated during *L. pneumophila* infection. After screening 70 effectors in *L. pneumophila*, we identified one effector that was modulating cell death pathways, cytokine production, and facilitating intracellular replication in BMDMs. The ectopic expression of this effector in epithelial cells showed its mitochondrial inner membrane localization. Expression of the Mitochondrial *Coxiella* effector protein F (MceF) in epithelial cells protected membrane integrity and enhanced mitochondrial functions. Proteomic analysis demonstrated that MceF increases the abundance of antioxidant proteins and we successfully affinity-purified Glutathione Peroxidase 4 (GPX4) from cells expressing MceF. In accordance, mitochondria isolated from epithelial cells expressing MceF showed increased mitochondrial localization of GPX4, and BMDM GPX4 CRISPR-cas9 KO cells lost the MceF protective effect during *L. pneumophila* infection. Importantly, overexpression of MceF during THP-1 infection protected against rotenone-induced cell death and decreased activation of caspases 1 and 3. Finally, we genetically engineered a clean deletion of the MceF encoding gene, which led to a more virulent phenotype than the WT in *Galleria mellonella*. Thus, we identified a unique bacterial effector that relocates host GPX4, which contributes to protect mitochondria against oxidative damage, and therefore, enhances oxidative phosphorylation capacity. Simultaneously, GPX4 relocation protects host cells against apoptosis, pyroptosis, and for the first time, ferroptosis. This study accounts for understanding how *C. burnetii* modulates leukocyte biology and provides insights for future targeting GPX4 to counteract bacterial infections or to modulate inflammatory processes during human diseases.

4

TRIM25 and DEAD-box RNA helicase DDX3X cooperate to regulate RIG-I-mediated antiviral immunity

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Tripartite motif protein 25 (TRIM25) is an E3 ligase that ubiquitinates multiple substrates within the retinoic acid-inducible gene-I (RIG-I)-like receptor (RLR) signalling cascade, with roles in IFN induction. Here, we show a novel interaction between TRIM25 and another protein in the RLR pathway that is essential for type I IFN induction, DEAD-box helicase 3X (DDX3X). In vitro assays and knockdown studies reveal that TRIM25 ubiquitinates DDX3X at lysine 55 (K55) and that TRIM25 and DDX3X cooperatively enhance *IFNB1* induction following RIG-I activation, but the latter is independent of TRIM25's catalytic activity. We also show that the influenza A virus non-structural protein 1 (NS1) disrupts the TRIM25:DDX3X interaction, abrogating both TRIM25-mediated ubiquitination of DDX3X and cooperative activation of the *IFNB1* promoter. Our results reveal a new interplay between two RLR-host proteins that cooperatively enhance IFN-β production. We also uncover a new and further mechanism by which influenza A virus NS1 suppresses host antiviral defence.

5

Bacterial self defence – *Haemophilus influenzae* S-oxide reductases as determinants of successful host-pathogen interactions

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Haemophilus influenzae is a major causative agent of acute respiratory diseases such as otitis media and pneumonia and drives exacerbations of chronic lung conditions including in COPD and COVID19 patients. Sites of *H. influenzae* infection are characterized by high levels of inflammation, and the resulting oxidizing environment causes damage to the bacterial cell. This oxidative damage particularly affects sulfur compounds such as methionine which is both a nutrient and essential component of proteins. Using a combination of enzymological, physiological and infection assays we have shown that *H. influenzae* strains possess a novel, periplasmic stress defence system that consists of one thiol-based methionine sulfoxide reductase, MsrAB, and up to two molybdenum-containing S-oxide reductases, MtsZ and DmsABC. Expression of the components of this system is triggered by exposure to hypochlorite, a reaction product of neutrophil myeloperoxidase, and the thiol-based MsrAB enzyme was required for physical resistance of the bacteria to hypochlorite and led to a small reduction in fitness during infection. Loss of MsrAB also had some immunomodulatory effects (BIRC3, antimicrobial peptides), which may be linked to its ability to repair damage to key Hi outer membrane proteins. In contrast, a loss of either of the Mo-containing S-oxide reductases, MtsZ and DmsABC, caused little or no apparent cellular defect *in vitro*, but reduced bacterial survival during infections in mice and primary human epithelia by up to 3 orders of magnitude compared to the wildtype. This suggests that these enzymes convert substrates that Hi encounters only during contact with host cells, and compared to related enzymes from non-pathogenic bacteria, both MtsZ and DmsABC showed altered substrate specificities. In the case of MtsZ, we identified methionine sulfoxide as a major substrate, which suggests a possible role of this enzyme in ensuring a supply of methionine for *H. influenzae* and redox balancing via the respiratory chain. Together, our data indicate that these highly conserved S-oxide reductases form an essential and so far underrecognized element of the adaptation of *H. influenzae* to survival in its human host.

6

Mechanisms of Survival of Latent *Toxoplasma*

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Toxoplasma latently infects one in four humans, residing in the brain and muscle, and where it can reactive into acute infection in immunocompromised patients and can cause progressive blindness in otherwise heatlh individuals. Little is understood about how

latent *Toxoplasma* manipulates host cells during latent infection. We have shown that latent *Toxoplasma* imparts a unique transcriptional signature on infected host cells as compared to acute stage tachyzoites. By generating parasites that cannot export proteins into the host cell we show that many of these transcriptional changes rely on effectors to suppress type I interferon (IFN) and IFNγ signalling. Loss of the protein export abrogates transcriptional remodelling and prevents suppression of IFN signalling. Among the exported proteins, the inhibitor of STAT1 transcription (IST) plays a key role in limiting IFNγ signalling in bradyzoites. Furthermore, bradyzoite protein export protects host cells from IFNγ-mediated programmed cell death, even when export is restricted to latent stages. These findings highlight the functional importance of host manipulation in *Toxoplasma's* bradyzoite stages.

7

A human turn of the TAP to more faithfully model herpes simplex virus infection

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Herpes simplex virus (HSV) evades immune surveillance by expressing ICP47, a protein that binds the transporter associated with antigen presentation (TAP), blocking peptide transport for presentation on MHC I and ultimately recognition by $CD8^+$ T cells. However, the full impact of this evasion on immunity to HSV has been impossible to assess in typical *in vivo* models because the binding of ICP47 to TAP is species-specific, with the affinity of this interaction being 100 times lower in mice than in humans. To address this gap we have made mice that express human instead of mouse *TAP1* and *TAP2* (HuTAP). HuTAP mice have a broadly similar immune profile to wild type mice and are able to control HSV infection. However, the ability of activated HSV-specific (gBT-I) $CD8^+$ T cells to protect against HSV replication and lesions was greatly compromised, but not entirely abrogated in HuTAP mice. Importantly, this difference in efficacy of gBT-I cells was not seen when mice were infected with an ICP47 deletion mutant of HSV. Remarkably, the difference in clearance of HSV by $CD8^+$ T cells in wild type and HuTAP mice was seen as early as one day after infection, demonstrating the capacity of these cells for rapid anti-viral activity. Taken together, this new mouse model faithfully renders the role of HSV ICP47 in evading $CD8^+$ T cell reponses and will be an invaluable tool for assessing the impact of cellular immunotherapies that rely on harnessing these cells to treat HSV infections.

8

Antibody immunity to SARS-CoV-2 by infection and vaccination

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Several serology tests for diagnoses of SARS-CoV-2 infection are currently in use which primarily assess Spike (S), and on occasion Nucleocapsid (N) antibodies. Most vaccines in use are Spike focused and inactivated whole virion vaccines are in limited use. Serology to detect infection is dependent on the retention of specific responses which have waning over time, contain antigenic changes, and specificity is dependent on low cross-reactivity with existing antibody responses. We have defined the antibody landscape of the SARS-CoV-2 response after infection. We evaluated the anti-SARS-CoV-2 antibody profiles to 15 antigens by cloning and expressing open reading frames (ORFs) in mammalian cells and screened antibody responses from COVID-19 patients using the Luciferase Immunoprecipitation System (LIPS). We assessed responses in patient plasma and a large set of pre-pandemic samples to define cut-offs, and calculated assay sensitivity and specificity. The LIPS technique allowed us to detect antibody responses in COVID-19 patients to 11 of the 15 SARS-CoV-2 antigens, identifying novel immunogenic targets. We found that antigens ORF3b and ORF8 allow detection of antibody early in infection in a specific manner and revealed the immunodominance of the N antigen in COVID-19 patients. Antibodies that target non surface proteins can mediate Fc receptor functions, we therefore assessed ORF8-specific antibodies in patients for FcR binding to mediate cellular cytotoxicity and phagocytosis function. Furthermore, the incorporation of non-structural proteins in inactivated vaccines was assessed by comparison of antibodies from mRNA BNT162b2 versus Coronavac vaccinees. These studies provide novel insights for SARS-CoV-2 replication, immunogenicity to identify key targets for specific diagnostics for breakthrough infections.

9

Unravelling the features of T cell Response to a dominant HLA B57-Restricted Gag epitope in HIV^+ Controllers and non-Controllers

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HIV infects and depletes $CD4^+$ T cells leading to severe immunosuppression. Currently almost 38 million people live with HIV worldwide. Rare individuals, termed HIV controllers, can control viral load and remain healthy while infected. Despite Human Leukocyte Antigen (HLA) gene diversity in the population, almost 50 % of HIV controllers express the HLA-B57 molecule which presents, among others, the Gag derived epitope, TW10. Strong T-cell responses to this epitope result in escape mutations in TW10 that reduce the viral fitness. Given its presentation in early infection, TW10, could therefore shape the long-term control of HIV. However, the mechanisms contributing to HIV control related to this epitope remain unclear. Here, we study the $CD8^+$ T cell responses to the TW10 epitope presented in HLA-B*57:01$^+$ HIV^+ individuals. We determine the αβ T cell receptor (TCR) repertoire in both HIV controller and non-controller individuals revealing similarities and the existence of a public TCR and public clonotypes in both groups. We further determine the polyfunctionality and avidity of selected T cell clones from each group that reveal strong CD8+ T cell responses, shaped by the specific TCR repertoire biases regardless of the viral load. Furthermore, affinity measurements of selected TCRs and the first crystal structure of HLA-B*57:01-TW10 in complex with a $CD8^+$ TCR reveal the molecular basis of TW10 recognition. The link between HIV viral load and T cell function driven by immunodominant epitopes may further our understanding of immunologic control of HIV.

10

Caspase-8 has dual roles in regulatory T cell homeostasis that balance immunity to infection and inflammatory pathology.

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FOXP3$^+$ regulatory T (Treg) cells suppress responses to maintain immunological tolerance, but can also impair immunity to infections and cancer. Despite their importance, mechanisms that control Treg cell survival during critical stages of inflammation remain unclear. We found that Treg cell-specific deletion of caspase 8 increased Treg cell number in steady-state, revealing a requirement for death receptor mediated apoptosis for their homeostasis. However, inflammation caused a precipitous drop in caspase-8-deficient Treg cell number, via the induction of the pseudokinase MLKL and subsequent necroptosis. This loss of Treg cells enhanced clearance of herpes virus infection and improved virological control in mice with overwhelming LCMV docile infection, at the expense of lethal autoimmunity in some animals. Furthermore, Treg cells from humans were more sensitive to pharmacological induction of necroptosis than other lymphocyte populations. Therefore, this dual role for caspase 8 in Treg cell apoptosis at steady-state versus protection from necroptosis during inflammation may represent a targetable switch to manipulate Treg cell numbers for therapeutic benefit.

11

Regulation of immunity to infection by emergency cDCpoiesis

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Innate and adaptive immunity work concertedly in vertebrates to restore homeostasis following pathogen invasion or other insults. Like all homeostatic circuits, immunity relies on an integrated system of sensors, transducers and effectors that can be analysed in cellular or molecular terms. At the cellular level, T and B lymphocytes act as an effector arm of immunity that is mobilised in response to signals transduced by innate immune cells that detect a given insult. These innate cells are spread around the body and include dendritic cells (DCs), the chief immune sensors of pathogen invasion. At the molecular level, DCs possess receptors that directly sense pathogen presence and tissue damage and that signal to control antigen presentation or to regulate a plethora of genes encoding effector proteins that regulate immunity. To what extent the complement of DCs in tissues is sufficient to detect infection and drive immunity remains unclear. The lecture will focus on understanding how the numbers of tissue DCs are regulated in homeostasis and during infection.

12

Analysis of the Dynamics and Composition of Lipid Droplets During Viral Infection

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The role of lipid droplets (LDs) in innate immune pathways remains relatively undescribed, however, recent research by us and others suggests that LDs may underpin the outcome of several signalling pathways.

We can now demonstrate that LDs are transiently upregulated as a host repose to multiple viral infections, both *in vitro* and *in vivo*, with this upregulation of LDs driving an increase in type I and III interferons. To understand if LDs enhance interferon responses through a bystander effect in virally infected cells, we generated GFP and mCherry labelled LD cell lines. A co-culture of these two cell lines revealed for the first, that LDs were able to cross between cells. Live-time microscopy trafficking analysis of individual LDs revealed that during viral infection, LD velocity and distance travelled increased significantly as early as 2 hrs post-infection, and remained upregulated for 72 hrs. As LDs are known to interact with other organelles to transfer lipids and proteins, we developed techniques to isolate pure LDs from cells to analyse their lipidome and proteome before and following viral infection. Lipidomic analysis revealed significant changes within the lipid profile of LDs during infection, with an upregulation of long-chain fatty acids, and bioactive lipid mediator precursors. Significant changes were also seen in the LD proteome, with 92 proteins upregulated 24 hrs post-infection, including multiple antiviral proteins, and members of the early innate antiviral signaling pathways, demonstrating for the first time that the LD 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 antiviral immune response, in particular, the production of IFN following viral infection. Here, we characterise the changing lipidome and proteome of LDs during an early antiviral response for the first time. 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.

13

Bromodomain proteins are essential regulators of gene expression and chromatin in *Plasmodium falciparum*

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Introduction. Emerging resistance to existing anti-malarials demands the discovery of new anti-malarial drugs. Novel anti-malarial targets are a priority to reduce the risk of cross-resistance. Bromodomains bind acetylated lysines, often on histones, and typically they recruit enzymes or transcriptional co-factors to chromatin where they participate in gene regulatory processes. Human bromodomain proteins have been pursued as drug targets for multiple diseases and several inhibitors are in late stage development. *Plasmodium falciparum* has seven novel bromodomain proteins (PfBDPs) unique to apicomplexan parasites and one that is conserved in eukaryotes but which carries a divergent bromodomain. We propose that these PfBDPs could furnish novel anti-malarial drug targets.

Aims. We aimed to validate the PfBDPs as anti-malarial drug targets and determine their role in parasite growth and development.

Methods. We created inducible knockout/knockdowns (KO/KD) of the PfBDPs and tested these for essentiality for blood and mosquito stage *P. falciparum*. We further assessed the function of PfBDPs by dissecting their roles in asexual growth and by characterising their associations with chromatin and gene regulation.

Results. Three PfBDPs are essential for asexual blood stage parasite growth and one is required for normal growth, two are required for development in the mosquito. The PfBDPs have diverse and overlapping genomic distributions and functions, with two involved in directly activating genes and three involved in chromatin structure regulation. Multiple PfBDPs are involved in critical processes including the tightly coordinated expression of proteins involved in erythrocyte invasion and sexual development.

Discussion. These results validate multiple PfBDPs as novel anti-malarial drug targets and establish novel functions for PfBDPs in asexual blood stage and mosquito stage parasites.

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Mitochondrial Shape Shifting in the T Cell Response

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Not yet available

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Eosinophils in Adipose Tissue Thermogenesis

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Beige adipocytes within white adipose tissue burn fuels to generate heat and therefore may reduce obesity by burning rather than storing excess fuels. Cells of the immune system – including eosinophils – appear to be essential in the beiging of white adipocytes.

Knockout mice for the transcriptional repressor Kruppel-like Factor 3 (KLF3) are lean and are protected from diet-induced obesity. Interestingly, these mice show evidence of an increased capacity for thermogenesis. We performed a bone marrow transplantation study and were able to confer the lean beige phenotype on wild type mice. This suggested that KLF3 deficiency in cells of the haematopoietic lineage may drive leanness in this mouse model. We interrogated different types of adipose-resident immune cells and discovered that there are three times as many eosinophils in KLF3-deficient adipose tissue.

We performed genome-wide expression analyses on eosinophils isolated from white adipose tissue and uncovered widespread gene expression differences within these primary cells in the absence of KLF3. Interestingly, we saw expression of a number of genes that encode secreted proteins known for their role in beiging. The eosinophils from KLF3 knockout mice, where we saw enhanced beiging, expressed higher levels of these secreted proteins.

Our data suggest that KLF3 is a master regulator of gene expression programs in adipose tissue-resident eosinophils and that adipose tissue-resident eosinophils secrete important factors that drive beiging of adipose tissue. We are now testing whether novel secreted proteins we have identified are able to induce beiging and energy expenditure and may present novel targets for obesity. We are also exploring how the gene expression programs in adipose tissue-resident eosinophils differ from eosinophils residing in other tissues and the transcription factors that drive this niche specification.

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Metabolic adaptations in ageing T cells

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Ageing leads to the accumulation of differentiated, unconventional T cell populations. One such cell type is the virtual memory T (T_{VM}) cell, which are semi-differentiated but antigen-naïve CD8 T cells. T_{VM} cells have increased survival compared to conventional naïve CD8 T cells (T_N) and accumulate in number with age, but become dysfunctional. Our work identified age-related shifts in T cell metabolism that did not correlate with function but may support the preferential survival of certain T cell subsets with age. With increased age, memory phenotype cells such as T_{VM} cells exhibited increased mitochondrial load and spare respiratory capacity, despite a marked reduction in classic T cell functions (proliferation, IFNg production and cytotoxicity). In both mouse and human CD8 T cells, increased SRC was associated with heightened sensitivity to IL-15 and blocking IL-15 could reduce SRC in T_{VM} cells. Our model suggests that IL-15 sensitivity and signalling increases in memory phenotype T cells in general and T_{VM} cells in particular with age, to support increased SRC and cell survival. However, SRC is not a consistent positive predictor of conventional T cell function across the lifespan. In our ongoing work, we are using our model of T cell survival, function, metabolism and ageing to optimise the development of T cell-based therapies for older patients.

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Development of a 3D cell culture model to study macrophage activation and metabolism

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Macrophages are present within all tissues where they exhibit a wide variety of functions, ranging from the phagocytosis and elimination of intracellular pathogens to homeostatic roles in organ function and development. Developing these varied, highly specialised functions is dependent on the macrophage sensing and correctly integrating cues from their complex, three-dimensional (3D) tissue niche. Yet, in the laboratory, macrophages are routinely cultured *in vitro* on stiff, flat, plastic cultureware. We set out to establish a 3D model that better mimics *in vivo* tissue niches and examine the influence of this microenvironment on the activation and metabolism of macrophages. Bone marrow derived-macrophages (BMDM) or RAW264.7 murine macrophage cell lines were suspended in 3D within collagen gels. These cells were compared to 2D controls (traditional plastic tissue-culture plates coated with a thin extracellular matrix (ECM) protein monolayer), and a 2D cushion model (cells cultured as a monolayer on top of a pre-set collagen gel). After 3 and 6 days in culture, the viability of BMDM cultured in 3D was significantly lower than 2D controls and RAW264.7 cells in 3D. We screened a variety of additional ECM proteins and identified that integration of vitronectin into 3D cultures restored BMDM viability in 3D. We next set out to examine the effect of 3D culture on macrophage activation and metabolism. In traditional 2D conditions, BMDM stimulated with pro-inflammatory cues (lipopolysaccharide (LPS) and interferon-gamma (IFN-γ)) upregulate the signature gene *Nos2,* concomitant with increased glycolytic flux and absent oxidative respiration, while stimulation with anti-inflammatory cues (IL-4 and IL-13) induces *Mrc1* expression and increases oxidative respiration. In 3D, BMDM activated to both pro- and anti-inflammatory phenotypes had significantly increased mitochondrial and glycolytic metabolism than 2D controls. This was associated with an increased expression of anti-inflammatory genes (*Mrc1, Arg1*) and reduced expression of *Nos2* and nitric oxide production. Taken together, our data shows that 3D culture influences macrophage activation into pro- and anti-inflammatory phenotypes and alters the cellular metabolism of macrophages, overall inducing them to be more energetic. This project is the first step on the road to better modelling macrophage biology in tissue microenvironments.

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CD98 controls the metabolic flexibility of low-density neutrophils mobilized by G-CSF

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Low density neutrophils (LDN) are rarely detected in healthy individuals but appear in the peripheral blood of individuals treated with granulocyte colony-stimulating factor (G-CSF) and in patients with inflammatory diseases and malignancies. LDN co-purify with mononuclear cells during density-gradient separation of peripheral blood cells. Using human G-CSF mobilized LDN, we show that LDN are transcriptionally, proteomically and metabolically distinct from other neutrophil populations. Whole cell proteomics revealed that CD98 was significantly upregulated in LDN. CD98 is a type II transmembrane protein which dimerizes with several L-type amino acid transporters to facilitate uptake of essential amino acids. We demonstrate that expression of CD98 is increased on LDN and can be used as a phenotypic marker to detect LDN in the blood of patients with system lupus erythematosus, and within the blood and tumour tissue of cancer patients. We present functional evidence that CD98 is responsible for the increased bioenergetic capacity of LDN. Upregulation of CD98 on LDN facilitates the uptake of amino acids that are subsequently used by the mitochondria as fuel to produce ATP in the absence of glucose. We show that pharmacological inhibition of CD98 reduces the metabolic flexibility of LDN, which may in turn limit the pathogenic capacity of this neutrophil subset.

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Deconvoluting host cell phosphorylation pathways during infection with a network-based modelling approach

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Phosphorylation-based signalling implicates a complex and intertwined ensemble of pathways that is critical to all domains of life. The interconnectivity between pathways results in the emergence of complex networks whose elucidation present a serious challenge. Many phosphorylation interactions that occur in human cells have been identified and constitute the basis for the known phosphorylation interaction network. In most traditional phosphorylation studies, a single phosphorylation interaction, or a single pathway, are interrogated. Though this is an effective strategy to address specific questions, it fails to reflect the complexity and size of the phosphorylation network within eukaryotic cells. In our system-wide approach, we use antibody microarrays to comprehensively identify changes in host cell phosphorylation networks following infection. To analyse the large datasets produced by these arrays, we have developed a pathway analysis tool that we called MAPPINGS (Mapping and Analysis of Phosphorylation Pathways Identified through Network/Graph Signalling). The program uses random walks based on the aforementioned known phosphorylation network to identify chains of phosphorylation events occurring much more or much less frequently than expected. MAPPINGS highlights pathways of phosphorylation that work synergistically, providing a rapid interpretation of the most critical pathways following infection. In our initial construction of the program, we interrogated the host erythrocyte's response to infection with the malaria parasite *Plasmodium falciparum*. This enabled us to confirm a number of previously described host phosphorylation events and, importantly, to identify several additional phosphorylation events and to suggest pathways in which such events are involved. The analysis strategy described here is widely applicable to comparative phosphorylation datasets in any context (e.g. response of cells to infection, treatment, or comparison between differentiation stages of any cell populations) and provides a rapid and reliable analysis to guide validation studies, which can reduce dataset analysis time dramatically.

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Targeting host-microbial interactions to develop therapies to prevent and treat otitis media

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Middle ear infections (Otitis Media, OM) are the most common reason for pre-school children to visit a doctor, be prescribed antimicrobials, or undergo surgery. Recent Cochrane reviews of clinical trials have identified that antibiotics and grommet surgery are only moderately effective in treating OM, with recurrent or persistent infection observed in one third of children. My team is harnessing host-microbe interactions to develop therapies to improve OM treatment and prevention. This includes development of a novel intranasal therapy that will soon be entering first-in-human trials. We are also leading a randomised controlled trial of a novel approach to disrupt middle ear biofilm that permits antibiotics to work more effectively in children with recurrent or chronic OM. This presentation will provide an update on our work on these novel OM therapies, which have the potential to improve health outcomes for children in Australia and worldwide.

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WNT signalling shapes inflammatory responses in patients with sepsis

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Publish consent withheld

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The transmembrane TLR adaptor SCIMP is a spatiotemporal Erk1/2 scaffold to drive pro-inflammatory responses in macrophages

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Innate immune and inflammatory responses are triggered by pathogen or damage activation of Toll-like (TLR) receptors. The MAPK extracellular signal-regulated kinase 1/2 (Erk1/2) contributes to signalling from many receptors, including TLRs, but how Erk1/2 is spatiotemporally recruited for TLR signalling is not known. SCIMP is an immune-specific transmembrane adaptor which regulates 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 pro-inflammatory cytokines including IL-6 and IL-12p40. Here, we reveal that SCIMP is a direct scaffold to recruit and activate Erk1/2 for pro-inflammatory signalling downstream of TLR4. Mass spectrometry, affinity pull downs and co-immunoprecipitation in macrophage lysates show that Erk1/2 is a novel binding partner of SCIMP which is recruited in response to TLR4 stimulation. Lattice light sheet live cell imaging shows that SCIMP spatiotemporally recruits Erk2 to TLR4 signalling domains on membrane ruffles and macropinosomes. BMMs from *Scimp*$^{-/-}$ mice display defective Erk1/2 recruitment to TLR4, reduced activation of transcription factor c-Fos and impaired production of pro-inflammatory cytokines IL-1ß, TNF, IL-6 and IL-12p40 consistent with Erk1/2 inhibition. Thus, we identify a mechanism by which SCIMP recruits Erk1/2 for c-Fos activation in pro-inflammatory TLR signalling in macrophages[3]. This positions SCIMP as the key scaffold for Erk1/2 kinase recruitment to TLRs 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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2. Luo et al. SCIMP is a universal Toll-like receptor adaptor in macrophages. Journal of Leukocyte Biology (2019) doi:10.1002/JLB.2MA0819-138RR
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Vδ2$^+$Vγ9$^+$ T-cells downregulate CCR6 following phosphoantigen-driven *in vivo* expansion in pigtail macaques (*Macaca nemestrina*)

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Introduction: Vδ2$^+$ T-cells, a subset of unconventional T-cells that recognise phosphoantigens, have garnered significant interest as immunotherapies against cancers and infectious diseases. These cells can be readily expanded pharmacologically and can mediate a wide variety of immune functions, including pro-inflammatory cytokine production, direct killing of target cells, and antigen presentation to conventional T-cells. Despite promising results in preclinical studies, the clinical efficacy of γδ T-cell immunotherapies has been limited. Improving Vδ2$^+$ T-cell immunotherapies will likely require refinements to the treatment protocols, along with an improved understanding of the tissue distribution and phenotype of expanded γδ T-cells.

Methods: We studied the impact of different antigenic stimuli and routes of administration on Vδ2$^+$Vγ9$^+$ T-cell expansion and tissue trafficking in 7 pigtail macaques (*Macaca nemestrina*). Macaques were treated with zoledronate, HMB-PP, or IPP intravenously (IV) or intratracheally (IT), along with recombinant human IL-2 (rhIL-2), to stimulate *in vivo* Vδ2$^+$Vγ9$^+$ T-cell expansion. γδ T-cell frequencies and phenotypes were monitored in blood, lymph nodes, and mucosal tissues, and compared to baseline samples.

Results: *In vivo* expanded Vδ2$^+$Vγ9$^+$ T-cells were readily identified the blood (13-28.5% of CD3$^+$ T-cells, 0.61-2.75% at baseline) and airway mucosa (6.09-14.6% of CD3$^+$ T-cells, 3.25-6.51% at baseline) following IV antigen administration. Expanded Vδ2$^+$Vγ9$^+$ T-cells were predominately confined to the airway mucosa following intratracheal antigen administration. Strikingly, Vδ2$^+$Vγ9$^+$ T-cells in the blood, and to a lesser degree in the airway mucosa, were predominately CCR6$^-$. Additionally, *in vitro* expanded Vδ2$^+$ T-cells were predominately CCR6$^-$, with CCR6 expression being negatively associated with rhIL-2 concentrations. No significant differences in the expansion capacity of sort-purified CCR6$^+$ or CCR6$^-$ Vδ2$^+$ T-cells were observed *in vitro*, suggesting that CCR6 is being downregulated during phosphoantigen mediated expansion. This was further supported by acute *in vitro* stimulations, where CCR6 expression decreased following cytokine stimulation.

Conclusion: Our findings indicate that tissue distribution of *in vivo* expanded Vδ2$^+$ T-cells can be modulated by changing the route of antigen administration. Furthermore, phosphoantigen and rhIL-2 driven Vδ2$^+$ T-cell expansion is associated with a loss of CCR6 expression in pigtail macaques. This work has implications for improving Vδ2$^+$ T-cell immunotherapies against both infectious and neoplastic diseases.

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Antifungal resistance – shaping the research agenda

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It is widely accepted that antimicrobial resistance is amongst the key health challenge of our times, although antifungal resistance is often left out of the conversation. However, fungal infections affect vulnerable patients and are associated with high morbidity and mortality – compounded by a paucity of available antifungal agents, outcomes are even worse when drug resistance is present. This talk will report on a WHO project, led by Australian researchers, to rank fungal pathogens according to their research and development needs and their public health impact. Using a combination of systematic reviews, discrete choice experiments, multi-criteria decision analysis techniques, and drug pipeline analyses, our project revealed that multi-drug resistance was a key driver behind perceived need for R&D. Preliminary

results from this project will be contextualised according to the under-recognised emerging and established health threats from drug resistant fungal pathogens.

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Mucormycosis in the COVID-19 pandemic era

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Not yet available

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Unravelling novel epigenetic mechanisms underpinning SARS-CoV-2 infection and implications for novel epi-therapies

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COVID-19 (SARS-CoV-2) is a persistent and aggressive pandemic, with over ~260 million cases to date and more than ~5 million deaths thus far globally. While vaccination can be effective not all patients are able to respond to vaccination (such as the elderly or immuno-compromised). Critically, asymptomatic patients who display no viral symptoms are capable of being highly contagious and represent a significant danger of spread of the virus. Therefore, there is an urgent need for new drugs, post-exposure prophylaxis drugs (PEPs), targeting COVID-19 at the either early or asymptomatic phase of the disease to combat the spread of the virus and the severity of the disease or at later stages to relieve disease severity. This is particularly important for those at most risk such as the health care works and the elderly within our community. Additionally, emergent strains with a variety of new mutations represent a challenging difficult for vaccine development.

Epigenetic Enzymes, Erasers and Writers, are moving toward the forefront of drug development for a plethora of diseases due to the plasticity and reversible nature of epi-therapeutics. Importantly, the combinatorial epigenetic codes or signatures laid down by these enzymes within the highly modifiable amino-terminal tails of histones eloquently regulates gene expression programs. Recent exciting findings show that these codes extend to non-histone proteins critical for protein stability of critical cellular factors.

My laboratory has been at the forefront of developing epi-based therapeutics. In this talk, I will present how we are harnessing this expertise to unravel the interplay between the epigenome and SARS-Cov-2 infection. Importantly, addressing potential novel opportunities for drug development for COVID-19.

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SARS-CoV-2 infection in children does not necessitate establishment of adaptive SARS-CoV-2-specific immunological memory

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Children are at lower risk of developing severe COVID-19, yet the underlying immune mechanisms are understudied. While children's innate immunity can drive rapid resolution of SARS-CoV-2 infection, the establishment of SARS-CoV-2-specific T-cell and B-cell memory in mild COVID-19 in children remains unexplored. We recruited a household cohort to understand SARS-CoV-2-specific $CD4^+$ and

$CD8^+$ T-cell immune responses at one month after mild SARS-CoV-2 infection in PCR-positive children, in comparison to their mothers. We analysed SARS-CoV-2-specific T-cell responses, together with B-cells, directly *ex vivo* using six SARS-CoV-2 T-cell HLA class-I tetramers (A1/ORF1a$_{1637}$, A2/S$_{269}$, A3/N$_{361}$, A24/S$_{1208}$, B7/N$_{105}$, B40/N$_{322}$), one class-II tetramer (DPB4/S$_{167}$), and Spike- and Receptor Binding Domain (RBD)-specific B-cell probes. Our in-depth profiling of epitope-specific T-cell responses at quantitative, phenotypic and clonal levels found that only children who seroconverted had prominent memory T-cell and B-cell profiles. These children had a high magnitude of SARS-CoV-2-specific T-cells displaying memory phenotypes and prevalent T cell receptor motifs, which were not observed in RBD IgG^- but PCR^+ children. This suggests that seroconversion but not PCR-positivity defines establishment of adaptive SARS-CoV-2-specific immunological memory in children, which is in contrast to adults with a mild SARS-CoV-2 infection. SARS-CoV-2-specific $CD8^+$ and $CD4^+$ T-cell responses in RBD IgG^+ children were comparable to those of their mothers, with more prominent tetramer-specific T-cell responses associated with seropositivity rather than PCR status alone. Our study suggests that COVID-19 vaccination of children with mRNA vaccines could be a major advantage in terms of establishing T-cell and B-cell immunological memory.

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ILRUN downregulates ACE2 expression and blocks infection of human cells by SARS-CoV-2

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The human protein-coding gene *ILRUN* (inflammation and lipid regulator with UBA-like and NBR1-like domains; previously C6orf106) was initially identified as a proviral factor for Hendra virus infection. More recently, ILRUN was characterized to function as an inhibitor of type I interferon (IFN) and proinflammatory cytokine expression *via* targeting of the IFNβ-enhancosome complex and inducing the degradation of the transcriptional coactivators CREB-binding protein (CBP) and p300.

In response to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, we sought to investigate the cellular pathways regulated by ILRUN in the context SARS-CoV-2 infection. Utilizing whole transcriptome sequencing (RNA-seq) we discovered that inhibition of ILRUN expression by RNA interference alters transcription profiles of numerous cellular pathways, suggestive of pleiotropic roles for ILRUN in cell biology beyond innate immunity. Notably, we observed upregulation of the SARS-CoV-2 entry receptor *ACE2* and several other members of the renin-angiotensin aldosterone system (RAAS), a key pathway involved in the regulation of blood pressure and inflammation. Additionally, transcripts of the SARS-CoV-2 coreceptors *TMPRSS2* and *CTSL* were also upregulated, and surface expression was ACE2 protein was also observed to increase in ILRUN-deficient cells using flow cytometry.

In the context of infection, inhibition of ILRUN resulted in increased SARS-CoV-2 replication at 6 and 24 h post-infection, while overexpression of ILRUN had the opposite effect, identifying ILRUN as a novel antiviral factor at the early stages of SARS-CoV-2 replication, consistent with its regulation of SARS-CoV-2 cell entry receptors. Together, these data firstly contribute to our understanding of biological pathways that regulate host factors critical to SARS-CoV-2 infection. Secondly, they identify ILRUN as a novel regulator of the RAAS, suggesting it may have a multifaceted approach to controlling inflammation through multiple cellular pathways.

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SARS-CoV-2 suppresses IFNβ production mediated by NSP1, 5, 6, 15, ORF6 and ORF7b but does not suppress the effects of added interferon

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Type I Interferons (IFN-Is) are a family of cytokines which play a major role in inhibiting viral infection. Resultantly, many viruses have evolved mechanisms in which to evade the IFN-I response. Here we tested the impact of expression of 27 different SARS-CoV-2 genes in relation to their effect on IFN production and activity using three independent experimental methods. We identified six gene products; NSP6, ORF6, ORF7b, NSP1, NSP5 and NSP15, which strongly (>10-fold) blocked MAVS-induced (but not TRIF-induced) IFNβ production. Expression of the first three of these SARS-CoV-2 genes specifically blocked MAVS-induced IFNβ-promoter activity, whereas all six genes induced a collapse in IFNβ mRNA levels, corresponding with suppressed IFNβ protein secretion. Five of these six genes furthermore suppressed MAVS-induced activation of IFNλs, however with no effect on IFNα or IFNγ production. In sharp contrast, SARS-CoV-2 infected cells remained extremely sensitive to anti-viral activity exerted by added IFN-Is. None of the SARS-CoV-2 genes were able to block IFN-I signaling, as demonstrated by robust activation of Interferon Stimulated Genes (ISGs) by added interferon. This, despite the reduced levels of STAT1 and phospho-STAT1, was likely caused by broad translation inhibition mediated by NSP1. Finally, wefound that a truncated ORF7b variant that has arisen from a mutant SARS-CoV-2 strain harboring a 382-nucleotide deletion associating with mild disease (Δ382 strain identified in Singapore & Taiwan in 2020) lost its ability to suppress type I and type III IFN production. In summary, our findings support a multi-gene process in which SARS-CoV-2 blocks IFN-production,with ORF7b as a major player, presumably facilitating evasion of host detection during early infection. However, SARS-CoV-2 fails to suppress IFN-I signaling thus providing an opportunity to exploit IFN-Is as potential therapeutic antiviral drugs.

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Interplay of type I and II IFN for the generation of CD8+ Stem-like memory T cells

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The timing and location of the interferon (IFN) response to viral infection is tightly coordinated. High expression or therapeutic administration of type-I IFN (IFN-I) enhances viral clearance, whereas deficiency or blocking of IFN-I prolongs infection. We have previously shown that deficiency in the IFN-I receptor, IFNAR promotes the formation of CD8+ T cell stem like memory cells (Tscm) due to cellular retention in the T cell paracortex where this fate is imprinted. This highlights the tension between effector and Tscm fates that can be leveraged by increasing IFN-I or blocking IFNAR. Here, we demonstrate that blocking IFNAR precisely at the start of LCMV infection results in potent Tscm formation, but unintuitively increases the levels of CXCR3 ligands. This suggests the mechanism of retention in the T cell paracortex, is due increased chemokine abundance and desensitization of CXCR3. Blocking IFNAR increased the recruitment of monocyte-derived inflammatory DCs in an IFNg-dependent fashion. In the absence of both IFNAR and IFNg chemokine expression was lost. In this setting Tscm fate was still enhanced and occurred along-side T cell exhaustion and persistent viral load. This study reveals the interplay of IFN-I and IFN-II that can be harnessed during vaccination to induce potent stem-like memory.

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Recognition of host Clr-b by the inhibitory NKR-P1B receptor provides a basis for missing-self recognition

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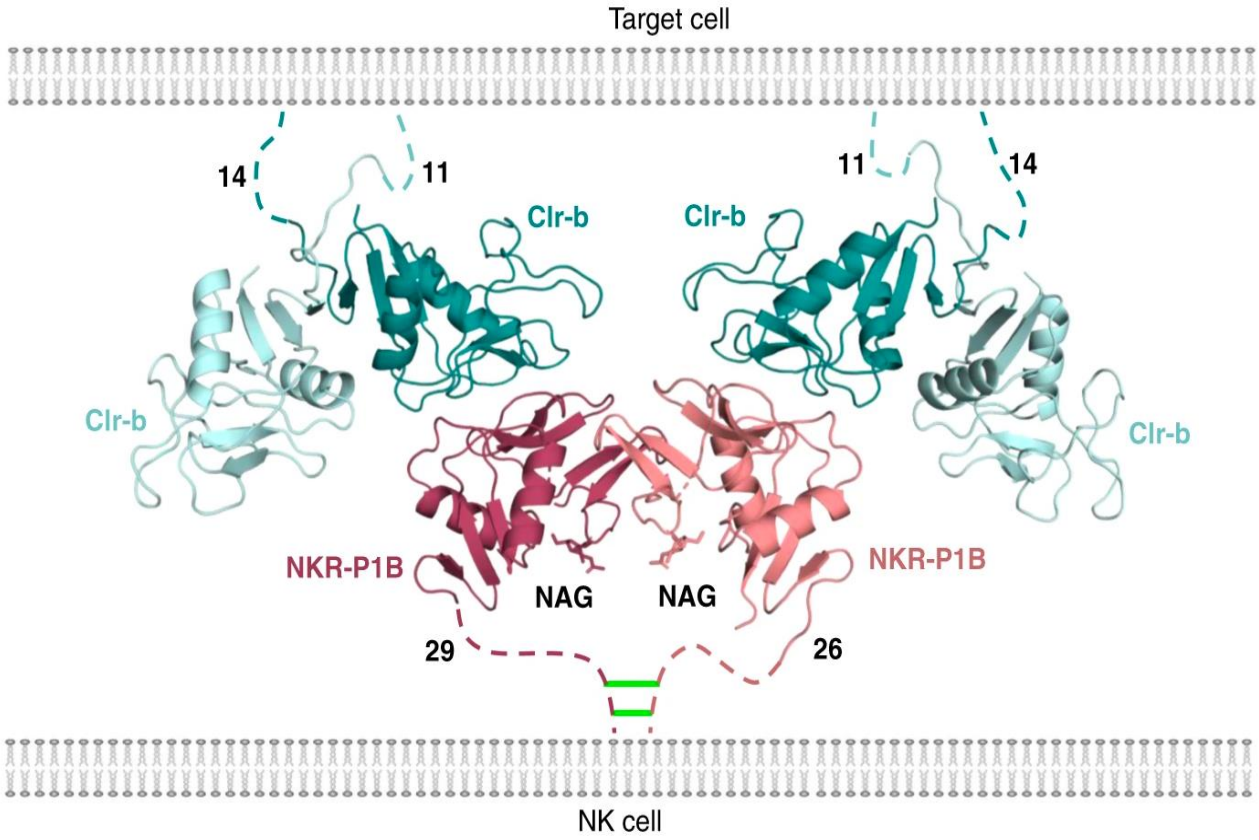
The interaction between natural killer (NK) cell inhibitory receptors and their cognate ligands constitutes a key mechanism by which healthy tissues are protected from NK cell-mediated lysis. However, self-ligand recognition remains poorly understood within the prototypical NKR-P1 receptor family. Here we report the structure of the inhibitory NKR-P1B receptor bound to its cognate host ligand, Clr-b. NKR-P1B and Clr-b interact via a head-to-head docking mode through an interface that includes a large array of polar interactions. NKR-P1B:Clr-b recognition is extremely sensitive to mutations at the heterodimeric interface, with most mutations severely impacting both Clr-b binding and NKR-P1B receptor function to implicate a low affinity interaction. Within the structure, two NKR-P1B:Clr-b complexes are cross-linked by a non-classic NKR-P1B homodimer, and the disruption of homodimer formation abrogates Clr-b recognition. These data provide an insight into a fundamental missing-self recognition system and suggest an avidity-based mechanism underpins NKR-P1B receptor function.



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Interferon epsilon limits ovarian cancer metastasis via tumour-extrinsic mechanisms

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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]. IFNε is known to be protective against FRT infections[1,2], however its role as an anti-tumour cytokine is under investigation. High grade serous ovarian cancer (HGSOC) is a cancer of the FRT which is frequently characterised by extensive metastasis throughout the peritoneal cavity, and carries a five year survival rate of ~45%. Preliminary research has suggested that IFNε is protective against HGSOC metastasis through both intrinsic action on tumour cells, and extrinsic action via immune/stromal cells. However, the relative contribution of these intrinsic and extrinsic effects to the observed anti-tumour efficacy of IFNε is unclear. Moreover, analysis of human HGSOC tumours indicates a potentially high prevalence of resistance/insensitivity to type I IFNs which may limit treatment response to IFNε in a clinical setting.

Here, the role of extrinsic anti-tumour activity by IFNε was investigated in a syngeneic mouse model of HGSOC. An IFN-insensitive ID8 mouse ovarian tumour cell line was generated via CRISPR-Cas9 knock-out of the type I IFN receptor subunit, IFNAR1. Mice were injected intraperitoneally with wild-type (WT) or IFNAR1$^{-/-}$ ID8 cells, and treated with PBS or IFNε thrice-weekly for 6 weeks. Analysis of disease scores, including tumour burden, ascites volume and peritoneal hemorrhaging, revealed that IFNε treatment effectively limited HGSOC metastasis and disease progression, with no loss of efficacy observed in mice bearing IFNAR1$^{-/-}$ tumours versus WT. Furthermore, IFNε treatment in both WT and IFNAR1$^{-/-}$ tumour-bearing mice was associated with marked alterations in peritoneal immune cells. PBS-treated mice displayed significant infiltration of immunosuppressive immune cells, alongside global suppression of immune cell proliferation - features which were reversed with IFNε treatment. Moreover, IFNε-treated mice displayed a greater frequency of activated immune cells such as CD8$^+$ T cells. Together, these results indicate that the primary mechanism of action of IFNε in HGSOC is tumour-extrinsic, mediated through activity in immune and stromal cells, and is sufficient for effective tumour control versus IFN-insensitive tumours.

1. Marks, Z. R. C. et al. PROPERTIES AND FUNCTIONS OF THE NOVEL TYPE I INTERFERON EPSILON. Semin. Immunol. 43, 101328 (2019).
2. Fung, K. Y. et al. Interferon-ε protects the female reproductive tract from viral and bacterial infection. Science (80-.). 339, 1088–1092 (2013).

33

CD4 T cells Sans Frontières: Division of labor in the lung

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Activated CD4 T cells have the remarkable ability to differentiate into many different types of effector subsets. This diversification is required for the generation of specialized and pathogen appropriate T cell responses, as well as long-lived and protective memory T cells. Although CD4 memory T cells are clearly important to control various infections (i.e. tuberculosis), vaccines targeting the induction of polyfunctional memory cells have had only limited success. In some cases, CD4 memory T cells can also induce host detrimental effects, for example during chronic viral infection or after organ transplantation. We hypothesize that these divergent effects are dependent on heterogeneity within the CD4 memory T cell compartment, the plasticity of these cells following recall, and their localization relative to other cells or environmental signals. Thus, a major goal of our work is to elucidate the specific factors regulating CD4 memory T cell diversification and their relationship to host immunity. We are using several infection models as well as complementary microscopy approaches to assess the dynamics and flexibility of T cell differentiation.

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Synergistic activity of antibodies in multivalent vaccines

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Vaccines based on multiple antigens often induce an immune response which is higher than that triggered by each single component, with antibodies acting cooperatively and synergistically in tackling the infection. In the case of meningococcal vaccines, bactericidal activity is generally mediated by antibodies that bind to antigens highly expressed on the bacterial surface and immunologically related. However, simultaneous binding of antibodies to various surface-exposed antigens can overcome the threshold density of antigen-antibody complexes needed for complement activation. An interesting example is the antibody response induced by the 4CMenB vaccine, currently licensed for the prevention of *Neisseria meningitidis* serogroup B (MenB). It contains four antigenic components: Factor H binding protein (fHbp), Neisseria adhesin A (NadA), Neisserial Heparin Binding Antigen (NHBA) and Outer Membrane Vesicles (OMV). Monoclonal and polyclonal antibodies raised by vaccination with 4CMenB show synergistic activity in complement-dependent bacterial killing. The interplay between antibodies targeting major and minor antigens and the effect on functionality provides the evidence of the added value of multicomponent vaccines.

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CARB-X after 5 years – lessons learned and future visions

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Over 700,000 deaths worldwide are attributed to resistant bacterial infections per year and this number is increasing. Resistance is spurred by overuse and misuse of antibiotics and the situation is then made worse by the lack of scientific innovation due to poor economic incentives. Founded in 2016, the Combating Antibiotic-Resistant Bacteria Biopharmaceutical Accelerator (CARB-X) is a global non-profit partnership dedicated to accelerating antibacterial research to tackle this global rising threat. Since inception it has provided non-dilutive funding and support to groups that are developing products to prevent, diagnose, and treat infections caused by drug-resistant bacterial pathogens. CARB-X has placed a high value on innovation, and this includes embracing non-traditional modalities for which there is neither a well-trodden preclinical nor a well-defined clinical history. The CARB-X portfolio supports a wide variety of novel programs to help the global antibacterial research ecosystem understand the potential that these modalities can play in the management or prevention of serious infections. As the CARB-X portfolio expanded, strategies to create a higher-level understanding of common issues facing product developers and to leverage efficiencies broadly across funded groups has evolved. This presentation will highlight some key lessons from the initial 5 years of CARB-X as well as some future opportunities that should positively impact the broader ecosystem of antibacterial drug development.

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Host-dependent resistance of Group A *Streptococcus* to antifolate antibiotics

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Described mechanisms of antimicrobial resistance (AMR) involve resistance to the direct effects of the antibiotic, and can be monitored by *in vitro* susceptibility testing and genetic methods. However, obligate human pathogens such as Group A *Streptococcus* (GAS) only experience antibiotic exposure in the context of an infection. The most widely-used treatment for GAS skin and soft tissue infections is a synergistic combination of trimethoprim and sulfamethoxazole (co-trimoxazole). Co-trimoxazole targets sequential steps in the bacterial folate biosynthesis pathway, with all known resistance mechanisms requiring individual resistance to each antibiotic. We have discovered a novel mechanism of co-trimoxazole resistance that involves an AMR protein (ThfT) that modifies the substrate recognition of a GAS ABC transporter, such that ThfT-positive GAS strains can acquire the end products of the folate synthesis pathway directly from the host. As these metabolites are abundant *in vivo* but lacking in susceptibility testing media, ThfT-mediated resistance is "host dependent" and not detectable by existing phenotypic or genetic methods. Evidence of transfer of *thfT* between GAS and related species suggests that resistance could disseminate widely without detection. Our study highlights the importance of understanding antibiotic activity in the context of the infections that they are used to treat, and should serve as a paradigm for investigating additional mechanisms of host-dependent AMR in medically-important bacterial pathogens.

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Dismantling *bla*$_{KPC}$ carbapenemase resistance: inhibiting the DsbA protein restores carbapenem susceptibility in KPC carbapenem-resistant bacteria.

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Carbapenems are beta-lactam antibiotics commonly used to treat severe infections by bacteria resistant to multiple antibiotic classes. The emergence and global spread of *Klebsiella pneumoniae* carbapenemase (KPC) enzymes among *Enterobacteriaceae,* which hydrolyse all beta-lactams including last line carbapenem antibiotics and are also resistant to beta-lactamase inhibitors, is a major threat to global public health. Consequently, carbapenemase-producing bacteria are listed by the WHO as 'Critical Priority' pathogens for new antimicrobial development. The KPC enzyme contains a disulfide bridge between Cys69 and Cys238, located close to the active site. However, the role of this disulfide bridge in maintaining KPC structural stability or enzyme activity remains unresolved. Here we report that disrupting the disulfide bridge in KPC by mutating the Cys69 residue to Ser, renders the enzyme less stable and less active for beta-lactam hydrolysis. In *Escherichia coli* the KPC C69S mutant confers reduced susceptibility to beta-lactams, including carbapenems. In addition, KPC-producing clinical *E. coli* isolates lacking the DsbA enzyme, which catalyses disulfide bond formation, are also significantly more susceptible to carbapenem antibiotics. Inhibition of DsbA in KPC-producing isolates with a small molecule inhibitor restores their clinical susceptibility to several carbapenem antibiotics. Our findings demonstrate the essential role of the disulfide bridge in the functional biogenesis of the most widespread class A carbapenemase and provide significant proof of principle for DsbA inhibition as an innovative antibiotic adjuvant strategy with a new mode of action for beta-lactamase inhibition.

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Investigating the mode of action of three compounds that block egress and invasion of malaria parasites

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Malaria is a devastating disease that kills nearly half a million people each year. Although a vaccine has recently been approved, its efficiency is limited and, together with increasing resistance to nearly all antimalarial drugs, the success of malaria elimination is threatened. New antimalarials with novel modes of action therefore urgently need to be developed. We aim to block the egress and invasion of *Plasmodium falciparum* parasites from human red blood cells (RBCs) thereby stopping parasite proliferation and symptomatic disease. A screen of the Medicines for Malaria Venture Pathogen Box identified several compounds that inhibited egress and invasion *in vitro* (Dans et al, IJP, 2020). From this screen, three compounds were selected for further characterisation: egress inhibitor MMV-A, and invasion inhibitors, MMV-B and MMV-C. To identify their targets, we attempted to select for parasites that were resistant to these compounds and to then find the genetic adaptations responsible for resistance. No resistance could be raised to MMV-A, however, as the compound resembles a cysteine inhibitor and blocks egress leaving parasites trapped within the old RBC, we reasoned MMV-A may inhibit SERA6, a key protease involved in egress. We have additionally shown the inhibitor also blocks the activity of falcipain-2A (a cysteine protease involved in both egress and haemoglobin digestion). Genome sequencing of parasites resistant to MMV-B revealed its target might be a serine protease involved in invasion. We are currently confirming this by introducing the resistance mutations into wild-type parasites with CRISPR-Cas9 to determine if the mutations confer resistance. We also aim to derive the molecular structure of MMV-B with the recombinant serine protease to design more potent inhibitors. Similarly, parasites resistant to MMV-C have been successfully selected and target identification is underway. Analogs of all compounds are currently being designed and tested to improve their potency and pharmacokinetic properties.

1. Madeline G. Dans, Greta E. Weiss, Danny W. Wilson, Brad E. Sleebs, Brendan S. Crabb, Tania F. de Koning-Ward, Paul R. Gilson, Screening the Medicines for Malaria Venture Pathogen Box for invasion and egress inhibitors of the blood stage of Plasmodium falciparum reveals several inhibitory compounds, International Journal for Parasitology, Volume 50, Issue 3, 2020, Pages 235-252, ISSN 0020-7519 doi.org/10.1016/j.ijpara.2020.01.002

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Phage-antibiotic combination is a superior treatment against multi-drug resistant *Acinetobacter baumannii*

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Phage therapy is becoming increasingly promising as a therapeutic strategy against antibiotic-resistant bacterial infections. Importantly, clinical phage therapy is often delivered alongside antibiotics. Therefore, to maximise their therapeutic effect, the biology of clinically-relevant phages needs to be better understood. Here, we assessed the *in vivo* bactericidal effect of a phage-antibiotic combination on *Acinetobacter baumannii* AB900 using phage FG02, which binds to capsular polysaccharides and leads to antimicrobial resensitisation *in vitro*. We performed a two-stage preclinical trial using a murine model of severe bacteraemia. Notably, our findings explain the mechanism through which the combination of these two agents results in a superior bactericidal effect. We confirm that, even in complex *in vivo* systems, treatment with a capsule-targeting phage can reliably and repeatably induce bacterial evolution towards a phenotype that is phage-resistant but resensitised to antibiotics. This study presents a preclinical trial, the first using a mammalian model, that demonstrates that a phage antibiotic combination has a superior bactericidal effect than each of these agents individually against severe *A. baumannii* infection.

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Translating lung "microbiomics" into clinical application for sgronic respiratory desease

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The use of next generation sequencing has revolutionised our detection and understanding of the airway microbiome in chronic respiratory disease including Chronic Obstructive Pulmonary Disease (COPD) and bronchiectasis. Targeted amplicon sequencing reveals important associations between the respiratory microbiome and disease outcome while metagenomics can elucidate functionality. How best to apply this information into patient care, monitoring and potentially treatment however remains challenging, and this talk summarizes how host and environmental microbiomes may be leveraged for clinical application in the era of precision medicine.

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Immunity, IBD and the gut-brain axis through the prism of functional mycobiota exploration

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Not yet available

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PfCERLI1, a novel rhoptry associated protein essential for rhoptry discharge and *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. *P. falciparum* merozoites have two types of secretory organelles (micronemes and rhoptries) whose sequential release is essential for invasion and intracellular development. During invasion, large bulb-like rhoptry organelles inject an array of invasion and virulence factors into the cytoplasm of the host red blood cell, but the molecular mechanism mediating rhoptry exocytosis is unknown. Here we identify a set of parasite specific proteins, termed *P. falciparum* Cytosolically Exposed Rhoptry Leaflet Interacting protein (PfCERLI) 1 and 2 that cap the extremity of the rhoptry. Reduction in CERLI expression interferes with proteolytic processing blocking secretion of key rhoptry antigens that coordinate merozoite invasion and therefore proliferation in *P. falciparum.* Lipid binding domains identified within CERLIs are likely contributors to assembling the machinery that docks the rhoptry to the plasma membrane prior to fusion. While further studies are needed to determine the fine detail of how PfCERLI 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 quantitative super-resolution microscopy, image analysis and computer vision to provide important mechanistic insight into a parasite specific exocytic pathway, essential for the establishment of infection.

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Single cell transcriptomics provides insights into innate and adaptive immune responses to malaria

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Malaria is caused by Plasmodium ssp. parasites, with 3.2 billion people estimated to be at risk of infection. The development of effective malaria vaccines has been hampered by poor understanding of naturally acquired immunity. Plasmodium spp. are complex organisms with potentially thousands of immune targets, hence a systems biology approach, combining immunology and "omics" tools i.e., transcriptomics, proteomics and bioinformatics can help to identify novel and potentially effective vaccine candidates. To obtain greater insights into human immune responses to malaria, we used of single-cell RNA sequencing (scRNA-seq) to characterise distinct immune cell subsets in PBMCs collected from patients during acute malaria (day0), and at convalescence (7 and 28 days post treatment). 14 major cell subsets were identified using canonical and lineage markers, with only small changes to cell proportions with infection. We found that during acute infection, genes involved in pattern recognition, TLR1, TLR7, TLR8 and phagocytosis including FCGR1A/CD64, CR1 and CD36 are upregulated during acute infection. In contrast, chemokines and cytokines TNF, IL1A and IL1B, CCL3 and antigen-presenting molecules HLA-DR/B were downregulated during infection, consistent with immunosuppressive monocyte phenotypes. The functional diversity of monocytes was highlighted by the downregulation of CCL2 (MCP-1) during acute infection in CD14 but not CD16 Monocytes. An enrichment analysis of cis-regulatory elements upstream of DEGs revealed IRF1 and P53 as potential upstream regulators of CD16 Monocytes; these TFs have previously been previously associated with protection from malaria disease. Among CD4 T cells, Th1-regulatory T (Th1 cells that co-produce IFNy and IL10 - Tr1) cells were the most transcriptionally active with genes coding for anti-inflammation proteins including, STING as well as co-inhibitory receptors (CIRs) such as PDCD1/PD1, CTLA4, and TIM3 upregulated during acute infection. LAG-3 a key inhibitory receptor on antigen activated T-cells, was highly upregulated in Th1 and Tr1 cells during acute infection, while the anti-inflammatory cytokine IL10 was upregulated in Tr1 cells during acute infection. Together, data highlights the major importance of anti-inflammatory responses in both innate and adaptive cell subsets in modulating immune responses during acute infection in uncomplicated malaria.

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Microbiota-derived outer membrane vesicles and their proinflammatory role in Parkinson's disease progression

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Introduction: Microbiotic dysbiosis of the gut is thought to facilitate the pathogenesis of neurodegenerative diseases, such as Parkinson's disease (PD) through the promotion of inflammation, however, the mechanisms involved remain unclear. We hypothesise that one of these unidentified mechanisms involves the release of Outer membrane vesicles (OMVs) from Gram-negative bacteria. We propose OMVs could drive PD inflammation, due to their enrichment in lipopolysaccharide (LPS) and other potent immune stimulators, their capability to disrupt gastrointestinal barrier integrity, and their potential to promote systemic and neural inflammation that typifies PD.

Methods: OMVs were isolated from *Escherichia coli* in culture and from faeces and characterized by density, size and morphology and LPS content and ability to stimulate immune cells *in vitro*. *E. coli* OMVs or LPS were orally administered to a PD neurotoxin murine model to determine if OMVs could accelerate and/or exacerbate Parkinson's disease motor and gastrointestinal dysfunction and inflammation.

Results: OMVs from *E. coli* cultures are shown to be more potent at promoting immune activation than an equivalent dosage of LPS in vitro. OMVs orally administered to PD model mice promote gastrointestinal dysfunction (reduced faecal pellet output and stool weight) and inflammation (increased ileal proinflammatory cytokines). OMV treated PD model mice had increased motor dysfunction compared

to sham or LPS treated mice, indicating that OMVs can cause impairments in the gastrointestinal tract which directly/indirectly promotes neurodegeneration.

Summary: This study is a first in uncovering a role for OMVs in PD related neurodegeneration and inflammation. We have shown that OMVs are potent immune stimulators *in vitro* and can exacerbate gastrointestinal dysfunction, inflammation and motor deficits in a PD mouse model. Our data has implications in discovering a functional link between microbiotic dysbiosis, inflammation and neurological dysfunction in many diseases, in particular PD.

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Breaking antibiotic resistance in *Streptococcus pneumoniae*

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Streptococcus pneumoniae is the primary cause of community-acquired bacterial pneumonia with rates of penicillin and multi-drug resistance exceeding 80% and 40%, respectively. The innate immune response generates a variety of antimicrobial agents to control infection, including zinc stress mediated by localized changes in the abundance of this metal ion. Here, we characterized the impact of zinc intoxication on *S. pneumoniae*, revealing disruptions in central carbon metabolism, lipid biogenesis and peptidoglycan biosynthesis. Characterization of the pivotal peptidoglycan biosynthetic enzyme GlmU revealed an exquisite sensitivity to zinc inhibition. Disruption of the sole zinc efflux pathway, *czcD*, rendered *S. pneumoniae* highly susceptible to multiple classes of antibiotics including β-lactams and tetracyclines. To dysregulate zinc homeostasis in the wild-type strain, we investigated the safe-for-human use ionophore PBT2. PBT2 mediated zinc intoxication rendered wild-type *S. pneumoniae* strains sensitive to a range of antibiotics. Using an invasive ampicillin-resistant strain, we demonstrate in a murine pneumonia infection model the efficacy of PBT2+ampicillin treatment. These findings present a novel therapeutic modality to break resistance of drug-resistant *S. pneumoniae*.

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Comprehensive mapping of innate and adaptive immune response dynamics across the blood and respiratory tract in COVID-19

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merging viral diseases such as viral encephalitis or COVID-19 drive a complex immune response where the inflammatory process intended to eliminate an invading pathogen contributes to significant immunopathology, both at the site of infection and more systemically. To understand this response, careful analysis using high-dimensional (HD) cytometry and single-cell technologies are required. As the size and complexity of HD data continue to expand, comprehensive, scalable, and methodical computational analysis approaches are essential. Yet, contemporary clustering and dimensionality reduction tools alone are insufficient to analyze or reproduce analyses across large numbers of samples, batches, or experiments. Moreover, approaches that allow for the integration of data across batches, experiments, and technologies are not well incorporated into computational toolkits to allow for streamlined workflows. Here we utilised our analysis tookit 'Spectre' to enable comprehensive mapping of the innate and adaptive immune response dynamics across the blood and respiratory tract in COVID-19. Our integrated analysis across the blood and respiratory tract reveal key changes in the myeloid lineage that drive disease severity over time. Using Spectre, we integrated our datasets with open source reference bone marrow datasets, revealing evidence of an inflammatory-derived acceleration of myelopoiesis and release of immature myeloid cells into the blood during severe disease. Additionally, carefully exploration of immune response in the respiratory tract allowed us to define a continuum of cellular infiltration from the blood into the airways, revealing key response patterns associated with disease severity and progression over time.

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Illuminating intracellular STING trafficking

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The cGAS-STING pathway is a key component of the innate immune response against viral and bacterial infection. Microbial double stranded DNA (dsDNA) within the host cell cytosol is recognised by the DNA sensor cyclic GMP-AMP synthase (cGAS). Once DNA-bound, cGAS generates a second messenger cyclic dinucleotide (CDN) molecule that binds and activates Stimulator of Interferon Genes (STING). STING can also be activated directly by bacterial CDNs during infection. In resting conditions, STING localises to the endoplasmic reticulum (ER) membrane as pre-formed dimers. Upon binding of CDNs, STING traffics to the Golgi and associates with signalling molecules, including the kinase TBK1. Activated TBK1 phosphorylates and activates the transcription factor IRF3, thus promoting the expression of type I interferons (e.g. IFNa/b) for an antiviral response. STING also induces the expression of numerous proinflammatory cytokines, further substantiating the immune response. Subsequently, STING traffics from the Golgi into endolysosomal regions which facilitates its degradation in a negative feedback manner, preventing sustained inflammation.

While STING is known to move through different organelle compartments, the kinetics and mechanisms controlling post-Golgi trafficking events remain poorly defined. To investigate STING translocation in more detail I have utilised both live-cell and super resolution imaging approaches, including spinning disk and Airyscan confocal systems. These high-level imaging modalities have enabled me to observe the dynamic spatiotemporal movement of STING within cells following its activation and gain insights into how STING trafficking mediates its cellular functions. In recent years it has emerged that dysregulation of STING localisation can promote aberrant STING activation and propagate autoimmune and autoinflammatory diseases. Therefore, there is considerable need to understand how STING trafficking events are controlled.

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Chlamydia pneumoniae infects the brain via olfactory and trigeminal nerves and triggers Alzheimer's disease pathologies

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Background: *Chlamydia pneumoniae* is a respiratory tract pathogen but can also infect the central nervous system (CNS). Recently, the link between *C. pneumoniae* CNS infection and Alzheimer's disease has become increasingly evident. In mice, *C. pneumoniae* infection of the CNS has been shown to occur weeks to months after intranasal inoculation, but the path of infection has not been determined.

However, other bacteria are known to rapidly (within 1-2 days) infect the brain via the olfactory and trigeminal nerves after intranasal inoculation. Understanding the path of *C. pneumoniae* infection and the cellular and molecular responses may reveal whether *C. pneumoniae* contributes to Alzheimer's disease.

Objective: To determine the path and timing of *C. pneumoniae* infection of the brain after intranasal inoculation and to determine the cellular and molecular responses.

Method: We investigated whether *C. pneumoniae* could invade the CNS via the olfactory and/or trigeminal nerves in mice, and if this resulted in any alterations in Aβ deposition or molecular pathways involved in Alzheimer's disease. We also determined whether injury to the nasal epithelium affected *C. pneumoniae* infection. Using in vitro cell cultures, we investigated whether *C. pneumoniae* could infect and survive in cultured primary mouse glial cells.

Results: By isolating live *C. pneumoniae* from tissues and using immunohistochemistry, we show that *C. pneumoniae* can infect the olfactory and trigeminal nerves, olfactory bulb and brain within 72 hours in mice. Injury to the nasal epithelium using a chemical insult resulted in increased peripheral, but decreased CNS infection. Amyloid beta accumulations were detected adjacent to *C. pneumoniae* in the olfactory system. 28 days after intranasal inoculation, analysis of gene expression at the transcriptional level revealed that multiple pathways associated with Alzheimer's disease were modulated. Examination of cellular responses to the bacteria using in vitro cultures revealed that *C. pneumoniae* was able to infect peripheral nerve and CNS glia.

Conclusion: The nerves extending between the nasal cavity and the brain constitute invasion paths by which *C. pneumoniae* can rapidly invade the CNS likely by surviving in glia, and leading to Aβ deposition and changes in gene expression associated with Alzheimer's disease

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A1 limits pathogen-induced macrophage and monocyte cell death and inflammatory responses

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Mitochondrial, BCL-2 family regulated, apoptosis plays a vital role in human health by promoting the safe clearance of damaged and infected cells by the innate immune system. However, our recent work has revolutionised this area by showing that activation of the mitochondrial effectors BAX and BAK, upon targeting pro-survival MCL-1 and BCL-XL, in LPS-primed macrophages can induce a complex series of events that culminate in NLRP3 inflammasome and IL-1β activation. Intriguingly, we found that LPS signalling delayed apoptosis, and postulated that the short-lived LPS-inducible pro-survival protein BCL2A1 might limit BAX/BAK activation. In line with this concept, we discovered that genetic deletion of *BCL2A1* renders macrophages sensitive to rapid killing and IL1β secretion upon loss of MCL1 and BCLxL activity. Even more remarkably, we found that in LPS-primed monocytes, loss of MCL-1 and A1 was sufficient for apoptosis and activation of IL-1β. In short, our data highlights that BCL2A1 protein may guard myeloid cells against death and inflammation during Gram-negative bacterial infections. Moreover, it highlights the need to further define how BCL2A1 protein is regulated during infection and its physiological significance during life-threatening infections, where repurposing anti-cancer BCL-2 family targeting drugs may represent an attractive approach to combat antimicrobial resistance.

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Differential immune detection of *Bacteroides fragilis* bacteria and their secreted outer membrane vesicles

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Outer membrane vesicles (OMVs) are secreted by Gram-negative bacteria and can package bacterial cargo including peptidoglycan, lipids and nucleic acids for their delivery to host cells. Therefore, the cargo composition of OMVs can determine their detection by host innate immune receptors and how they modulate host immune responses. Recently, the release of OMVs by the intestinal microbiota, including the commensal *Bacteroides fragilis*, have emerged as novel mechanisms to dampen inflammation in the host. In this study, we aimed to delineate the pathways by which *B. fragilis* OMVs mediate host innate immune responses compared to their parent bacteria.

To do this, the size and composition of purified *B. fragilis* OMVs was characterised, revealing that immunostimulatory products including peptidoglycan and nucleic acids were associated with *B. fragilis* OMVs. Additionally, we observed the enrichment of specific protein and LPS cargo into OMVs compared to their parent bacteria. Furthermore, the ability of OMVs to enter and deliver their cargo intracellularly to intestinal epithelial cells was determined using confocal microscopy, and their potential to activate innate immune receptors compared to their parent bacteria was determined using HEK-Blue reporter cell lines. Whilst *B. fragilis* bacteria could only activate Toll-like receptor (TLR)-2, *B. fragilis* OMVs induced the activation of TLR2 as well as TLR4, TLR7 and NOD1 that detect bacterial LPS, RNA and peptidoglycan, respectively. Currently, we are elucidating the mechanisms underpinning the differences in innate immune receptor activation between *B. fragilis* OMVs and their bacteria, and the subsequent immunological outcomes.

Collectively, our results demonstrate that *B. fragilis* OMVs activate different immune signalling pathways compared to their parent bacteria, revealing novel roles for OMVs secreted by the intestinal microbiota in activating host immune responses.

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DURABILITY OF B-CELL MEMORY TO SARS-CoV-2 INFECTION AND VACCINATION

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Background: Lasting immunity following SARS-CoV-2 infection or vaccination is questioned because serum antibodies decline in convalescence. However, functional immunity is mediated by long-lived memory T and B (Bmem) cells, which we hypothesise are more accurate markers of long-term immunity.

Objective: To determine the immunophenotype and durability of SARS-CoV-2-specific Bmem cells in individuals after infection or vaccination for SARS-CoV-2.

Methods: Recombinant Spike receptor binding domain (RBD) and Nucleocapsid (NCP) proteins were produced for ELISA-based serology, and biotinylated for fluorescent tetramer formation to identify SARS-CoV-2-specific Bmem cells by flow cytometry. Cells were obtained from 29 convalescent patients and repeat samples were taken from individuals up to one-year post-infection. In addition, samples were collected from healthy adults immunised with the Pfizer mRNA (n=30) and AstraZeneca vector (n=35) SARS-CoV-2 vaccines at three time points: pre-vaccination, 3-4 weeks post-dose 1 and 4-weeks post-dose 2.

Results: All recovered COVID-19 patients had serum IgG that specifically recognised RBD and NCP proteins, with levels declining beyond 20 days post-infection. Vaccination induced anti-RBD antibodies, which were increased after dose 2, whereas no anti-NCP antibodies were formed. In recovered COVID-19 patients, RBD- and NCP-specific Bmem cell numbers remained stable at 1.25-170 cells/ml of blood in all patients for >240 days post-infection and predominantly expressed IgM or IgG1. Individuals immunized with the Pfizer mRNA vaccination generated RBD-specific Bmem cells at 16-85.4 cells/ml blood 1-month post-dose 2 and also predominantly expressed IgM or IgG1.

Conclusion: Detailed immune profiling revealed durable RBD- and NCP-specific Bmem cells in COVID-19 convalescent individuals, and RBD-specific Bmem cells upon Pfizer vaccination. We will now quantify the serological and antigen-specific Bmem cell response in AstraZeneca vaccinated individuals. This will allow us to compare the generation of durable immunological memory between natural infection and vaccination, as well as between mRNA and vector-based SARS-CoV-2 vaccinations. This could inform on the need for future booster vaccinations and levels of protection to emerging variants of concern.

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Proteomics biomarker discovery for the early diagnosis of neonatal sepsis

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Introduction: Sepsis is a global burden affecting 3 million infants and accounts for >400,000 deaths annually. Infection-related inflammation contributes to long-term adverse neurodevelopmental outcomes in infants that survive sepsis. Sepsis is a frequent complication among extremely preterm infants (<28 weeks gestational age), with ~25% developing sepsis during their NICU stay. Early diagnosis of sepsis is critical to minimise inflammation and facilitate antibiotic therapy, but diagnosis is complicated by slow (24-36 hours) and variable diagnostic tests. Consequently, 2/3 of uninfected infants receive antibiotic therapy, which is in preterm infants is associated with unintended adverse events, including death. Thus, there is an urgent and unmet need for accurate and more rapid adjunct diagnostics to reduce the high prevalence of antibiotic use in this vulnerable population. Proteome differences in response to infection can be used to identify protein biomarkers to improve the current diagnostic approach.

Aim: To use targeted and untargeted approaches to explore the plasma proteome for biomarker discovery in infants with sepsis.

Methods: Untargeted label-free quantitative diaPASEF mass-spectrometry based proteomics and targeted immunoassays were used to explore the plasma proteome of preterm human infants born <29 weeks gestational age with and without sepsis. Plasma was processed from peripheral blood samples collected at first suspicion of sepsis.

Results: In very preterm infants with and without sepsis (n=15 and n=39, respectively), >500 plasma proteins were identified. Over 70 differentially expressed proteins are associated with sepsis. A panel of 7 protein biomarkers can identify sepsis with high accuracy.

Conclusion: We identified known and novel proteins that are associated with sepsis. On-going analysis suggests that a subset of proteins may have clinical utility as biomarkers for early diagnosis of sepsis in very preterm infants.

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Polymicrobial infection interferes pathogen-specific neutrophil responses and impairs bacterial clearance.

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Polymicrobial infections are common in wound infections, especially in diabetic lower-limb wounds. Wounds infected with multiple pathogens are often associated with worse prognoses, delayed recovery, and higher complexity treatments. Despite their clinical significance, little is known about the mechanistic interactions between the polymicrobial community and host immune cells. Using *Staphylococcus aureus* and *Enterococcus faecalis*, two pathogens commonly isolated from diabetic wounds, we established *in vitro* and *in vivo* models to investigate the responses of neutrophils upon polymicrobial infection and their impact on bacterial clearance. Upon exposure to *E. faecalis in vitro,* neutrophils increased intracellular ROS production but did not induce neutrophil extracellular trap (NET) formation, and chemical inhibition of ROS leads to significantly impaired *E. faecalis* killing. Co-infection of *S. aureus* with *E. faecalis* resulted in a significant reduction both intracellular ROS levels and *E. faecalis* clearance, suggesting that *S. aureus* can inhibit intracellular ROS production which promotes *E. faecalis* survival. Conversely, upon exposure to *S. aureus*, neutrophils underwent NETosis in the absence of detectable intracellular ROS. Co-infection with *E. faecalis* resulted in a significant reduction of *S. aureus*-induced NET formation, indicating that *E. faecalis* can interfere with S. aureus-induced NETosis. Using an *in vivo* mouse wound model, we also observed that co-infection is associated with augmented bacterial burdens for both *E. faecalis* and *S. aureus*, compared to either single species infection. Ongoing work will determine whether the *in vivo* synergy in bacterial burden that we observe upon co-infection arises via cross-species neutrophil modulation. Together these results suggest that optimal pathogen-specific neutrophil responses can be subverted in a reciprocal manner during mixed species infection, thus resulting in attenuated neutrophil-mediated clearance and augmented bacterial growth of both co-infecting organisms.

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Nonsense-mediated decay is highly divergent in the malaria parasite *Plasmodium falciparum*

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Nonsense-mediated decay (NMD) is a conserved mRNA quality control process that eliminates transcripts bearing a premature termination codon. In addition to its role in removing erroneous transcripts, NMD is involved in post-transcriptional regulation of gene expression via programmed intron retention in metazoans. The apicomplexan parasite *Plasmodium falciparum* shows relatively high levels of intron retention, but it is unclear whether these variant transcripts are functional targets of NMD. In this study, we use CRISPR-Cas9 to disrupt and epitope-tag two core NMD components: *Pf*UPF1 (PF3D7_1005500) and *Pf*UPF2 (PF3D7_0925800). Using RNA-seq, we find that NMD in *P. falciparum* is highly derived and requires UPF2, but not UPF1 for transcript degradation. Furthermore, our work suggests that the majority of intron retention in *P. falciparum* has no functional role and that NMD is not required for parasite growth *ex vivo*. We localise both *Pf*UPF1 and *Pf*UPF2 to puncta within the parasite cytoplasm, which may represent processing bodies - ribonucleoparticles that are sites of cytoplasmic mRNA decay. Finally, we identify a number of mRNA-binding proteins that co-immunoprecipitate with the NMD core complex and propose a model for a divergent NMD that does not require *Pf*UPF1 and incorporates novel accessory proteins to elicit mRNA decay in the human malaria parasite.

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Dimeric IgA as a novel biomarker of acute measles infection

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Despite tremendous progress in reducing measles incidence through universal vaccine coverage, elimination efforts rely on improved surveillance. Detection of measles-specific immunoglobulin M (IgM) by ELISA is the standard laboratory diagnosis, however, true infection is rare, with most positives being false positives and IgM “true” positivity may also arise following MMR vaccination. This results in low positive predictive values of assays in elimination settings and necessitates confirmatory testing. Improved tests are a WHO research priority. We aimed to determine whether dimeric immunoglobulin A (dIgA), the predominant antibody produced in mucosal immunity, may be a marker of recent or acute measles infection. We examined a commercial panel of IgM positives (confirmed acute infected, n=9), a WHO measles IgM proficiency test panel comprising measles IgM positives (which may be either vaccinees or acute infected, n=6), parvo/rubella/dengue IgM positives (n=7), rubella/measles IgM negatives (n=6), provisional diagnosis confirmed measles IgM negatives (n=12) and a panel of blood donors (n=88) on Euroimmun anti-measles virus lysate (VL) and nucleoprotein (NP) IgM kits, then modified for dIgA using an in-house protocol based on a recombinant chimeric secretory component protein and anti-secretory component monoclonal antibody. We report for the first time high levels of anti-measles VL dIgA in the acutely infected group (median S/Co: dIgA: 2.61, IgM: 1.48) and low correlation with IgM levels (R^{2}: 0.019, p value:0.723), although the dIgA ELISA requires further optimisation to reduce false positives (4/88 blood donors). Interestingly, the IgM positives in the WHO proficiency panel had very low levels of anti-measles VL dIgA compared to IgM (median S/Co: dIgA: 0.74, IgM: 1.66) which may represent relative absence of dIgA after vaccination compared to infection, or collection of samples later after acute infection. Among acutely infected individuals of whom 8/9 were anti-measles NP IgM positive, only 1/9 had detectable anti-measles NP dIgA. In one acutely infected donor with multiple time points, anti-measles VL dIgA declined over time while anti-measles VL IgM remained constant. These data suggest that dIgA is an early and transient response in acute measles that warrants further investigation.

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Stromal cell heterogeneity in the lungs supports epithelial cell regeneration

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The appropriate maintenance and regeneration of the lung epithelium involves complex interactions between epithelial cells and resident stromal cells. In diseases like IPF, abnormal epithelial regeneration results in fibrosis, where (stromal) myofibroblasts accumulate and basal cells which are usually restricted to the airways appear adjacent to alveoli (i.e. honeycombing). Our work investigates whether myofibroblast accumulation and ectopic basal cell hyperplasia occur concomitantly, or have a cause and effect relationship. We used flow cytometry screening for bimodal distribution of surface markers to identify 8 distinct stromal populations in mice, which we then confirmed and characterised with RNA sequencing gene expression analyses. Three stromal populations highly expressed either Cthrc1, Cxcl12 or FGF10 and were termed mesenchymal stem cells, Cxcl12+ auxiliary stromal cells or FGF10+ auxiliary stromal cells, respectively. These 3 stromal populations also expressed markers for stem cell activity and exclusively supported epithelial cell proliferation *in vitro.* Additionally, in an influenza-induced lung injury model (recapitulating honeycombing lung injury), the same 3 stromal cell types were highly proliferative 7-14 days post-infection and this coincided with basal cell expansion. Importantly, proliferation of the 3 epithelial-supporting stromal populations and basal cells occurred before myofibroblast proliferation indicating that reciprocal signalling between stromal progenitor populations and ectopic basal cells occurs prior to, and likely drives, myofibroblast accumulation and fibrotic lung disease. Understanding which of these 8 newly identified stromal populations appropriately support epithelial regeneration, rather than impair it, is key in advancing mesenchymal/stromal stem cell therapies.

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A new *in vitro* colon simulating model to examine *Candida albicans* in the presence of human GI microbiota.

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Candida albicans is a commensal fungus inhabiting the gastrointestinal (GI) tract of >60% of humans. It can however cause serious, life-threatening systemic infections in people immunocompromised or otherwise seriously ill. The source of these infections is most often the endogenous *C. albicans* living in the patient's own GI tract. Despite antifungal intervention, 40% of systemic *C. albicans* infections are lethal. Understanding how *C. albicans* is kept in check through competition with the GI microbiota is therefore important. The most commonly used mouse model of *C. albicans* GI commensalism involves treating mice with a cocktail of antibiotics, precluding the ability to introduce and study the effects of human GI microbiota on *C. albicans* colonisation. We have therefore developed a novel, *in vitro* colon model to allow us to study the effects of human GI microbiota on *C. albicans*.

In order to characterise our *in vitro* colon model, we designed, optimised, and carried out competitive fitness assays utilising deletion mutants for five genes, *WOR1, EFG1, ECE1, CRZ2,* and *SAP6*, the deletion or overexpression of which had been shown to affect the commensal fitness of *C. albicans* in the mouse GI tract. We also carried out transcriptomic analyses comparing *C. albicans* gene expression in our *in vitro* colon model to that in two parts of the mouse GI tract. Consistent with recent *in vivo* findings, we observed the expression of key hypha-specific "virulence" genes in our *in vitro* colon model, despite *C. albicans* growing in the yeast morphology. This supports a role for the products of these hypha-associated "virulence" genes in commensalism.

Overall, the similarities and differences identified through these two comparative studies have allowed us to define where our *in vitro* colon model sits relative to the mouse model of GI commensalism. This will be considered as we take our investigations forward and examine the effect of human GI microbiota on *C. albicans* in a colon-simulating environment, with a view to identifying potentially therapeutic bacterial species residing in the GI tract of humans.

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Wollamide cyclic hexapeptides synergise with tuberculosis antibiotics to inhibit growth of *Mycobacterium tuberculosis*

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Tuberculosis (TB) remains a leading cause of mortality, currently responsible for 1.4 million deaths annually. The TB global health crisis is aggravated by the rise of multi- and extensively drug-resistant *Mycobacterium (M.) tuberculosis*. The World Health Organisation has declared multidrug-resistant TB both a security threat and a public health concern, and urges the development of shorter, safer, more effective TB therapies. Treatment of TB currently requires an extensive course of multiple antibiotics with diverse mechanisms of action. Thus, pre-clinical evaluation of new TB drug leads requires assessment of potential interactions with existing TB antibiotics. We recently reported a new class of *Streptomyces*-derived cyclic hexapeptides, wollamides, which exert anti-mycobacterial activity against drug-

sensitive and drug-resistant *M. tuberculosis*, in the absence of mammalian cytotoxicity. Here, we assessed synergistic and antagonistic effects of wollamides, in combination with approved anti-TB antibiotics, in inhibiting the growth and survival of *M. tuberculosis*. Our data indicate that wollamides do not antagonise current first-line TB antibiotics, alone or in currently used TB treatment regimens. Additionally, wollamides demonstrated synergy with a select subset of current TB antibiotics. These data add to the profile of desirable characteristics of wollamides and encourage further exploration of the utility of the wollamide pharmacophore as a lead for new TB antibiotics.

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Investigating the trans-synaptic transfer mechanism of rabies virus.

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Rabies is a lethal neurotrophic virus which spreads exclusively within interconnected neurons in the host nervous system by transferring across neuronal junctions (synapses). This ability to trans-synaptically transfer between neurons is also shared by other neurotropic viruses such as herpes simplex virus, varicella zoster virus, measles, nipah, west nile virus and poliovirus. Spread of viruses in the nervous system by trans-synaptic transmission cause acute as well as chronic long-term impact on the neurological functioning, if not fatal. Alternatively, the specific trans-synaptic transfer ability of attenuated rabies virus is also exploited beneficially as an anatomical neuronal circuit tracer and as a potential vector system to carry therapeutics to the brain. However, the mechanisms that facilitate the transfer of rabies and other neurotropic viruses across the synapse is unknown. While the envelope protein (glycoprotein) of rabies virus is responsible for this trans-synaptic transmission, the specific neuronal receptors and associated ultrastructural changes occurring at the neuronal synapse to facilitate viral transfer remains unknown.

In this study, we employed advanced confocal imaging techniques in high-containment PC3 laboratories to study trans-synaptic transfer of rabies virus in neurons. Using ex-vivo neuronal models, we performed ultrastructural investigation of rabies virus transfer between neurons in live and fixed cultures. In these studies, we identified novel trans-synaptic transfer mechanisms utilised by highly-neuroinvasive and low-neuroinvasive rabies strains in neurons. Interestingly, we also discovered novel abilities of rabies virus glycoprotein to control the signalling pathways of cell adhesion molecules and modify synaptic architecture in neurons to enable virus transfer. These studies identified new information about how rabies spreads through the nervous system enabling future approaches for rabies treatment and design next-generation rabies-derived vector systems for brain research and drug delivery. In addition, the findings from this study provide valuable and transferable knowledge about how viruses could manipulate the biology of synapses in the nervous system to enable efficient transmission. Targeting these mechanisms could identify treatment strategies to protect the nervous system during viral infections.

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STING is a critical sensor in CD4$^+$ T cells during malaria that stimulates type 1 IFN production, that in turn, promotes the development of IL-10-producing Th1 (Tr1) cells.

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Malaria is a significant public health concern that caused 409,000 deaths in 2019. During malaria, Th1 cells produce pro-inflammatory cytokines to promote parasite clearance. However, IL-10-producing Th1 (Tr1) cells develop to protect tissues, but also suppress Th1 cell-mediated immune responses, contributing to the persistence of infection. To try and better understand the development of Th1 and Tr1 cells during malaria, we performed an RNAseq on Th1 and Tr1 cells from human volunteers participating in controlled human malaria infection (CHMI) studies with *Plasmodium falciparum* and identified *TMEM173* (coding STING) to be more up-regulated in Tr1 cells than Th1 cells. We then employed CRISPR-Cas9 technology to knock down *TMEM173* transcription in human CD4$^+$ T cells. We found that STING activation was critical for the development of Tr1 cells, promoting the expression of interferon (IFN) β1 and IL-10, as well as co-inhibitory receptors (CIRs). In contrast, activation of STING in CD4$^+$ T cells suppressed the generation of Th1 cells *in vivo*. Moreover, we confirmed that STING-mediated development of Tr1 cells required type I IFN signalling, but also discovered that the inhibition of Th1 cell development occurred through a STING-intrinsic but type I IFN-independent mechanism. Importantly, we found that malaria parasites stimulated the expression of IL-10 by CD4$^+$ T cells and increased the sensitivity of Tr1 cells to STING activation. Finally, we found that the JAK1/2 inhibitor ruxolitinib inhibited the STING-dependent generation of Tr1 cells, identifying it as a potential host-directed therapy for improving anti-parasitic immunity during malaria. Together, this data identifies a key CD4$^+$ T cell innate signalling pathway that drives anti-inflammatory immune responses during malaria, as well as a drug to modulate this response.

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Identification and characterisation of the pH-dependent antifungal saltwater crocodile defensin CpoBD13

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Crocodilians are an order of ancient reptiles that have adapted throughout evolution to inhabit microbial-laden environments. Despite commonly receiving wounds during territorial disputes, the likelihood of developing a systemic infection is rare for these animals, indicating a potent immune system. Defensins, a class of cysteine-rich cationic host defence peptides, contribute to the innate immunity of all eukaryotes. These peptides, which permeabilise microbial cell membranes through the direct binding of negatively charged phospholipids, have been well characterised in humans[1] and plants[2], however, the defensins of reptiles are poorly understood.

In this study, to better define the structure-function of crocodilian defensins, *Crocodylus porosus* (saltwater crocodile) β-defensin 13 (CpoBD13) was recombinantly expressed in the methylotrophic yeast *Pichia pastoris*. CpoBD13 was shown to inhibit the growth of the pathogenic fungus *Candida albicans* through the permeabilisation of the cell's plasma membrane. Phospholipid-binding experiments

revealed that CpoBD13 specifically bound the membrane lipid phosphatidic acid (PA). The protein structure of CpoBD13 in complex with PA was determined using X-ray crystallography and revealed that protein-lipid interactions were mediated by arginine and histidine residues. Membrane permeabilisation assays at a range of physiologically relevant pH levels showed that the antifungal activity of CpoBD13 was greater at pH <6.0 due to the increase in charge, and therefore the affinity for PA, accredited to the protonation of the peptide's histidine residues.

These results indicate that the membrane-targeting mechanism, established in the studies of human and plant defensins, has been evolutionarily conserved in the crocodilian defensin CpoBD13. This study has also uncovered that CpoBD13's ability to bind PA and permeabilise fungal cell membranes is regulated by changes in pH, an ability which has not been observed in previous defensin studies.

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Characterising the functional diversity of the gastrointestinal microbiota

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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. There is limited knowledge on how nutrient availability impacts the growth of individual bacterial species and underpins bacterial community structures within the gastrointestinal tract. This has restricted our ability for targeted modulation of the microbiome composition and structure, through dietary interventions and therapeutics, for the treatment of microbiome-related diseases.

Applying a novel high-throughput screening technique, we aimed to undertake functional analysis of members of the microbiota to enhance understandings of how nutrient availability impacts microbiome community structure. Initial phenotypic analysis of 23 common gastrointestinal isolates was performed to assess the nutrient requirements of these bacteria. This analysis has identified growth variation, carbohydrate dependencies and antibacterial activity of phenolic compounds in these isolates for the first time. This provides fundamental knowledge to understand how specific nutritional changes may cause species-level microbiome alterations. Future community level, multi-omic analysis, in the context of this understanding, will provide the potential to elucidate the role of dietary interventions in manipulating the microbiome.

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Single-cycle influenza virus vaccine generates lung CD8$_+$ Trm with a diverse TCR repertoire that preclude the emergence of virus escape mutants

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Current influenza vaccines protect by evoking strain-specific neutralising antibodies, a form of immunity which leaves the population vulnerable to drifted seasonal and newly emerging pandemic strains. Developing vaccines that provide universal protection against both circulating and newly emerging influenza strains remains a health issue of utmost global importance. Vaccines that induce influenza reactive tissue-resident memory CD8$^+$ T cells (Trm) along the respiratory tract have the potential to provide this coveted long term, cross strain immunity. Here, we tested the capacity of a single cycle candidate influenza vaccine (S-FLU) to evoke cross-reactive lung CD8$^+$ Trm. We show that S-FLU immunisation of mice results in the deposition of influenza-specific lung CD8$^+$ Trm which afforded protection against heterosubtypic infection. S-FLU vaccination induced lung CD8$^+$ Trm of reduced antigen sensitivity compared to influenza specific CD8$^+$ Trm generated following natural infection, and this was partly driven by an increased diversity within the T cell receptor (TCR) repertoire of S-FLU-generated CD8$^+$ Trm. Interestingly, this diverse TCR repertoire within this S-FLU-generated memory T cell population, which was in part associated to the limited inflammatory profile evoked by this vaccination regime, was beneficial in thwarting the development of CD8$^+$ T cell-mediated escape mutants. Our results show that S-FLU vaccination generates lung CD8$^+$ Trm with broad TCR repertoire diversity, and this immune profile can protect against severe disease without driving the virus to rapidly evolve and escape vaccine-induced immunity.

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Investigating the immune control of herpesvirus infection in marine molluscs

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We have a limited understanding of the specificities of innate immune protection against viral pathogens in non-model invertebrate species, and comparative immunological approaches may offer opportunities to protect agriculturally significant species against viral threats. Haliotid herpesvirus (HaHV-1) is a re-emerging viral pathogen of abalone, with a high mortality rate in wild and farmed species in Australia. This study sought to examine immune priming strategies against this pathogen, and to describe mechanisms of innate immune antiviral protection in this species.

Immune priming with poly(I:C) offered significant protection against HaHV-1 challenge in Australian hybrid abalone a minimum of 72hrs prior to viral challenge. To investigate the antiviral immune gene landscape in this species, the recently available abalone draft genome of the economically important Australian greenlip abalone was subjected to gene mining strategies and domain database analysis, to gain a putative representation of potential key players involved in the greenlip abalone immune response. This was then compared to the better studied mollusc, the oyster. We highlight that these immune counterparts, mainly from the TLR, cytosolic RNA/DNA and RNA interference signalling pathways are highly divergent between the two molluscs, with the oyster closely reflecting that of the mammalian immune response. Interestingly, we identified that STING, a member of the dsDNA sensing pathway is absent in both the greenlip abalone and in a transcriptome assembly of the Australian blacklip abalone, however, is present in the oyster genome, and two additional abalone species (*Haliotis rufescens, Haliotis discus hannai*). We hypothesise that this may alter susceptibility to HaHV-1 challenge *in vivo*, however further work needs to be performed to determine this.

This work provides a better understanding of the key features of the abalone antiviral innate immune system, providing key information towards the development of immune priming strategies in these animals.

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Tryptase regulates the epigenetic modification of core histones in mast cell leukemia cells

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Mast cells are immune cells that store large amounts of mast cell-restricted proteases in their secretory granules, including tryptase, chymase and carboxypeptidase A3. In mouse mast cells, it has been shown that tryptase, in addition to its canonical location in secretory granules, can be found in the nuclear compartment where it can impact on core histones. Here we asked whether tryptase can execute core histone processing in human mast cell leukemia cells, and whether tryptase thereby can affect the epigenetic modification of core histones. Our findings reveal that triggering of cell death in HMC-1 mast cell leukemia cells is associated with extensive cleavage of core histone 3 (H3) and more restricted cleavage of H2B. Tryptase inhibition caused a complete blockade of such processing. Our data also show that HMC-1 cell death was associated with a major reduction of several epigenetic histone marks, including H3 lysine-4-mono-methylation (H3K4me1), H3K9me2, H3 serine-10-phosphorylation (H3S10p) and H2B lysine-16-acetylation (H2BK16ac), and that tryptase inhibition reverses the effect of cell death on these epigenetic marks. Further, we show that tryptase is present in the nucleus of both viable and dying mast cell leukemia cells. In line with a role for tryptase in regulating nuclear events, tryptase inhibition caused an increased proliferation of the mast cell leukemia cells. Altogether, the present study emphasizes a novel principle for how epigenetic modification of core histones is regulated, and provides novel insight into the biological function of human mast cell tryptase.

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Restricted translation of antiviral cytokines and transcription factors by SARS-CoV-2.

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Post-transcriptional regulation plays an important role in infection and immunity. Regulation of translation and mRNA stability allows tight control over potent responses by both the host and the invading virus. Here, we apply ribosome profiling to assess post-transcriptional regulation of host genes after 24 hours of SARS-CoV-2 infection in the human lung epithelial cell line (Calu-3). We show restriction of numerous transcription factors (JUN, ZBTB20, ATF3, HIVEP2 and EGR1) as well as select antiviral cytokine genes, namely IFNB1, IFNL1,2 and 3, IL-6 and CCL5, at the post-transcriptional level by SARS-CoV-2, consistent with the delayed IFN response seen in COVID19. Upstream open reading frames were not protective against translational down-regulation suggesting a mechanism independent of the integrated stress response. Unstable mRNAs were more sensitive to translational down-regulation, suggesting an early step such as nascent mRNA recruitment to the ribosome may be affected. We know that the SARS-CoV-2 Non-structural protein 1 (Nsp1) acts in this way. An early phase restriction of antiviral transcripts in the lungs may allow high viral load and consequent immune dysregulation typically seen in SARS-CoV-2 infection.

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Preterm infants exhibit a reduced pro-inflammatory immune response to RSV

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Respiratory syncytial virus leads to 33.1 infections and 60,000 in-hospital deaths each year in children under 5 years. The most at-risk demographic to severe RSV disease are infants born preterm, being more likely to require hospitalisation, admission to the intensive care unit and ventilatory assistance. The reason for their increased susceptibility is multi-factorial, however, a major contributor is thought to be an immature immune system. In this study, we have collected cord blood from 25 preterm infants and 25 term infants and stimulated the cord blood mononuclear cells with RSVA or RSVB for 24 and 96h to observe innate and adaptive immune responses. Multiplex assays and flow cytometry were performed at both timepoints whilst RNA-sequencing was only performed at 24h. We found a consistently elevated inflammatory response in term infants following RSVA stimulation through cytokine secretion (IL-1β, IL-6 and IL-17A) chemokine secretion (IL-8 and eotaxin) and gene expression (XCL1, CXCL1, CXCL2, GZMB, NLRP6, IL-1β and IL1R1). Flow cytometry revealed a higher proportion of transitional B-cells, Th2 and Treg populations in preterm infants suggesting promotion of anti-inflammatory immune responses. Overall, preterm infants exhibit a diminished inflammatory response that may contribute to their increased susceptibility to severe RSV disease. These data may aid the development of therapeutics or vaccines to reduce the burden of disease in this highly vulnerable group.

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Molecular surveillance of asymptomatic *Plasmodium falciparum* malaria in high-transmission regions in the context of interventions

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Molecular surveillance is pivotal to fully grasp the impact of malaria control interventions in high-transmission settings. However, the majority of *Plasmodium falciparum* infections in these regions are asymptomatic with low gDNA and are multiclonal. Molecular tools have been created to monitor *P. falciparum* populations yet are seldom validated on the reservoir of asymptomatic infection and in high-transmission settings. We present a population genetic study that compares the performance of a biallelic 20 single nucleotide polymorphisms (SNPs) barcode and 10 polymorphic microsatellite markers on asymptomatic *P. falciparum* isolates in a high-seasonal transmission setting in northern Ghana, West Africa. *P. falciparum* multilocus infection haplotypes were constructed from SNPs and from microsatellites for the same isolates across two age-stratified cross-sectional surveys before and after an indoor residual spraying (IRS) intervention which led to a >90% reduction in transmission intensity and 35.7% reduction in the *P. falciparum* prevalence. The multiplicity of infection (MOI) and genetic diversity parameters were compared between the two markers. Strikingly, 10 SNP loci (50%) had minor allele frequencies ≤10% in the population at both time points (i.e., pre- and post-IRS). Using *THE REAL McCOIL* method to estimate MOI from the SNP-genotyped isolates, we found that it could not reliably estimate isolate MOI when compared to other methods, including *msp2* typing. Population genetic analyses of the SNP infection haplotypes showed low expected heterozygosity, high genetic relatedness, and the presence of clones in the population. However, microsatellite analysis revealed that infection haplotypes were highly diverse with low genetic relatedness, as all multilocus haplotypes were unique. This SNP barcode originated from surveillance in Senegal and yet has proven to be unsuitable for this location in northern Ghana. These data posit that this proposed SNP barcode is not suitable to assess MOI or genetic diversity in this high-transmission setting. This leads us to question the utility of SNP barcode in high-transmission if it cannot deal with multiclonal infections and must be customised for parasite populations at local geographic scales. This study further highlights the utility of microsatellites with multiple alleles per locus as a neutral marker in high-transmission settings.

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Cytomegalovirus infection drives an atypical human Vδ2+ T cell compartment

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Human Vδ2$^+$ T cells form the predominant human γδ T cell population in peripheral blood and possess a semi-invariant Vγ9 and Vδ2 T cell receptor (TCR) chain pairing. This TCR repertoire allows Vδ2$^+$ T cells to function in an innate-like manner towards pyrophosphate metabolites (PAg) and mount TCR-dependent immune responses to bacterial and parasitic pathogens. Here, we explored the heterogeneity of this Vδ2$^+$ T cell immune response in human *Plasmodium falciparum* and cytomegalovirus (HCMV) infection. We find that subpopulations of Vδ2$^+$ T cells express alternate and often expanded TCR clonotypes and often these expanded TCRs define a population of PAg unresponsive Granzyme (Gzm) K$^-$ CD16$^+$ Vd2$^+$ T cells (atypical Vd2$^+$ T cells). Transcriptional profiling of Vγ9/Vδ2$^+$ T cells indicated that clonally expanded atypical Vδ2$^+$ T cells possess a natural killer cell-like transcriptome compared to PAg reactive GzmK$^+$ Vγ9/Vδ2$^+$ T cells. Using a controlled human malaria infection (CHMI) we found that an individual with a predominant clonally expanded atypical Vδ2$^+$ T cell population fails to mount a response to *in vivo P. falciparum* infection in humans. Vδ2$^+$ T cells are classically responsive to both PAg and inflammatory cytokines, we found that neither stimuli could induce the clonal selection of the Vδ2$^+$ TCR. Moreover, four repeated CHMIs over two years in individuals with a predominant PAg reactive GzmK$^+$ Vδ2$^+$ T cell population failed to drive clonal selection. However, cytomegalovirus infection in individuals undergoing lung transplant or hematopoietic stem cell transplantation drove the emergence and selection of atypical Vδ2$^+$ T cells. Together, we reveal an alternative compartment of human Vδ2$^+$ T cells that are selected after acute viral infection and that may be an important avenue for harnessing these cells for anti-microbial immunotherapies.

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Dissecting the role of programmed cell death and inflammation in SARS-CoV-2 infection in vivo

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The SARS-CoV-2 pandemic has resulted in millions of infections and deaths worldwide. Severe COVID-19 is associated with a dysregulated hyperinflammatory immune response, in part, characterized by the release of pro-inflammatory cytokines through inflammasome mediated pathways. This has led to a huge interest in targeting inflammatory mediators and their key activation pathways as COVID-19 therapeutics. Programmed Cell Death is known to drive inflammatory processes via caspase-mediated activation cascades, however the molecular mechanisms underlying these events during SARS-CoV-2 infection remain poorly understood. To address this, we developed and characterized a mouse adapted strain of SARS-CoV-2, derived from a clinical isolate. Multiple passaging of the virus in C5BL6/7 mice resulted in a more virulent strain (JCP21) that causes weight loss, inflammation and lung pathology in young mice and is deadly in old animals, thus reflecting key aspects of human COVID-19 disease. We used a gene-targeted approach to understand the role of cell death and inflammation in SARS-CoV-2 pathogenesis and disease. Infection of mice lacking the pro-inflammatory cytokine IL-1β displayed mitigated disease and decreased viral burden. However, loss of lytic cell death by deletion of pyroptosis initiators Caspase1/11/12 or effectors Gasdermin D, E, C or A or the necroptosis initiator RIPK3 or effector MLKL alone had no effect on disease or viral burden. Interestingly, the loss of Caspase-8-driven apoptosis rescued weight loss, but had no impact on viral load. Combined deficiency of pyroptosis, necroptosis and apoptosis pathways (Caspase1/11/12/8/Ripk3$^{-/-}$) caused significant amelioration of both disease and viral burdens in mice, demonstrating the functional redundancy of the main cell death pathways during SARS-CoV-2 infection. Together, our data suggests that Caspase-1 and Caspase-8 can both drive disease during SARS-CoV-2 infection via the release of pro-inflammatory cytokines through the NLRP3 inflammasome pathway. We are currently exploring pharmacological approaches targeting these key caspases as therapeutic to ameliorate disease in wild type mice.

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Programmable macrophages understanding the role of nrg1 and macrophage plasticity through synthetic biology

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Neuregulin-1 (NRG1) is a pleiotropic growth factor that signals via EGF receptors. It is essential for healthy organ development, cell maturation and metabolism, with defined roles in the heart, skeletal muscle, neurons and the gut. NRG1 belongs to a complex family of transmembrane proteins that are regulated transcriptionally through differential use of promoters and alternative exon splicing, and post-transcriptionally via enzymatic processing of propeptides. This structural complexity makes it hard to discern how NRG1 functions in different tissues, and constitutive loss is lethal in rodent knock-out models. We hypothesize that NRG1 is one of the missing links in *in vitro* developmental protocols of multicellular organoids. Through nanopore long-read sequencing, we have been able to identify and sequence the NRG1 isoforms expressed in human macrophages, which include a myeloid specific isoform that was previously uncharacterised. Applying principles of synthetic biology and genetic engineering to stem-cell derived macrophages, we aim to design a model in which we can track and control NRG1 signalling. This system will allow better understanding of NRG1 dynamics, the influence of its signal in the receiving cell, and the processes that happen prior to its expression in the signalling cell. Developing a macrophage with a controllable and trackable synthetic NRG1 would enable deeper understanding of macrophage roles during organogenesis, as well as deeper understanding of the functions of NRG1 in development and disease processes.

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New insights into the immunological roles of macrophages adjacent to the rete testis and tunica albuginea

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The rete testis and subcapsular regions of the testis are sites of disease onset in murine experimental auto-immune orchitis, but macrophages in these compartments are not well-characterised. Macrophages were localized by immunohistochemistry using an anti-F4/80 antibody in testis sections from adult wild-type mice (n=10-17) and mice with a GFP-expressing transgene at the locus of the macrophage receptor, CX_3CR_1 (*$Cx_3cr_1^{gfp/+}$*;n=6). Sections were also co-labelled by immunofluorescence for the anti-inflammatory marker, CD206, and the antigen-presenting MHC class II molecule (I-A/1-E) to identify activated macrophages. Sections were scanned (Olympus VS120 slide scanner), and macrophages were enumerated by stereology. Compared with the parenchyma surrounding the seminiferous tubules, the volume density of interstitial macrophages (30 macrophages/μm^3) was 10-fold higher in the rete testis, and the density of peritubular macrophages (3.5 macrophages/μm^3) was 3-fold higher. Macrophage density between the tunica albuginea and tubules (subcapsular region) was similar to the rest of the parenchyma, and macrophages were observed within the tunica albuginea itself. In contrast to interstitial macrophages between the seminiferous tubules, which have low MHCII expression, most interstitial macrophages in the rete testis and subcapsular region were $CX_3CR_1^+F4/80^+MHCII^{high}$. Peritubular macrophages in the rete testis were $CX_3CR_1^+F4/80^+MHCII^{high}$, but also expressed CD206, unlike peritubular macrophages of the seminiferous tubules. These data indicate that the majority of macrophages within the rete testis and subcapsular regions differ from macrophages in the rest of the parenchyma in that they have an activated, but possibly anti-inflammatory, phenotype. Accumulation of these macrophages and the phenotype of the peritubular macrophages within the rete testis suggest that they may play a role in recognizing sperm antigens and inducing tolerance to mature sperm leaving the testis, in addition to providing protection against ascending infections of the genital tract. We also predict that alterations in the function of these macrophages during inflammatory disease may cause sperm autoimmunity.

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Pathophysiological changes in a large animal model of COPD

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Background

Chronic obstructive pulmonary disease (COPD) is a serious chronic disease of the airways that affects many people worldwide and currently has only limited treatment options. Sheep have a respiratory system that is in many ways similar to that of humans and can be used as a valid model for the study of airway diseases. The aim of this study was to gain a better understanding of disease pathology in an experimental sheep model of COPD.

Methods

COPD was induced in n=7 sheep following exposure of the lungs to porcine elastase (PE, 50 mg/ml in 5 ml sterile saline) and chronic weekly lung exposures (over 8 weeks) to lipopolysaccharide (LPS, 100 µg/ml in 5 ml saline), delivered via bronchoscopy to the two large caudal lung lobes. The smaller right apical lung lobe in each sheep received saline administrations and served as a healthy control lobe. Control sheep (n=4) that received no PE/LPS lung exposures were also included. Peripheral blood, bronchoalveolar lavage (BAL) and lung function measures were performed throughout the experiment, with lung tissue samples collected and histopathology assessed at post-mortem, 2 weeks following LPS exposures.

Results

Blood and BAL sampling showed significant increases in systemic and local neutrophil levels during the course of disease development. There was a concurrent decrease in lung ventilation within COPD lungs indicative of a decline in breathing capacity over time. Examination of tissue histopathology in Masson's Trichrome-stained lung sections showed only modest changes in lung airspace and tissue percentages as assessed by mean linear intercept measures. However, there was extensive chronic inflammation of the airways, characterised by neutrophilic and macrophage infiltration, with significant increases in both the airway and parenchymal tissue inflammation scores in COPD compared to saline treated lung lobes.

Conclusions

These studies confirmed chronic airway inflammation and pathophysiological lung changes in a sheep model of COPD, providing similarities to that seen in the lungs of COPD patients. This opens up the opportunity for translational studies using this unique large animal model of COPD.

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Methods of vesicle production and quantification can influence immunological outcomes.

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Bacterial membrane vesicles (BMVs) are naturally secreted by all bacteria and can facilitate host-microbe interactions in a multitude of ways via their biologically active cargo. Quantification of BMVs is a critical step that underpins all subsequent assays examining their composition and functions. Historically, protein assays have been the preferred method of BMV quantification. However, BMV protein content can varying with bacterial growth stage, growth conditions and BMV size, suggesting that BMV protein amount may not directly correlate with BMV number.

To investigate this, we compared the enumeration of BMVs from Gram-positive and Gram-negative bacteria using different protein assays, and nanoparticle tracking analysis (NTA). We showed that different protein assays vary significantly in their quantification of BMVs, and

the species from which BMVs originate influence their quantification by different protein assays. Moreover, stimulation of epithelial cells with an equivalent amount of BMVs quantified by different protein assays resulted in significant differences in IL-8 responses. Quantification of BMVs by NTA and normalization of BMV cargo to particle number revealed BMV protein, DNA and RNA content were variable between strains, species and throughout bacterial growth. The effect of strain differences in BMV protein content on their ability to activate NF-κB and induce IL-8 responses were evident when epithelial cell stimulations were based on particle number, but not when stimulations were based on protein amount. Similarly, strain differences in the ability of BMVs to activate TLR7 and 9 by HEKBlue reporter cell lines were evident when stimulations were based on particle number but not protein amount.

In summary, our findings show that standardization of BMVs by protein amount can reduce the ability to distinguish differences in their immunological functions. In contrast, species, strain and growth stage-dependent differences in BMV cargo content and immunogenic functions are evident when BMVs are enumerated by particle number. These findings highlight that standardization of BMV enumeration is required in the BMV field to bring uniformity and comparability, and ultimately advance our understanding of BMV functions.

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Tissue-specific contributions of WNT production and signalling to cytokine and chemokine responses during *E. coli* infection

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Publish consent withheld

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Targeting T follicular helper cells to improve immunity to malaria

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Immunity to malaria is mediated by antibodies that block parasite replication to limit parasite burden and prevent disease. T follicular helper (Tfh) cells play critical roles in the induction of functional and protective antibody responses in malaria. In humans, Tfh cells have been observed in the circulation and can be differentiated into various subsets including Th1-, Th2-, and Th17-. We have previously shown in volunteer infection studies of naïve adults infected with *Plasmodium falciparum* that Th2-cTfh (circulating Tfh) cells are strongly associated with induction of functional antibodies, but not Th1- or Th17-cTfh cells. Our group is now focused on developing Th2-Tfh targeting approaches that may be used to boost immunity in at risk populations. However, current knowledge of the pathways driving the heterogeneity in cTfh cells is limited. We have applied single-cell RNA sequencing on cTfh cells isolated during experimental human malaria to delineate pathways underlying the differentiation of specific Tfh subsets and to characterize the transcriptomic dynamics of cTfh cells in malaria. In addition, within the experimental human malaria infection model, we are testing whether host-directed therapy can boost Tfh cell responses in human malaria by blocking Type I Interferon signalling. Excessive Type I Interferon signalling has been linked to sub-optimal Tfh cell responses via activation of different immunoregulatory pathways in malaria. Together, our research will improve upon strategies to better target Tfh cell responses for enhancing vaccine efficacy in at-risk populations of malaria.

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Apicoplast derived metabolites are essential for the biosynthesis of glycophosphatidylinositol anchors and egress of asexual stage Plasmodium falciparum.

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The addition of glycophosphatidylinositol (GPI) anchors represents an important post-translational modification which enables proteins associate with biological membranes and is the predominant glycoconjugate in *Plasmodium* parasites. During the intraerythrocytic asexual cycle the GPI-anchored proteome is predominantly expressed in the later trophozoite and schizont stages and is almost exclusively localised to the merozoite surface where they constitute greater than 50% of the total surface protein coat. Notable among these is merozoite surface protein 1 (MSP1), which has an established function in egress whereby it contributes to the destabilization of the host red blood cell cytoskeleton, leading to cell rupture and the release of daughter parasites. An essential component to GPIs is the sugar residue mannose which is donated by dolichol-phosphate in the endoplasmic reticulum. Dolichols themselves are believed to derive from isoprenoid precursors synthesised in the *Plasmodium* apicoplast, a relict plastid organelle of Prokaryotic origin. We found that treatment of *Plasmodium* parasites with apicoplast inhibitors decreases the abundance of isoprenoid and GPI intermediates resulting in GPI-anchored proteins becoming untethered from their normal membrane association. This detachment results in the mis-localisation of surface GPI-anchored proteins to the parasitophorous vacuole. Following the loss of surface bound MSP1, these GPI-deficient parasites experienced an egress defect, though surprisingly also exhibited aberrant membrane morphology and unsuccessful segmentation. Our data provides further evidence for the importance of GPI biosynthesis during the asexual cycle of *P. falciparum*, and indicates that GPI biosynthesis, and in extension egress, is dependent on isoprenoids synthesised in the apicoplast.

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Charting elimination in the pandemic: a SARS-CoV-2 serosurvey of blood donors in New Zealand

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New Zealand had a strategy of eliminating SARS-CoV-2 that resulted in a low incidence of reported coronavirus-19 disease (COVID-19). The aim of this study was to describe the spread of SARS-CoV-2 in New Zealand via a nationwide serosurvey of blood donors, prior to vaccination roll-out. Samples (n=9806) were collected over a month-long period (December 3rd, 2020 - January 6th, 2021) from donors aged 16-88 years. The sample population was geographically spread, covering 16 of 20 district health board regions. A series of Spike-based immunoassays were utilized, and the serological testing algorithm was optimized for specificity given New Zealand is a low prevalence setting. Samples were first screened with a widely used and well-validated 2-step ELISA that comprises a single point dilution assay against the RBD followed by titration against trimeric S protein. Samples above the cut-off were tested on two further immunoassays – the EuroImmun SARS-CoV-2 IgG ELISA and the cPass surrogate Viral Neutralization Test and deemed seropositive if above the cut-off on both commercial assays. Of the 9806 samples, eighteen were seropositive for SARS-CoV-2 antibodies, six of which were retrospectively matched to previously confirmed COVID-19 cases. A further four were from donors that travelled to settings with a high risk of SARS-CoV-2 exposure, suggesting likely infection outside New Zealand. The remaining eight seropositive samples were from seven different district health regions for a true seroprevalence estimate, adjusted for test sensitivity and specificity, of 0.103% (95% confidence interval, 0.09-0.12%). The very low seroprevalence is consistent with limited undetected community transmission, providing robust, serological evidence to support New Zealand's successful elimination strategy for COVID-19 prior to the emergence of the delta variant in the community in August 2021. This study also demonstrates the ability for blood donor services to be used to rapidly conduct seroprevalence studies, and highlights the ongoing need for vaccine-induced protection in the New Zealand population.

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Long-read RNA sequencing identifies polyadenylation elongation and differential transcript usage of host transcripts during SARS-CoV-2 *in vitro* infection

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Improved methods to interrogate host-pathogen interactions during Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infections are crucial to help understand and prevent this disease. Here we implemented RNA-sequencing (RNA-seq) coupled with the Oxford Nanopore Technologies (ONT) long-reads to measure differential host gene expression, transcript polyadenylation and isoform usage within various epithelial cell lines permissive and non-permissive for SARS-CoV-2 infection. SARS-CoV-2-infected and mock-infected Vero (African green monkey kidney epithelial cells), Calu-3 (human lung adenocarcinoma epithelial cells), Caco-2 (human colorectal adenocarcinoma epithelial cells) and A549 (human lung carcinoma epithelial cells) were analysed over time (0, 2, 24, 48 hours). Differential polyadenylation was found to occur in both infected Calu-3 and Vero cells during a late time point (48 hpi), with Gene Ontology (GO) terms such as viral transcription and translation shown to be significantly enriched in Calu-3 data. Poly(A) tails showed increased lengths in the majority of the differentially polyadenylated transcripts in Calu-3 and Vero cell lines (up to ~136 nt in mean poly(A) length, padj = 0.029). Among these genes, ribosomal protein genes such as *RPS4X* and *RPS6* also showed downregulation in expression levels, suggesting the importance of ribosomal protein genes during infection. Furthermore, differential transcript usage was detected in Caco-2, Calu-3 and Vero cells, including transcripts of genes such as *GSDMB* and *KPNA2*, which have previously been implicated in SARS-CoV-2 infections. Overall, these results highlight the potential role of differential polyadenylation and transcript usage in host immune response or manipulation of the host by the virus during SARS-CoV-2 infection.

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Inflammatory bowel disease remodels circulating and tissue-resident populations of human Vd1^{+} T cells.

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γδ T cells have evolved as a third lymphocyte lineage alongside αβ T cells and B cells over millions of years of vertebrate evolution[1]. Human gd T cells constitute only a small proportion of all the circulating T lymphocytes in the blood but are enriched at mucosal barriers[2].

Inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease (CD) is thought to involve a dysregulated response from the mucosal immune system to the intestinal microbiota resulting in chronic inflammation of the gastrointestinal tract[3]. Here, we investigated the dynamics of human paediatric gd T cell subsets in the intestinal tissue and circulation at the early stages of IBD. Using immunophenotyping and T cell receptor (TCR) repertoire profiling we found that children at the first diagnosis of IBD displayed clonal populations of $Vd1^+$ TCRs in the blood compared to age and gender matched healthy children. Moreover, circulating $Vd1^+$ T cells in IBD children had shifted their phenotype from a $CD27^+CD28^+$ naïve-like population to a cytotoxic $CD27^{neg}$ effector population. We then explored the relationship of circulating and intestinal resident populations of $Vd1^+$ T cells. Firstly, we found that expanded $Vd1^+$ T cell clonotypes in circulation, duodenum, terminal ileum and rectum contained unique tissue-location dependent TCR repertoires. Secondly, chronic Crohn's disease associated inflammation drove the remodelling of terminal ileum resident $Vd1^+$ T cells. Our study indicates that tissue-resident $Vd1^+$ T cells in discrete niches of the intestine display unique TCR repertoires. Morevoer, the onset of IBD has a major impact on intestinal and circulating populations of $Vd1^+$ T cells. These findings show that the γδ T cell repertoire is highly responsive to the early stages of chronic intestinal inflammation

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Inhaled pirfenidone reduces transforming growth factor-beta-enhanced lung viral infection and inflammation as effectively as oral pirfenidone

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Introduction & Aims: Patients with respiratory diseases are both more susceptible to viral infection and developing more severe symptoms. These exacerbated symptoms often lead to deleterious effects such as irreversible disease progression. Emerging evidence suggests that the elevated transforming growth factor-beta (TGF) seen in patients with respiratory disease likely plays a crucial role in suppressing the immune response. Oral pirfenidone (PFD), an anti-fibrotic used to treat patients with pulmonary fibrosis, has previously been shown to reduce TGF-enhanced viral infection. This study aimed to determine if inhaled PFD was as beneficial as oral PFD.

Methods: Transgenic C57Bl/6 mice with inducible over-expression of TGF were treated with intranasal vehicle (control), oral PFD (oPFD, 100mg/kg) or intranasal PFD (iPFD, 6.7mg/kg) daily, starting 2 days prior to infection with IAV ($1x10^2$ PFU, HKx31). At 3 days post infection, mice were culled. Tissue and bronchoalveolar lavage (BAL) were collected for assessment of infection, inflammation and immune response.

Results: oPFD afforded protection against IAV-induced weight loss ($p<0.01$), while both oPFD and iPFD reduced lung viral load ($p<0.05$) as measured by plaque assay of lung homogenates. TNFa and KC in BAL fluid were also reduced by both oPFD and iPFD ($p<0.05$), as measured by ELISA.

Conclusion: This study demonstrated that a 15-fold lower dose of iPFD was able to afford protection against TGF-enhanced viral infection and inflammation. These promising findings present the possibility of treating patients with respiratory disease with low dose inhaled PFD, protecting them from worse infection outcomes with fewer side effects.

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Benchmarking transcriptional profiles of immune cells online at Stemformatics

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Stemformatics is an established online gene expression data portal containing hundreds of carefully curated and annotated datasets, which include many haematopoietic cell types. One of Stemformatics' key features is the integrated data atlas where the user can project their own data and classify their cell types against a reference.

The website has recently been completely redesigned using latest web technologies to provide a smooth experience for the users. Many other features are also available at Stemformatics, including gene expression plots for each dataset and tools for finding interesting genes across all datasets.

Stemformatics is a fully open source resource, available at www.stemformatics.org.

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Specific gut microbiota-epithelial cell interactions induces the unfolded protein response and ER stress pathways

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Recent advances in novel culturing methods and high-resolution sequencing have provided significant insight into the composition and function of the human gut microbiota. Despite the known associations between the microbiota and the host immune response, there is an unmet need to define these interactions, particularly in disorders with a complex pathophysiology such as inflammatory bowel disease (IBD). Site-specific mucosal culturing of intestinal biopsies has enabled the establishment of a human gut microbiome culture collection from patient cohorts. Host gene transcriptional analysis and bacterial metagenomic sequencing identified key gut microbiota subclades that are associated with an inflammatory gene signature. To experimentally validate these findings *in vitro*, mucosal bacterial isolates were co-cultured with intestinal epithelial cells which resulted in a cell cytotoxic phenotype and transcription of host genes belonging to the unfolded protein response (UPR) and endoplasmic reticulum (ER) stress pathways. Epithelial cell cytotoxicity was subsequently observed following bacterial co-culture in Transwell assays and similarly in response to conditioned media from bacterial-stimulated epithelial cells. This indicates that there may be specific microbial factors or metabolites that function as mediators of innate immune signalling and highlights the importance of microbiota-host cell crosstalk. Further studies will provide a better understanding of microbiota-host interactions in IBD and other microbiota-associated disorders, unlimited to the gastrointestinal tract.

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Australian Aboriginal children with otitis media produce high avidity serum IgG to potential non-typeable *Haemophilus influenzae* vaccine antigens at lower titres when compared to non-Aboriginal children

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Introduction: Australian Aboriginal children have the highest rates of otitis media (middle ear infections) globally. This infection is predominantly caused by the bacteria non-typeable *Haemophilus influenzae* (NTHi). Previously we demonstrated that Aboriginal children with otitis media have antigen-specific reduction in serum IgG specific for NTHi antigens rsPilA, ChimV4 and Protein D compared to non-Aboriginal children with and without OM. However there were no difference in serum IgG specific for NTHi antigen Outer Membrane Protein 26 (OMP26) in Aboriginal and non-Aboriginal children.

Objective: To assess natural serum anti-rsPilA, anti-ChimV4, anti-PD and anti-OMP26 IgG avidity in Aboriginal, non-Aboriginal children with otitis media and non-Aboriginal children without otitis media.

Methods: Sera were collected from 77 Aboriginal and 70 non-Aboriginal children with otitis media and 36 non-Aboriginal children without otitis media. Antigen-specific serum IgG avidities were measured using an in-house multiplex immunoassay. IgG avidities were compared between groups using ANOVA.

Results: Aboriginal children with otitis media had similar proportions of high avidity IgG specific for rsPilA, ChimV4 and Protein D compared to non-Aboriginal children with and without otitis media (rsPilA: 56-62%, ChimV4: 50-61% and Protein D: 87-90%; $p>0.05$). However Aboriginal and non-Aboriginal children with otitis media had a higher proportion of high avidity IgG to OMP26 compared to children without otitis media (73% and 70% vs 61% respectively; $p<0.05$). IgG avidity had no correlation with IgG titre or age for any antigen.

Conclusion: Australian Aboriginal children produce high avidity IgG to important NTHi antigens rsPilA, ChimV4 and PD, but at reduced levels. Boosting antibody titres through vaccination strategies using these antigens may impact the chronicity and high burden of otitis media in Australian Aboriginal children.

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Whole-body analysis of tissue-resident immune cells

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Tissue-resident memory T (TRM) cells are a non-circulating lymphocyte population that are principally located in peripheral tissues. TRM cells provide rapid protection against a wide range of infections and cancer; hence, enhancing TRM cell formation and persistence is an attractive means for establishing durable immunity. While many studies have dissected the properties of TRM cells within peripheral tissues in mice, our knowledge of human T cells has been largely derived from blood sampling. In collaboration with Austin Health, we have established the first Australian Donation and Transplantation Biobank that provides access to a wide range of healthy human organs. Using this resource, we performed a whole-body analysis of TRM cells across barrier and non-barrier tissue sites. We employed multiparameter flow cytometry and scRNAseq to resolve distinct TRM cell populations across the gut, skin, liver and spleen. We observed intra- and inter-organ TRM cell heterogeneity based on the expression of tissue residency markers CD69 and CD103, and inhibitory molecules such as PD-1 and CD244. Furthermore, we have demonstrated how the tissue microenvironment influences various TRM cell functional capabilities. Together, this holistic characterisation of TRM cells across solid organs underscores the importance of investigating local tissue immunity which cannot be discovered by conventional blood sampling. The results of this study will direct novel tissue-specific immunotherapies aimed to promote and establish durable tissue immunity.

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Developing antimicrobial zinc ion embedded polymers for One Health applications

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Antimicrobial resistance is one of the foremost threats of the 21st century, with a disruptive impact on global health and economies estimated to be on the scale of climate change. The World Health Organisation has called for innovative solutions to the threat posed by multi-drug resistant 'superbugs' with new, multifaceted approaches to control bacterial infections being urgently required. Prior work from our group and others have shown the potential of first-row transition metal ions to prosecute antimicrobial activity against a broad range of bacterial pathogens of animals and humans. Here, we worked with Ascend Performance Materials using their Acteev™ technology platform to investigate antimicrobial applications of ionic zinc incorporated into polyamide polymer matrices in an active form. We report that zinc-embedded Acteev™ materials exert antimicrobial activity against a broad range of high priority bacterial pathogens including *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae*. Using the industry standard ISO20743 testing framework the antimicrobial efficacy of Acteev™ materials was shown for laboratory reference strains and multidrug resistant strains of *S. aureus*, *K. pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *E. coli, Acinetobacter baumannii* and *Enterococcus faecium*. Time to kill analyses reveal that Acteev™ exerts potent antibacterial activity shortly after contact, with potent activity within 2-6 hours. Further, elemental analyses of Acteev™ materials show that embedded zinc ions are retained after multiple wash cycles highlighting the potential for re-use, where appropriate. Collectively, these data show that zinc-embedded Acteev™ materials have a range of potential applications across a breadth of settings, such as in hospitals and in the community, to enable control of bacterial pathogen transmission in an antibiotic independent manner.

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Novel fluorescent TNF reporter systems for characterisation of TNF expression

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Tumour Necrosis Factor (TNF) is a pro-inflammatory cytokine with a diverse range of biological functions, and dysregulation of TNF expression is associated with various autoimmune and inflammatory diseases including rheumatoid arthritis and inflammatory bowel disease. TNF expression is dependent upon both transcriptional and post-transcriptional mechanisms, but a reliable reporter of TNF expression *in vivo* is still lacking. We therefore sought to develop a fluorescent TNF translational reporter mouse where functional TNF protein is preserved, permitting accurate visualisation and observation of TNF-producing cells by a variety of approaches including intravital and live imaging.

We have generated two knock-in fluorescent TNF reporter mouse strains utilising tdTomato-TNF fusion protein expression constructs we have previously characterised and validated *in vitro*. In cell lines, the positioning of tdTomato within the TNF open reading frame significantly altered TNF expression between our two reporter constructs, however, similar patterns of TNF expression were observed *in vivo* between our two reporter mouse strains. Our TNF reporter animals, while expressing robust fluorescence, exhibit reduced TNF protein expression in a gene dose-dependent manner where both heterozygosity and homozygosity for the reporter allele lead to clear reductions in the amount of TNF protein produced. Despite successful expression *in vitro*, preliminary results also suggest homozygosity for the reporter allele yields near-complete loss of TNF expression, however, characterisation of homozygote reporter animals is ongoing. Collectively, our results highlight the complexity of developing reporter models for the study of TNF *in vivo*, and we are now investigating alternatives to the TNF fusion protein approach to develop our translational TNF reporter.

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The sulfonylpiperazine MMV020291 prevents red blood cell invasion through interference with actin-1/profilin dynamics in the malaria parasite *Plasmodium falciparum*

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With emerging resistance to frontline treatments, it is vital that new antimalarial drugs are identified to target *Plasmodium falciparum*. A critical process during the parasite's asexual lifecycle is invasion of red blood cells (RBCs), a mechanism that requires many unique parasite proteins that could be exploited as druggable targets. We have recently reported the identification of a compound, MMV020291, as a specific inhibitor of RBC invasion, and successful drug resistance selection was performed. Whole genome sequencing on three MMV020291 resistant populations revealed three non-synonymous single nucleotide polymorphisms (SNPs) in two genes; two populations had SNPs in a gene encoding *profilin* (N154Y, K124N) and the third had a SNP present in the *actin-1* gene (M356L). Using CRISPR-Cas9, we engineered these SNPs into wildtype parasites, reproducing parasite resistance to MMV020291. Monomeric actin-1 is polymerised to form filamentous strands, which are crucial for generating the force required for RBC invasion, with profilin acting to regulate this process by binding monomeric actin-1. MMV020291 appears to inhibit actin polymerisation and we are seeking to understand how

the compound engages its target proteins. We have developed a series of MMV020291 analogues, achieving potency with an EC_{50} <100 nM, indicating the possible development for invasion blocking drugs. In addition, MMV020291 may be utilised a research tool to study the complex actin dynamics in the invading malaria parasite.

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PKR is a sensor of proteotoxic stress via accumulation of cytoplasmic IL-24

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Proteasome dysfunction can lead to an autoinflammatory disease associated with elevated type I interferon (IFNαβ) and NF-κB signalling, however the innate immune pathway driving this is currently unknown. Here, we identify Protein Kinase R (PKR) as an innate immune sensor for proteotoxic stress. PKR activation was observed in cellular models of decreased proteasome function and in cells from proteasome-associated autoinflammatory disease (PRAAS) patients. Furthermore, genetic deletion or small molecule inhibition of PKR ameliorated inflammation in these contexts. Similarly, proteasome inhibitor induced inflammatory gene transcription was blunted in PKR deficient mice compared to littermate controls. Appropriately, PKR is a rheostat for proteotoxic stress, its activation triggers phosphorylation of eIF2α which prevents the translation of new proteins, thereby promoting a cell's return to homeostasis. Although traditionally known as a sensor of RNA, under conditions of proteasome dysfunction PKR senses the cytoplasmic accumulation of a known interactor, interleukin 24 (IL-24). Importantly, blocking misfolded IL-24 egress into the cytosol via the endoplasmic reticulum degradation pathway blunted PKR activation and subsequent inflammatory signalling. Cytokines such as IL-24 are normally secreted, therefore cytoplasmic accumulation of IL-24 represents a danger associated molecular pattern for the cell. Thus, we have identified a novel mechanism by which cells sense proteotoxic stress, causing inflammation observed in the disease PRAAS.

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The good, the bad and the ugly: The functional IgA response in convalescent COVID-19 patients.

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Mutations in the receptor binding domain (RBD) (e.g.N501Y) of SARS-CoV2 has resulted in emergence of variants of concern (alpha). Following infection, virus-specific antibodies are generated that can neutralise and clear the virus via Fc effector functions (phagocytosis). The importance of IgG antibodies for protection and control of SARS-CoV2 has been extensively reported. In comparison, other antibody isotypes including IgA have been poorly characterized, especially to variants. Here we endeavoured to determine the functional contribution of plasma IgA from convalescent COVID-19 subjects.

IgA and IgG was purified from the plasma of convalescent COVID-19 patients (n=58) and healthy controls (n=25). IgA and IgA+IgG depleted plasma fractions were also collected. SARS-CoV2-specific antibody responses were characterized via multiplex assay. Neutralization was assessed via a multiplex ACE2-RBD binding inhibition assay. Samples were also characterized for their Fc functional capacity using a THP-1 cell bead-based phagocytosis assay and a cell association assay. Multivariate analysis was used to compare purified antibody binding to different RBD mutants.

Convalescent patients induced SARS-CoV2-specific IgG (100%) and IgA (91.38%) with 85.19% of patients able to inhibit ACE2-RBD binding. IgA depletion from plasma significantly increased neutralization (median=62.12%, $p=0.0013$) compared to plasma (median=39.62%). Interestingly, purified IgG and IgA exhibited differential antibody binding to 15 RBD mutants e.g. alpha (N501Y) ($p<0.05$) and neutralization to 5 mutants ($p<0.05$). Finally, IgA depletion resulted in similar Fc function as plasma, however, IgA+IgG depletion drastically reduced the phagocytosis ($p<0.0001$) and cell association ($p<0.0001$) compared to plasma.

We show SARS-CoV2-specific IgA responses are induced in most convalescent COVID-19 individuals, with negligible Fc functional capacity in comparison to IgG. Furthermore, potent IgA neutralisation was observed within a small subset of these individuals. Surprisingly, depletion of IgA from plasma increased neutralizing capacity of plasma in certain individuals, suggesting that IgA may block the binding of other neutralising antibody isotypes. Notably, purified IgG and IgA displayed differential binding to RBD mutants, such as alpha variant, suggesting convalescent antibody class switching could impact the capacity of plasma to neutralise different COVID variants. Understanding the mechanisms behind IgA neutralization and IgA mediated blocking is warranted to provide insights for improved vaccination and antibody therapies.

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Uncovering resident macrophages in the mammary gland by 3D and intravital imaging

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The mammary gland is a unique organ that develops predominantly after birth and undergoes dramatic remodelling in reproductive phases. The immune system is intimately tied to mammary gland function and disease, with key roles in morphogenesis, tissue remodelling, pathogen clearance and cancer. Although macrophages have been implicated in mammary gland function and remodelling, their diversity has not been fully addressed. Through whole organ-clearing and high-resolution 3D imaging, we have identified a unique population of dendritic-shaped tissue-resident ductal macrophages (DMs) that form a contiguous network between the luminal and basal layers of the entire mammary gland throughout post-natal development. The distinct localisation of DMs allowed characterisation of marker expression and subsequent isolation by flow cytometry. We show that DMs are uniquely dependent on the epithelium and are long-lived tissue-resident macrophages. While they initially originate from embryonic precursors, DMs are generated from monocytes as they increase in parallel with the epithelium during puberty. We observed DM interaction with the epithelium using a novel intravital imaging approach. This revealed that DMs do not migrate but constantly survey the epithelium through dendrite movement. DMs undergo proliferation in pregnancy to maintain complete coverage of the epithelium in lactation, where they are poised to phagocytose milk-producing cells post-lactation and facilitate remodelling. DMs strongly resemble mammary tumour-associated macrophages and increase throughout tumorigenesis to form a network that pervades the tumour epithelium. Thus, the mammary epithelium programs specialised resident macrophages in both physiological and tumorigenic contexts. This knowledge of a new breast macrophage population will help future studies on how breast-resident immune cells respond to infection and cancer.

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Interferon ε as a novel regulator of intestinal homeostasis

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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 an incomplete understanding of T1IFN signalling in the intestine. 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 decreased in inflamed conditions. Furthermore, our results show IFNε limits 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.

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. Interestingly, neutralisation of IFNε in IFNAR2-/- mice resulted in ameliorated colitis severity. This indicates that while conventional IFNε signalling is protective, non-canonical signalling by IFNε at this site is pathogenic.

These findings show the importance of IFNε in mucosal immunity and IBD, where non-canonical signalling may be pathologically relevant. As IFNε is expressed constitutively in mucosal epithelium, this makes it an appealing target for therapy in IBD.

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The efficacy and safety of pinocembrin in a sheep model of bleomycin-induced pulmonary fibrosis

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Background

The primary flavonoid, pinocembrin, is thought to have a variety of medical uses which relate to its reported anti-oxidant, anti-inflammatory, anti-microbial and anti-cancer properties. Some studies have reported that this flavonoid has anti-fibrotic activities. In this study, we investigated whether pinocembrin would impede fibrosis, dampen inflammation and improve lung function in a large animal model of pulmonary fibrosis.

Methods

Fibrosis was induced in two localised lung segments in each of the 10 sheep participating in the study. This was achieved via two bronchoscopic infusions of bleomycin into the left and right caudal lobes at a two-week interval. A third lung segment in the same sheep was left untreated and served as a healthy control segment. The animals were kept for 5 weeks after the second bleomycin infusion. Pinocembrin, isolated from *Eucalyptus* leaves, was administered to one of the two bleomycin-damaged lung segments at a dose of 7 mg dissolved in 5 ml 10% DMSO solution. This dose was given once-weekly over 4 weeks, starting one week after the second bleomycin infusion.

Results

Lung compliance (as a measure of stiffness) was significantly improved after four weekly administrations of pinocembrin to bleomycin-damaged lung segments. There were significantly lower numbers of neutrophils and inflammatory cells in the bronchoalveolar lavage of bleomycin-infused lung segments that were treated with pinocembrin. Compared to bleomycin-damaged lung segments without drug treatment, pinocembrin administration was associated with significantly lower numbers of $CD8^+$ and $CD4^+$ T cells in the lung parenchyma. Histopathology scoring data showed that pinocembrin treatment was associated with significant improvement in inflammation and overall pathology scores. Hydroxyproline analysis showed that the administration of pinocembrin did not reduce the increased lung collagen content induced by bleomycin in this model. Analyses of Masson's Trichrome stained lung tissue sections showed that pinocembrin treatment significantly reduced the connective tissue content in lung segments exposed to bleomycin when compared to bleomycin-infused lungs that did not receive pinocembrin.

Conclusions

The striking anti-inflammatory and modest anti-fibrotic remodeling effects of pinocembrin administration were likely linked to the compound's ability to improve lung pathology and functional compliance in this animal model of pulmonary fibrosis.

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Developing inhibitors against fungal inositol polyphosphate kinases (IPK) as a novel class of antifungal drug

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Background:

Invasive fungal infections (IFIs) kill ~1.5 million people each year. Despite this, few antifungal drug classes are available to treat IFIs and they have poor efficacy and/or significant toxicity. Furthermore, drug-resistant fungi have emerged. New drug classes are urgently required to address this unmet global medical need.

Aim:

Our aim is to develop a novel class of antifungal drug targeting inositol polyphosphate (IP) kinases (IPK). These drugs would have a different mode of action to current drugs, which predominantly block cell wall and ergosterol biosynthesis. We and others showed that genetically ablating the kinase that produces IP_3 (IP3K) in the major human fungal pathogens, *Cryptococcus neoformans* and *Candida albicans,* leads to multiple cellular defects and avirulence or lethality [1-3]. These data suggest that inhibition of IP3K would clear a fungal infection by inhibiting numerous cellular functions as opposed to a single function inhibited by the current drugs.

Methods:

We used 2 approaches to develop IPK inhibitors:

(1) rational design from two molecular starting points; dibenzylpurines (DBP), which we showed have selectivity for fungal over human IPKs (Monash collaboration) and a novel scaffold provided by University of North Carolina (UNC);

(2) fragment-based drug discovery (FBDD).

To complete each approach, we purified recombinant IP3K from *C. neoformans* and *C. albicans* and developed assays to assess inhibitor potency.

Results:

We synthesised 44 DBP analogues. However, the half-inhibitory concentration (IC_{50}) of these analogues remained too high against *Cryptococcus* IP3K (μM IC_{50}s) and none inhibited *Candida* IP3K. In contrast, UNC analogues had IC_{50}s of 30nM/400nM for *Cryptococcus/Candida* IP3K. FBDD identified 3 new molecular scaffolds that inhibited both fungal IP3Ks.

Conclusion:

(1) The UNC compounds are a promising scaffold for developing potent, pan fungal IP3K inhibitors. (2) FBDD identified additional novel lead scaffolds with scope for development into pan fungal IPK inhibitors.

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The calcineurin and TOR signaling pathways are critical for mitigating stress caused by phosphate overload in invasive fungal pathogens

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Invasive fungal diseases cause more than 1.5 million deaths each year. The essential nutrient, phosphate, is critical for invasive fungal pathogens to cause disease. Phosphate levels are regulated by the PHO pathway, which consists of a CDK complex: the Pho85 kinase, the Pho80 cyclin and the CDK inhibitor Pho81. We and others have shown that phosphate deficiency achieved by blocking the activation of the phosphate acquisition (PHO) pathway, attenuates disseminated infection in mouse models of candidiasis and cryptococcosis. By creating a Pho80 cyclin mutant (*pho80*Δ), we now demonstrate that constitutively elevating phosphate in *Cryptococcus neoformans* also abrogates pathogenicity. To investigate the impact of elevated phosphate on cellular function, we compare the transcriptomes of WT and *pho80*Δ using RNAseq and use the data to select a range of conditions to test *pho80*Δ growth. The results demonstrate that *pho80*Δ accumulates metal ions, including calcium, has compromised mitochondrial function and ER stress response and is sensitive to inhibitors of the energy-sensing TOR pathway and inhibitors of the Ca^{2+}-dependent phosphatase, calcineurin. The calcineurin pathway promotes growth during heat, ER, and oxidative stress. Phosphate deprivation rescues *pho80*Δ sensitivity to both TOR and calcineurin inhibition, suggesting that excess phosphate or excess phosphate-cation complexes are cytotoxic and that the calcineurin and TOR pathways are essential for mitigating the stress associated with phosphate overload, especially at 37°C. Using fluorescent reporter strains, we also demonstrate that the calcineurin pathway is activated not only by heat stress, but also by phosphate deprivation; and that the calcineurin response is reduced in the phosphate-rich *pho80*Δ mutant. Our data provide novel mechanistic insight into how calcineurin pathway activation is critical for alleviating the stress associated with phosphate overload.

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Functional overlap of different cell death pathways ensures host protection against intracellular bacterial pathogens

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Caspases play central roles in the removal of unwanted cells through the control, initiation and execution of diverse forms of cell death, including apoptosis, pyroptosis and necroptosis and thereby contribute to the host's ability to control intracellular pathogens by removing their replicative niche. However, how the diverse forms of cell death are coordinated, inter-connected and provide back-up for each other upon infection of the cell with an intracellular pathogen remains unclear.

To explore this systematically, we ablated key components of all relevant cell death pathways genetically both *in vivo* and *in vitro* and infected mice and macrophages with *Salmonella*. Individual loss of pyroptosis, caspase-8-driven apoptosis, or necroptosis had minor impact on *Salmonella* control and cell death. However, combined deficiency of these cell death pathways caused loss of bacterial control in mice and their macrophages, demonstrating that host defense can employ varying components of several cell death pathways to limit intracellular infections. This flexible use of distinct cell death pathways involved extensive cross-talk between initiators and effectors of pyroptosis and apoptosis, and CRISPR/Cas9 whole genome screening identified that caspases-1 and -8 also functioned as executioners when all known effectors of cell death were absent. We are now employing novel CRISPR/Cas activator tools to further unravel the molecular control mechanisms underlying this highly coordinated and flexible cell death system with in-built fail-safe processes that protect the host from intracellular infections.

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Rab6b localises to the Golgi complex in murine macrophages and promotes TNF release in response to mycobacterial infection

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Mycobacterial infections remain leading causes of morbidity and mortality globally. The pro-inflammatory cytokine TNF is paramount to effective host immune responses against mycobacterial infections. Expression and release of TNF are tightly regulated, yet the molecular mechanisms that control the release of TNF by mycobacteria-infected host cells, in particular macrophages, remain to be defined. Rab

proteins are small GTPases that direct the transport of intracellular membrane-enclosed vesicles and are important regulators of macrophage cytokine secretion. The Golgi-associated GTPases Rab6a and Rab6a' are known to positively regulate the trafficking of TNF in LPS-activated macrophages. Unlike Rab6a and Rab6a' which are ubiquitously expressed, the related family member, Rab6b, is highly expressed in human and mouse neuronal tissues. Functional studies in brain-derived cells have reported Rab6b contributions to retrograde vesicular transport and suggested a role for this protein in secretory trafficking for exocytosis. Whether Rab6b executes similar functions in the context of immune responses is unknown. Here we show that Rab6b is expressed by primary mouse macrophages and localises to the Golgi complex in a fashion similar to Rab6a. Infection with *Mycobacterium bovis* Bacille Calmette Guérin (BCG) led to dynamic changes in *Rab6b* expression in primary mouse macrophages *in vitro* as well as organs of infected mice *in vivo*. Rab6b facilitated TNF release by BCG-infected macrophages, in the absence of discernible impact on *Tnf* mRNA and intracellular TNF protein expression. Similarly, Rab6a promoted BCG-induced TNF release by macrophages without impacting TNF protein expression. Our observations identify a positive regulatory role for Rab6a and Rab6b in BCG-induced TNF secretion by macrophages, and positions both proteins among the molecular machinery that direct macrophage functions in the context of infection and inflammation.

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Activation of cytotoxic Vγ9Vδ2 T cells during primary *Plasmodium falciparum* infection

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Previous research has repeatedly associated the expansion of the Vγ9Vδ2 T cell population during *Plasmodium falciparum* infection with malaria protection, however the cellular mechanisms behind this association are not fully characterised. We propose that increased cytotoxic function during *Pf* infection, is one mechanism by which Vγ9Vδ2 T cells confer malaria protection. Vγ9Vδ2 T cells have innate cytotoxic potential and can execute direct cytotoxic killing of blood-stage *Pf in vitro*. Clinical studies in malaria-endemic children have also correlated higher $CD16^+$ Vγ9Vδ2 T cell frequencies and increasing age with clearance of *Pf* infection. In our study we aimed to characterise the emergence of this cytotoxic phenotype during primary *Pf* infection and investigate age-related differences in the cytotoxic potential of Vγ9Vδ2 T cells. Firstly, we investigated the activation of cytotoxic Vγ9Vδ2 T cells during a Controlled Human Malaria Infection trial (CHMI) of malaria-naive adults. In this CHMI, participants were intravenously inoculated with 2800 intact *Pf* parasitised red blood cells, and treated with an anti-malarial drug at 8-days post-infection. Peripheral blood was drawn from participants (n = 8) at 0-, 8-, 15- and 42-days post-infection. Surface and intracellular staining of cryopreserved PBMCs revealed the activation of a highly cytotoxic Vγ9Vδ2 T cell at 15-days post-infection. The expanded, cytotoxic Vγ9Vδ2 T cell population had increased frequencies of CD16 and CD56 surface expression, as well as, increased intracellular production of perforin, granzyme-B and granulysin. Next, we performed ex vivo and in vitro phenotyping of PBMCs from malaria-naive children, to delineate associations of age and *Pf* exposure with the expansion of cytotoxic Vγ9Vδ2 T cells. We correlated increased age with decreased $CD16^+$ Vγ9Vδ2 T cell frequency and reduced intracellular production of granzyme-B after stimulation with blood-stage *Pf*. Overall, we showed the expansion and activation of cytotoxic Vγ9Vδ2 T cells 15-days after primary *Pf* infection of malaria-naive adults *in vivo*, and decreased cytotoxic Vγ9Vδ2 T cell frequency with age in malaria-naive children. Further analysis aims to investigate the association of prior cytomegalovirus infection and the augmentation of the cytotoxic Vγ9Vδ2T cell phenotype within these cohorts.

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Rapid multiplex PCR to detect and characterise polymicrobial sepsis

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Sepsis is a huge global health burden, with a recent Lancet paper reporting 11 million deaths in 2017 (1). Each year approximately 7 million sepsis cases (14%) are triggered by more than one pathogen (polymicrobial sepsis), which often leads to significantly worse patient outcomes, including longer hospital stays and higher mortality rates compared to single-pathogen sepsis (2). Despite polymicrobial sepsis being a more severe manifestation of sepsis, recent epidemiological data is lacking. Most of the data available is based on the routine culture-based method for diagnosing sepsis. In addition to slow turnaround times (24-72 hours), the method often yields inaccurate results due to contamination, low pathogen load, unculturable organisms, and interference from antibiotics administered prior to sample collection (1). When sepsis is polymicrobial, only the pathogen present at the highest abundance is usually detected in the first instance, leading to delayed identification of all causative organisms and incorrect initial antimicrobial therapy (3–5). Therefore, the development of an alternative culture-independent tool for detecting polymicrobial infections is highly warranted. Our group is currently validating a highly sensitive and rapid multiplex polymerase chain reaction (PCR) test that detects the 12 most common sepsis pathogens. This project aims to optimise this PCR test for the detection of polymicrobial infections, which will then be used to retrospectively analyse positive blood cultures frozen at PathWest Laboratories (diagnostic laboratory). Concordance between our PCR test and the routine method (PathWest data) will be assessed to determine whether PCR can detect polymicrobial infections missed by routine testing. The clinical characteristics of the patients will also be assessed in relation to bacterial composition. Ultimately the project will allow us to assess the true prevalence and clinical significance of polymicrobial sepsis.

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Targeted HBeAg seroclearance in a chronic-like HBV mouse model using chimeric bionanoparticles.

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Introduction: Chronic hepatitis B (CHB) contributes to more than 880,000 deaths each year, and despite existing prophylactic and therapeutic advances, there is currently no cure. An important viral protein, the hepatitis B e antigen (HBeAg) is essential for initial establishment of CHB by modulating the host's immune responses. HBeAg seroconversion is a current treatment endpoint and a preceding step for functional cure. This project utilises HBeAg-epitope expressing bio-nanoparticles (eBNPs) to program the immune responses for detection and clearance of HBeAg at earlier phases of infection, as a groundwork step towards a potential novel curative approach.

Methods: Our bionanoparticles are non-infectious, sub-viral particles composed of the HBsAg, making excellent presentation platforms by providing high antigenic density of exposed epitopes. In this project, eBNPs candidates were bioengineered and characterised against the epitopes of interest. Then, the most immunogenic eBNP candidates were used to challenge a chronic-like HBV mouse model and stimulate immune responses against HBeAg and HBsAg.

Results: In this project, biochemical characterisation of eBNPs has demonstrated their immunogenic against the inserted HBeAg epitopes whilst also maintaining some immunogenicity against the HBsAg backbone protein. Challenging a chronic-like HBV mouse model with eBNPs resulted in seroclearance of HBeAg in 43% of eBNP3 group (3/7 mice) and 67% of eBNP6 group (8/12 mice). In addition, 17% of eBNP6 group (2/12 mice) serocleared HBsAg, achieving functional cure.

Discussion: The immune factors contributing to HBeAg seroconversion in CHB patients remain unclear. This project is the first to describe assembly-competent, immunogenic eBNPs capable to selectively trigger immune responses against the native HBeAg and induce its seroclearance. The successful production of eBNPs forms the basis of initiating a novel experimental approach to target HBeAg, further investigate the immune factors contributing to its seroclearance and progress towards a potential curative outcome.

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HCV infection induces prolonged inflammation and elevated chronic disease risk biomarkers even after curative direct-acting antiviral treatment

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Around 58 million people globally have Hepatitis C virus (HCV) with an estimated 1.5 million new infections annually. Untreated chronic infection often leads to liver damage and can increase risk of inflammatory diseases including cardiovascular disease (CVD) and diabetes. We investigated whether HCV-associated inflammation/immune dysfunction persisted following viral clearance by direct-acting antiviral (DAA) treatment.

Plasma samples were collected from people who inject drugs with viremic HCV infection at baseline, and 12 and 48 weeks after DAA therapy (n=32, median age 39 years [range 25-57], 75% male). Control individuals were HCV antigen and antibody negative and of a comparable age and sex (n=29, mean age 40 [range 25-60], 72% male). Levels of soluble biomarkers of inflammation/immune activation and chronic disease risk were measured in plasma samples using Luminex immunoassay (R&D Systems) or commercial ELISA.

Biomarkers of inflammation (IL-6, soluble TNF- receptor II (sTNF-RII), high sensitivity (hs) CRP), endothelial activation (VCAM) and coagulation (d-dimer) were elevated in untreated HCV+ individuals compared to controls ($p<0.05$ for all variables measured). Levels of the endothelial activation biomarker VCAM were decreased after DAA treatment compared to pre-treatment samples ($p=0.003$). In contrast, IL-6, d-dimer and hsCRP, which have all been independently associated with increased risk of CVD, as well as sTNF-RII were not significantly altered by viral clearance and remained elevated 48 weeks after DAA treatment in HCV+ people as compared to controls ($p>0.05$ for all). The blood glucose marker of diabetes risk, HbA1c, was also significantly elevated in HCV+ individuals 48 weeks post-treatment as compared to controls ($p<0.001$).

This study showed persistent elevation of biomarkers associated with increased risk of CVD and diabetes in HCV+ people despite curative DAA therapy. These findings suggest people who have current or past HCV infection, particularly those who inject drugs, should be closely monitored for CVD and diabetes risk.

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Screening of a panel of FDA-approved drugs to identify CCL17-inhibiting candidates

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Introduction/Aim:

Rheumatoid arthritis (RA) is an inflammatory and destructive autoimmune polyarthritis which affects approximately 2% of Australians. While clinical trials targeting GM-CSF in RA are showing promise, the potential side effects of anti-GM-CSF therapy highlight the need for identifying downstream mediators of GM-CSF-activated pathway. CCL17, a downstream inflammatory mediator of GM-CSF, has been shown to mediate GM-CSF-driven inflammatory arthritis in animal models. Unlike CCL22, which has a role in resolving inflammation, CCL17 levels are elevated in RA synovial fluid and plasma. Here, a panel of FDA-approved drugs were screened to identify candidate CCL17-inhibiting drugs

Methods:

Human monocytes and mouse macrophages were pre-treated with FDA-approved drugs followed by treatment with either PBS or GM-CSF for 16h. Culture supernatants were subjected to CCL17 and CCL22 ELISA. The effect of drugs on cells viability was confirmed by MTT assay.

Results:

Among 1,508 FDA-approved drugs, 14 drugs inhibited the levels of CCL17 but not CCL22 expression robustly in human monocytes without cytotoxicity. Among 14 drugs, seven drugs inhibited the levels of CCL17 but not CCL22 expression in mouse macrophages.

Conclusions:

Identifying CCL17-inhibiting drugs and repurposing them would be both time-saving and cost-effective. The potency of identified CCL17-inhibiting drugs in blocking arthritis pain and disease development will be assessed in preclinical models.

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An integrated transcriptional atlas of human dendritic cells

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Dendritic cells (DCs) are rare cells that are essential regulators of anti-tumour, anti-viral and vaccine responses by the immune system. DC populations are functionally diverse and present in most adult tissues, however human models of DC biology rely on differentiation from peripheral blood monocytes or cord blood progenitors. A critical gap for development of DC-based immunotherapies is understanding how faithfully these capture the repertoire of DC subsets or behaviors.

We built a computational platform to compare DC subtypes including freshly isolated and cultured subsets, by combining 342 samples from 15 expression profiling studies derived from 10 laboratories. The resulting combined transcriptional atlas revealed that the most common *in vitro* model, CD34+ HSC-derived DCs, are missing key immunoregulatory factors when compared to primary DCs, and further, that these can be partially rescued by DCs differentiated *in vivo* in humanized mouse models.

The atlas is implemented in the Stemformatics.org platform. Users can visually compare gene expression of DC samples categorized by tissue, sample source and disease or activation status. Also, users are allowed to project external single cell datasets for annotation of DC subsets, or other transcriptome platforms to benchmark new models of DC biology.

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

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Receptor interacting serine/threonine protein kinase (RIPK) 1 and 3 are key signaling factors in cellular inflammation and programmed death. In particular, the interaction of RIPK1 and RIPK3 through their conserved RHIM domains results in necroptosis, where membrane pore formation releases danger-associated molecular patterns that drive an inflammatory response. Consequently, many viruses and bacteria have evolved pathogenic mechanisms that target RIPK for inactivation. The diarrhoeagenic gut bacteria enteropathogenic *Escherichia coli* (EPEC) has recently been described to carry an effector that cleaves the RHIM domains of RIPK1/3, however the physiological significance of this action to host immunity remains to be examined.

Using *Citrobacter rodentium* – the model organism for EPEC, evaluation of disease in a panel of RIPK knockout mice revealed that the absence of both RIPK1 and RIPK3 greatly heightened the diarrhoeal severity and bacterial burden experienced at peak infection. Notably, only RIPK3 had a significant role in moderating local gut pathology and in a manner independent from necroptosis. More interestingly,

flow cytometry analysis in RIP1/3-deficient mice also found a marked reduction in the T-helper 17 and T-regulatory cell populations in the colonic lamina propria, which are important for mediating bacteria-induced colitis. These results are consistent with the improved disease phenotype exhibited by wildtype mice infected with an *espL* deleted *C. rodentium* mutant. Here, qPCR evaluation of inflammatory cytokine levels found an increased expression of *Il22*, but not *Il17a* in the colons of mutant *C. rodentium* infected mice, suggesting RIPK1/3 skews towards an anti-inflammatory response.

Here we show for the first time, a novel link between RIPK1/3 (innate immunity) and T cell responses (adaptive immunity) that is involved in host mucosal protection. Further characterisation of the underlying signaling pathways will be important to inform future management and treatment of serious enteric diseases.

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Molecular mechanisms of apoptosis induction during *Salmonella* Typhimurium infection

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Programmed cell death (PCD) assures the removal of infected cells and is therefore crucial for the host defence against intracellular pathogens. It has become apparent that the PCD pathways pyroptosis, apoptosis and necroptosis are tightly interconnected and regulated by a remarkable level of redundancy. The generation of mice lacking caspases-1/-11/-12/-8 and receptor-interacting protein kinase 3 (RIPK3) in various combinations allowed us to study the individual roles of different caspases in PCD processes under conditions where other key caspases required for the host response are absent and to define new mechanisms of apoptosis induction during *Salmonella* Typhimurium infection.

To investigate the importance of caspase-2 in apoptosis regulation during *Salmonella* Typhimurium infection, we compared wild-type with caspase-2 deficient and caspases-1/-11/-12/-8/-2/RIPK3 deficient mice to prevent the compensatory effects of other caspases. Our findings revealed that the absence of caspase-2 caused no major impairments in infection control and therefore argue against a significant role for caspase-2 in PCD induction and bacterial clearance during *Salmonella* infections.

We next analysed if apoptosis of infected cells is induced via IFN-γ-driven mechanisms, because *Salmonella* stimulates a robust IFN-γ-producing CD4 T cells host response. Our findings that IFN-γ and interferon regulatory factor 1 (IRF1) deficient mice have comparably elevated bacterial titres suggest that IRF1 is the main transcription factor downstream of IFN-γ signalling during *Salmonella* infections. In line with this, the observed impaired infection control in mice lacking the IRF1-regulated factors TNF-α, Fas ligand and TNF-related apoptosis-inducing ligand (TRAIL) indicates their importance for apoptosis induction. Additionally, T cell depletion in pyroptosis deficient mice, which rely on apoptotic PCD pathways to control intracellular pathogens, resulted in significantly elevated bacterial titres highlighting the major role of CD4 T cells in apoptosis induction and *Salmonella* control.

Based on our findings we hypothesise that IFN-γ production of T cells activated by cytokines released during pyroptotic cell death can sensitise macrophages via IRF1-driven upregulation of TNF receptor superfamily members to CD4 T cell-induced extrinsic apoptosis.

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Circulating microRNA biomarkers enable accurate identification of COVID-19 patients

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COVID-19 continues to extensively impact every facet of our lives. Central to our efforts to slow the spread of SARS-CoV-2 is accurate and sensitive diagnostic tests. Unfortunately, news reports continually highlight examples of individuals initially deemed uninfected, only to test positive later, contributing to the spread throughout the community. Conventional diagnostic tests target the viral genome, which may not be readily detectable in the early, particularly pre-symptomatic, stages of infection. Host-encoded microRNAs (miRNA) have shown promise as biomarkers of infection; they respond rapidly and are easily detectable within biofluids. While other host responses, especially inflammatory cytokines, have been extensively studied, the host-encoded miRNA response to SARS-CoV-2 remains poorly defined.

Here, we used next-generation sequencing to profile the circulating miRNAs from 10 COVID-19 patients, sampled longitudinally, and age and gender matched controls. We found 55 differentially expressed miRNAs (FDR adjusted p-value <0.05) during early-stage disease, with several miRNAs having previously established roles in inflammation. We then employed a multivariate machine learning approach to identify a three-miRNA biomarker signature (miR-423-5p, miR23a-3p, miR-195-5p) that identifies COVID-19 cases with 99.9% accuracy, 99.8% sensitivity, 99.8% recall, and a receiver operator characteristic area under the curve (ROC AUC) of 1. This signature is associated with early-stage disease; as the patients recovered, their samples clustered with the healthy controls. Further validation was conducted

in a ferret model, where these three miRNAs identified SARS-CoV-2 infection with 99.7% accuracy, and distinguished SARS-CoV-2 infection from influenza A (H1H1) infection and uninfected animals with 95% accuracy.

This study, recently published in PLoS Pathogens [1], gives insights into the host miRNA response to SARS-CoV-2 infection and highlights the use of these molecules as circulating biomarkers for the detection of COVID-19. This biomarkers signature has great potential to compliment existing diagnostic tests and help to identify infections that may otherwise be missed.

1. 1. Farr, R.J., et al., Altered microRNA expression in COVID-19 patients enables identification of SARS-CoV-2 infection. PLoS Pathog, 2021. 17(7): p. e1009759.

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Rabies against the machine: using artificial intelligence to find cellular and exosomal microRNA signatures of lyssavirus infection in a human stem-cell derived neural culture

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Rabies (lyssavirus) continues to have a significant impact on global mortality, especially in children under 15 years of age. This virus is extremely well-adapted to avoid host detection while travelling through the nerves to the brain. Until recently, research to fully understand the role of non-coding RNA in the pathogenesis of this neurotropic virus has been hampered by a lack of human models. Recently, we described the use of human stem cell-derived neural cultures to model lyssavirus infection [1]. Here, we have utilised this model to investigate the effect of lyssavirus infection on microRNA (miRNA) expression in human neural cells and the exosomes they secrete. We found 25 cellular and 16 exosomal miRNAs that were differentially expressed (FDR p-value <0.05) in response to infection with different lyssavirus strains. Supervised machine learning was then used to find combinations of cellular and exosomal miRNAs that are indicative of lyssavirus infection. A signature of 6 cellular miRNAs (miR-99b-5p, miR-346, miR-5701, miR-138-2-3p, miR-651-5p, and miR-7977) or 4 exosomal miRNAs (miR-25-3p, miR-26b-5p, miR-218-5p, miR-598-3p) could distinguish infected neural cells with almost 100% accuracy, giving an insight into the molecular pathogenesis pathways and highlighting potential biomarkers for rabies detection. Many of the miRNAs identified through machine learning, such as miR-5701 and miR-99b-5p, have established roles in regulating neuronal cell death, neuroprotection and neuroregeneration, and some have been found to be dysregulated in other neuronal disorders, such as Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (ALS). Interestingly, other miRNAs, such as miR-138-2, correlate with behavioural changes that are also seen in rabies infection, including panic, anxiety, confusion, impulsivity, and cognitive impairment. Together, this data lays the foundation for a more complete understanding of rabies molecular pathogenesis pathways, and highlights the use of exosomal miRNAs as biomarkers of rabies infection for improved detection and patient outcomes.

1. 1. Sundaramoorthy, V., et al., Modelling Lyssavirus Infections in Human Stem Cell-Derived Neural Cultures. Viruses, 2020. 12(4).

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Exploring TRIM proteins as targets for broad spectrum antivirals

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Acute respiratory tract infections are a major cause of severe lower respiratory tract infections, resulting in significant morbidity and mortality, particularly in paediatric, elderly and immunocompromised populations. Despite this, prophylactic and therapeutic treatment options are limited. While viral specific inhibitors are being actively researched, they are often not broad spectrum and can lead to antiviral resistance. Host targeted therapies are a promising alternative, as they have the potential to be highly effective against a broad range of viruses.

This project aimed to investigate whether members of the Tripartite Motif (TRIM) family of proteins, which are intracellular E3 ubiquitin ligases, might represent targets for the development of antiviral host targeted therapies. To this end, a selection of 11 different human TRIM proteins (from a family of ~80) were experimentally over-expressed in human epithelial cells to test their potential as antiviral factors. Cells were then infected with either: respiratory syncytial virus (RSV), human metapneumovirus (HMPV) or parainfluenza virus type 3 (PIV-3). If a reduction in viral growth was observed in cells overexpressing a particular TRIM protein compared to a control cell line (overexpressing a protein with no antiviral activity), the role of the TRIMs was explored further. The results from this screen indicated that TRIM16 was able to restrict the growth of all three viruses tested, while TRIM69 was able to restrict the growth of RSV and HMPV. Subsequent analysis showed that both TRIM proteins restricted virus replication at the early stages of infection (i.e prior to synthesis of viral proteins within infected cells). Knockdown approaches further confirmed the antiviral role of endogenous TRIM16 and TRIM69 proteins. Currently, experiments are underway to determine their mechanism of antiviral action.

These findings are significant as they will equip us with the knowledge needed to design therapeutic compounds targeting antiviral TRIM proteins.

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Programming Vγ9Vδ2 T cells for HIV immunotherapies

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Introduction: Vγ9Vδ2 T cells offer promising potential for immunotherapies and could be employed to target HIV upon latency reversal. Vδ2 T cells can be expanded to great magnitudes through the application of aminobisphosphonate drugs and express multiple cytotoxic surface receptors enabling recognition of a range of self-stress molecules. Vδ2 T cell-based immunotherapies have been well tolerated in clinical trials against cancers but have achieved limited efficacy. Inducing more potent cytotoxic functions and defining mechanisms of antigen recognition may be key to improving future Vδ2 T cell-based immunotherapies against diseases such as HIV.

Methods: Using a series of redirected LDH cytotoxicity assays, we evaluated lysis of target cells by individual Vδ2 T cell surface receptors. In addition, we examined the impact of stimulation with certain combinations of cytokines during *in vitro* expansion on Vδ2 T cell mediated cytotoxicity.

Results: We found that Vδ2 T cells were capable of mediating lysis through NKG2D, CD16, CD3, and CD26. We also identify various cytokine combinations which were capable of enhancing Vδ2 T cell mediated cytotoxicity through a variety of these receptors.

Conclusion: We profiled Vδ2 T cell surface receptors capable of mediating direct lysis of target cells and identify several factors capable of increasing this lysis. These finding may have key implications for future Vδ2 T cell-based immunotherapies to target HIV, as many ligands for these cytotoxic surface receptors are present during infection.

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The GPR183/oxysterol axis controls macrophage infiltration and inflammation in the lung during influenza infection and SARS-CoV-2 infection

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Infectious diseases such as influenza and coronavirus disease 2019 (COVID-19) cause a significant burden to global health. Severe forms of these respiratory infections are often characterized by hyper-inflammation due to excessive immune cell infiltration to the site of infection and pro-inflammatory cytokine production, also referred to as cytokine storm. However, the immunological mechanisms underlying the development of a cytokine storm remain elusive. We have identified a novel player and important regulator of immune cell recruitment to the lung, the oxidized cholesterol receptor GPR183, which is expressed on cells of the innate and adaptive immune system. Oxidized cholesterols, so called oxysterols, have emerged as important signalling molecules of immune function. The oxysterol 7a,25-hydroxycholesterol (7a,25OHC) is the endogenous high affinity ligand for the oxysterol-sensing receptor GPR183, which is expressed on cells of the innate and adaptive immune system. GPR183 expressing immune cells migrate towards a gradient of the 7a,25OHC to position them to secondary lymphoid organs. However, little is known about oxysterols and GPR183 in the lung.

Influenza A virus infected GPR183KO mice had lower pro-inflammatory profile upon severe IAV infection compared to WT C57BL/6 mice. WT mice treated with GPR183 antagonist NIBR189 demonstrated lower pro-inflammatory cytokine production and reduced infiltration of macrophages, with neutrophil and T cell subsets not being affected. We have further demonstrated in a mouse model of SARS-CoV-2 infection that GPR183 KO mice had less severe weight loss compared to WT mice. Infected WT mice treated with GPR183 antagonist had less severe SARS-CoV-2 infection characterised by less drastic weight loss and symptoms and faster recovery compared to infected WT mice given vehicle. Therefore, we provide preclinical translational evidence that modulation of GPR183 activity shows promise for improving viral respiratory infections outcomes.

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Drug screening to uncover novel aspects of *Salmonella* infection

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Salmonella species are among the most common foodborne pathogens. As an intracellular pathogen, *Salmonella* employs multiple virulence factors to interfere with critical host cell pathways to achieve successful colonization of the host. Past studies have provided many molecular insights into *Salmonella*-host cell interactions. However, many aspects of *Salmonella* pathogenesis are still not well understood. Furthermore, emerging antibiotic-resistant *Salmonella* strains represent a significant clinical threat, and it is crucial to develop novel anti-*Salmonella* therapeutics.

Previous studies have established that IFNγ plays a pivotal role driving the clearance of *Salmonella* from infected hosts. Although several elegant examples of IFNγ-triggered host defense mechanisms have been elucidated, the activities of many IFNγ-regulated genes remain elusive. In this project, we aimed to uncover novel aspects of IFNγ-regulated, *Salmonella*-host cell interactions via conducting a macrophage-based drug screen. A drug library of 3,088 compounds, mainly comprised of FDA approved drugs, were used to treat RAW264.7 cells before cells were stimulated with IFNγ and subsequently infected with *Salmonella*. The infection was examined by confocal microscopy and analyzed through the CellProfiler data analysis pipeline. From the primary screen, we identified 86 drugs that

inhibited *Salmonella* intracellular replication with IFNγ treatment when compared to untreated samples, while 121 drugs appeared to enhance *Salmonella* intracellular replication. Our preliminary data suggests that IFNγ stimulation alters *Salmonella* host cell infection outcomes from various drug treatments.

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High frequency human MLKL mutation causes innate immune response defects and hematopoietic dysfunction in CRISPR-cas9 generated mouse model.

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Programmed cell death has long been implicated in the progression of human disease. Recently, there has been a major focus on the inflammatory lytic form of programmed cell death, necroptosis, in human pathogen responses. Necroptotic signaling is mediated by the terminal executioner protein, pseudokinase mixed lineage kinase domain-like (MLKL), and its upstream activating kinase, receptor interacting protein kinase 3 (RIPK3). These integral necroptotic proteins have been comprehensively revealed as potent drivers and suppressors of human disease in pre-clinical murine models. The investigation of MLKL's role in human disease within the 'real world' of diverse environmental challenges and genetic backgrounds is limited. Here, we present investigations of a high frequency missense polymorphism in human MLKL that is carried by 2-3% of the general population. This gene variant encodes a serine to proline substitution at position 132 within MLKL's regulatory brace region and is enriched in trans with similar MLKL polymorphisms in a cohort of Chronic Recurrent Multifocal Osteomyelitis patients. Primary patient cells heterozygous for *MLKL*^{S132P} and exogenous expression systems in immortalized cell lines were examined for their response to necroptotic and inflammatory stimuli *in vitro.* To study the potential disease modulating effects of MLKL^{S132P}, on a systemic level, we have generated a mouse model that expressed the mouse equivalent variant, *Mlkl*^{S131P}. *Mlkl*$^{S131P/S131P}$ mice exhibit innate immune cell defects in the bone marrow at steady state. Following challenge with *Salmonella*, Mlkl$^{S131P/S131P}$ mice exhibited impeded bacterial clearance and innate immune cell defects in peripheral blood. Furthermore, *Mlkl*$^{S131P/S131P}$ mice were susceptible to bone marrow failure characterized by hematopoietic dysfunction following sublethal irradiation or transplantation. Our work highlights that this human MLKL polymorphism may be an important modulator of disease progression under everyday environmental challenge and raises important questions about the historical circumstances that have led to its high frequency.

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Investigating IL-37 in the setting of bronchopulmonary dysplasia (BPD)

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Introduction. BPD is a lung disease of premature babies characterised by reduced lung function that is primarily mediated by inflammation. The interleukin (IL)-1 family of cytokines/receptors has regulatory roles in both driving and opposing inflammation but remains largely undescribed in BPD. The broadly acting anti-inflammatory cytokine IL-37 is of particular interest, as it reduces proinflammatory factors, such as IL-1β, that are elevated in infants with BPD.

Aims. To subject wild-type (Wt) and IL-37 transgenic (IL-37tg) mice to a 28-day model of murine BPD to investigate changes in alveolar structure, airway remodeling and expression of IL-1 family members.

Methods. In an established murine "double hit" model of BPD, pregnant dams were injected with 150 µg/kg LPS i.p. at embryonic day 14. At birth, Wt and IL-37tg pups were randomised into normoxia (air; 21% O_2) or hyperoxia (hyp; 65% O_2) to give 4 groups (1) air Wt (2) air IL-37tg (3) hyp Wt and (4) hyp IL-37tg. Lungs were prepared for alveolar and airway histology and morphometry (day 28). Changes in lung IL-1 family mRNA expression relative to air Wt were determined by real time PCR (day 5 and 28).

Results. Hyp Wt pups developed a severe BPD-like lung disease, with fewer, larger alveoli, reduced surface area for gas exchange and increased alveolar epithelial thickness. Hyp IL-37tg mice were not protected against these changes. Of note, IL-37 mRNA expression levels were reduced at day 28 of the model in air IL-37tg when compared to day 5 air IL-37tg (62 fold, $p<0.05$). Additionally, at day 28, a trend for lower mRNA expression of the IL-37 co-receptor SIGIRR was observed in the lungs of air IL-37tg pups, as compared to air Wt pups.

Discussion. IL-37tg mice were not protected against the hyperoxia-induced changes in lung morphology observed at day 28 in Wt mice, potentially due to insufficient levels of IL-37 or downregulation of its receptors. Daily administration of recombinant IL-37 to achieve steady state levels may be required to establish the protective role of IL-37 in murine BPD.

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CD8$^+$ T cell cross-reactivity towards conserved influenza A and B epitopes

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Influenza A, B and C viruses (IAV, IBV and ICV respectively) circulate globally, infecting humans and causing widespread morbidity and mortality. IAV-NP_{265} is a known HLA-A*03:01-restricted immunodominant epitope, albeit not fully characterised. We identified novel IBV and ICV homologue IAV-NP_{265} peptides and assessed their immunogenicity, and the presence of cross-reactive $CD8^+$ T cells able to recognise peptides from different influenza virus types. Our study further characterised the $CD8^+$ T cell response to IAV-NP_{265}, and of the newly identified IBV-NP_{323} and ICV-NP_{270}. We provided evidence of T cell cross-reactivity between those epitopes derived from different influenza types, and its molecular basis by solving the crystal structures of IAV-NP_{265} and IBV-NP_{323} peptides in complex with HLA-A*03:01. Altogether, our study provides evidence behind T cell cross-reactivity across a universally conserved epitope from different types of influenza viruses, which has an important impact for vaccine consideration.

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The broccoli-derived antioxidant sulforaphane changes the growth of bacteria within the gastrointestinal microbiota.

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Sulforaphane is a naturally occurring, potent antioxidant and anti-inflammatory compound, found in broccoli that is broken down and absorbed primarily in the gut. Sulforaphane has an established clinical safety profile and is effective in managing hypertension, and the vascular dysfunction that underlies preeclampsia. As sulforaphane would be consumed as a nutritional supplement primarily absorbed in the gut, there is the potential for sulforaphane to impact the gut microbiome. The human gut microbiome has been implicated in both health and disease. However, nutritional components such as prebiotics, have been shown to change microbial composition and improve outcomes for patients with metabolic diseases such as prediabetes and obesity. Therefore, we have screened the effect of sulforaphane on the growth of a panel of 47 common gastrointestinal bacterial isolates, in both rich media (YCFA) and media lacking thiamine and riboflavin. The 47 isolates were chosen as they represented the four main phyla, and the proportions of each, found in the human gastrointestinal microbiome; Firmicutes (24 strains), Bacteroidetes (10 strains), Proteobacteria (9 strains) and Actinobacteria (4 strains). From this group, 31 isolates showed significant changes in growth, with 12 showing significant changes in both media types. These isolates are members of the Firmicutes (6 isolates), Proteobacteria (5 isolates) and Bacteroidetes (1 isolate) phyla and contain 10 commensals and two pathogenic isolates. Further work will aim to investigate the metabolic changes that underpin these growth changes and determine if these bacteria are impacting the efficacy of sulforaphane or if sulforaphane is changing microbiome composition.

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Immunodominance in malarial antibody responses

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Malaria is a parasitic disease that causes significant disease burden, with >2 million cases and 400,000 deaths annually. Vaccination remains a promising avenue for reducing malaria burden, however efforts to create a highly efficacious vaccine have been hampered by a poor understanding of how malarial immunity develops. To improve the understanding of malarial immunity, we aimed to identify targets of protective antibody responses in naturally acquired and experimentally induced malaria. Specifically, we studied epitope level antibody responses to an intrinsically unstructured blood-stage malarial antigen, merozoite surface protein 2 (MSP2) via peptide array ELISA. This was completed for IgM, total IgG, IgG1, IgG2, IgG3, and antibody dependent complement responses, and the results compared between age groups (adults or children) and infection type (naturally acquired or experimentally induced). We found specific epitopes were more immunodominant than others, but that these immunodominant epitopes changed with age, infection type and antibody class. Further, the epitopes that were immunodominant for antibody binding weren't always the immunodominant epitopes for antibody dependent complement fixation. We also noted that participants mostly demonstrated "discrete recognition", where the antibody classes each targeted different epitopes. This raises several questions about the relationship between epitopes, B cell class switching, and affinity maturation. Results inform our understanding of how antibodies develop to specific antigen targets in individuals.

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DPP9 deficiency: an Inflammasomopathy which can be rescued by lowering NLRP1/IL-1 signaling

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The NLRP1 inflammasome is activated upon viral infection or danger-associated signals, resulting in secretion of pro-inflammatory cytokines IL-1b and IL-18, as well as pyroptosis an inflammatory form of cell death. NLRP1 is maintained in an inactive state by the serine protease, DPP9. Whilst inhibition of DPP9 activates the NLRP1 inflammasome; however, these findings have been established *in vitro* using mouse and human cell lines. How DPP9 impacts NLRP1 inflammasome regulation *in vivo* has not yet been demonstrated.

Here we have identified patients displaying immune-associated manifestations, skin pigmentation abnormalities and neurological deficits that harbor loss-of-function mutations in DPP9. Patient keratinocytes display spontaneous activation of the NLRP1 inflammasome. We employed a mouse model harbouring a mutation in DPP9 that renders the protein catalytically inactive (*Dpp9*$^{S759A/S759A}$) to model this condition. *Dpp9*$^{S729A/S729A}$ mice die within one day of birth through an unknown mechanism. Excitingly we have found that crossing *Dpp9*$^{S759A/S759A}$ mice to NLRP1 knockout mice (*Nlrp1*$^{-/-}$) rescues neonate lethality. *Dpp9*$^{S759A/S759A}$ *Nlrp1*$^{-/-}$ mice appeared runted but are outwardly healthy and prove fertile. This dramatic amelioration of phenotype suggests that a major homeostatic role of DPP9 is to prevent NLRP1 inflammasome assembly. Additionally, we show that the *Dpp9*$^{S759A/S759A}$ neonate lethality can also be rescued by deletion of downstream components of the NLRP1 inflammasome, including ASC, GSDMD and IL-1R, with the phenotype of the rescued mice mirroring that of the *Dpp9*$^{S759A/S759A}$ *Nlrp1*$^{-/-}$ mice. In contrast, IL-18 deletion does not rescue *Dpp9*$^{S759A/S759A}$ lethality. Overall, we have shown the importance of DPP9 restraint of the NLRP1 inflammasome activation *in vivo*, with DPP9 deficiency resulting in NLRP1 driven inflammatory disease in both humans and mice.

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The naturally derived cytokine peptide LAT8881 limits respiratory virus replication and severe disease

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Severe and fatal influenza type A (IAV) infections are associated with significant viral replication and damaging hyperinflammatory immune responses in the lung. LAT8881 (is a 16 amino acid synthetic form of the naturally occurring C-terminal fragment of growth hormone (GH). LAT8881 has been previously shown to act independently of the GH receptor to reduce inflammatory damage and promote tissue repair in a rabbit model of arthritis. Additionally, LAT8881 has been investigated in several clinical trials in healthy volunteers and has an established safety record. Considering its effects in improving inflammatory damage in animal models, we investigated the potential of LAT8881 and related compounds as a treatment in a mouse model of severe IAV infection (10^4 PFU HKx31 H3N2). Daily, intranasal (IN) delivery of synthetic GH fragment LAT8881 (≤ 20mg/kg) from 1 day following IAV infection significantly reduced disease susceptibility and resulted in a dose dependent reduction in infectious viral loads, innate immune cell infiltrates and proinflammatory cytokines in the lungs. The efficacy of LAT8881 IN treatment was comparable to that of daily, oral administration of neuraminidase inhibitor, oseltamivir, however, LAT8881 did not hinder IAV or SARS-CoV-2 replication *in vitro*, which suggests against a direct antiviral role for the peptide. Intriguingly, similar results are achievable using a small peptide derived from the C-terminus of prolactin, a structural homolog of GH. LAT8881 and similar peptides are potent modulators of influenza disease and are novel candidate therapeutics that limit viral replication and reduce airway hyperinflammation. Furthermore, the demonstrated clinical safety profile of LAT8881 warrants future clinical studies of influenza disease and may facilitate its rapid deployment as a treatment for respiratory virus infections.

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Investigating antimicrobial activity by bacterial members of the human gastrointestinal microbiome

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The discovery of novel antimicrobial molecules is vital to combat the increasing prevalence of antimicrobial resistance in bacterial pathogens around the world. The ability to now cultivate the previously “unculturable” members of the human gastrointestinal microbiota, together with computational evidence of putative antimicrobial molecules within the gut microbiome, offers a promising approach in exploring antimicrobial production by the human gut microbiome.

A panel of human gastrointestinal commensal bacteria were screened for antimicrobial activity against eight multi-drug resistant strains of gastrointestinal pathogens: *Clostridiodes difficile, Escherichia coli, Enterococcus faecium*, and *Klebsiella pneumoniae*. Of the 95 bacterial isolates screened, 75% exhibited inhibition of at least one of the pathogens tested. Specifically, a *Bacteroides faecis,* was selected for further investigation. To identify the inhibitory molecule produced by the *B faecis* candidate, assays with the cell-free supernatant of the isolate were performed. However, inhibition was unable to be replicated in the cell-free supernatant, and further exploration of the environment and stimuli required for secretion of a bioactive antimicrobial in *B. faecis* was conducted. The *B.*

faecis candidate did not exhibit antimicrobial activity in these assays, and additional experiments are required to confirm the source of the pathogen inhibition caused by the *B. faecis* candidate, and the environment needed for potential production of antimicrobial molecules.

This study demonstrated that members of the human gut microbiota have inhibitory effects against multi-drug resistant gastrointestinal pathogens. Further investigation is required to determine the requirements for antimicrobial production in these commensal strains.

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Whole-genome CRISPR/Cas9 screen to identify host factors playing a role in *Plasmodium falciparum* cell traversal

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Malaria is a leading cause of mortality causing more than 600,000 deaths in 2020. It is caused by parasites of the *Plasmodium* genus with most deaths due to *P. falciparum* infection. The control of malaria is complicated by the lack of a widely effective vaccine, the spread of mosquito resistance to insecticides, and *Plasmodium* parasite resistance to available drugs. Thus, there is an urgent need for novel treatment strategies to continue to combat the devastating effects of malaria globally.

The infection of red blood cells with *Plasmodium* parasites causes the typical clinical symptoms of malaria. However, before the parasite transitions into this erythrocytic life stage, *Plasmodium* sporozoites, injected into the human body after the bite of an infected mosquito, first travel from the skin via the bloodstream to the liver where the initial step of malaria infection occurs. The remarkable sporozoite's journey from the skin to final invasion of hepatocytes in the liver is associated with cell traversal which describes the migration of parasites through host tissue by entering and rupturing host cells. Stopping this step of infection would prevent onward transmission and the development of the malaria disease. However, there is only little knowledge about the molecular interactions involved in this process, specifically about potentially involved host proteins which the parasite may interact with to successfully migrate through a cell.

Here, we established a protocol that combines a whole-genome CRISPR/Cas9 knock-out screen with a newly designed positive selection cell traversal assay to study host factors involved in *P. falciparum* cell traversal. The first step of this pipeline, a pooled CRISPR screen, identified more than hundred host factors potentially associated with this mechanism. Interestingly, many of these genes are also involved in infection by other pathogens. In a second step, single-gene knockouts were generated to validate the screening results which is currently underway. The identification of essential host factors involved in the first step of the malaria lifecycle would not just provide more leverage for future prophylactic malaria treatments but might also give insights into migration processes of other pathogens that demonstrate similar migratory behaviors.

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Rare catastrophes and evolutionary winners – Human gene variation and inflammatory programmed cell death

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Necroptosis, a form of programmed cell death, can be induced directly by pathogens or by cytokines released as part of the innate immune response. The downstream effector protein Mixed Lineage Kinase-domain Like (MLKL) is phosphorylated by RIPK3, oligomerises, and associates with membrane phospholipids to promote the release of pro-inflammatory cytokines and the lytic destruction of cells. MLKL's two-helix 'brace' forms an essential conduit between its regulatory pseudokinase domain and membrane-busting four-helix bundle (4HB) effector domain. Our team recently showed that a single substitution of a conserved residue in this two-helix brace, D139V, randomly introduced by ENU mutagenesis, causes RIPK3-independent hyper-activation of mouse MLKL. This resulted in a lethal, postnatal systemic inflammatory syndrome.

Paradoxically, the most commonly inherited human *MLKL* missense gene polymorphisms map to the vicinity of the very same two-helix brace as the deadly D139V mouse MLKL substitution. This clustering is apparent not only in the MLKL monomer but also following daisy chain oligomerisation. The summed allele frequency for polymorphisms mapping to this region is 0.05, or 5 % of all human *MLKL* alleles sequenced in hundreds of thousands of individuals of diverse ancestry. As carriers of two *MLKL* alleles, we each have a 10% chance of being heterozygous for one of these *MLKL* variants when averaged across the global population. Have these human *MLKL* gene variants achieved such high frequencies by chance 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 for present-day humans? We have recently shown that combinations of these *MLKL* gene 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 that they confer a context specific 'gain' of cell death function *in vitro*. At a systems level, we have shown that genetically modified mice show signs of defective hematopoiesis and pathogen clearance, and we are actively investigating their potential influence on a wide array of complex polygenic human traits and diagnoses using targeted Phenome-Wide Association Studies (PheWAS).

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Loss of β-ketoacyl acyl carrier protein synthase III sensitise multidrug-resistant *Escherichia coli* to previously ineffective antibiotics

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Antibiotic resistance is one of the most prominent threats to modern medicine. In the latest World Health Organisation (WHO) list of bacterial pathogens that urgently require the development of new antibiotics, nine out of 12 are Gram-negative, with four of those listed under Critical Priority. One important barrier restricting antibiotic efficacy against Gram-negative bacteria is their unique cell envelope. Fatty acids are a shared constituent of all structural membrane lipids and in bacteria, the Type II fatty acid synthesis pathway (FAS II) is a promising target for antibiotic development, as it is distinct from that of eukaryotes. However many FAS II components have evolved redundancy, and deemed unsuitable drug targets. Here, we interrogated the redundant components of FAS II, showing that disrupting FAS II homeostasis by deletion of *fabH* damages the cell envelope of clinical *Escherichia coli* isolates. The *fabH* gene encodes the β-ketoacyl acyl carrier protein synthase III (KAS III) which catalyses the initial condensation reactions during fatty acid biosynthesis. We found *fabH* null mutation potentiated killing of multi-drug resistant *E. coli* by a broad panel of previously ineffective antibiotics, despite the presence of relevant antibiotic resistance determinants ($bla_{CTX\text{-}M\text{-}15}$ and *kpc2*). This sensitivity were transferrable to both biofilm eradication and the treatment of pre-established bladder cell monolayer infections. Our findings showcase the potential of FabH as a promising target that may rescue failing last-resort antibiotics.

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Characterization of a novel Group A Streptococcus virulence factor Spy0433

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Group A Streptococcus (GAS) consists of a vast variety of virulence factors that help the bacterium from evading its host's immune response, making it a relatively successful pathogen. Spy0433, one of the uncharacterized GAS proteins, was found to bind a few innate immune components including complement 1 subcomponent subunit C (C1qC) and myeloperoxidase (MPO), postulating its potential as an immune evasion factor. To investigate the functional effect of these interactions, Wieslab complement system screen, chlorination, and peroxidation assays were performed. The interactions of Spy0433 with plasma proteins and leukocytes were respectively examined via pull-down assay and flow cytometry. Western blot was also carried out to identify the expression of Spy0433 during the course of GAS infection. Spy0433 was shown to inhibit the chlorination activity of MPO that has an antimicrobial effect, where it reached nearly 100% inhibition at 15.27 μM. The protein also specifically binds granulocytes and monocytes that play an important role in innate immunity. However, no inhibition of Spy0433 was detected against the three complement pathways. The protein did not bind to any plasma proteins in the pull-down assay. The result for Western blot was inconclusive to confirm that Spy0433 was expressed during GAS infection. Overall, my project displayed that Spy0433 is an MPO inhibitor with a possible evasion mechanism against the innate immune cells.

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Characterisation of a novel non-canonical interferon pathway in macrophages

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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, including TREM1 [3]. Activation of this pathway using *Ifnar2-/-* mice has been shown to result in lethality following lipopolysaccharide (LPS) induced septic shock and reduce neuronal cell death in ischaemic stroke models [3, 4]. This project aims to characterise this novel, non-canonical IFN pathway using *in vivo* and *in vitro* methods to better understand its activation and involvement in pathogenesis, as well as its potential for therapeutic intervention.

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. As the murine *in vivo* and *in vitro* work identified macrophages to be a candidate for non-canonical signalling, RNAseq was performed on iPSC macrophages to translate these findings into a human model. *IFNAR2-/-* iPSC macrophages were stimulated with IFNβ and LPS *in vitro*, with transcriptomic analysis revealing the induction of non-canonical genes identified in the mouse model. This finding suggests the physiological relevance of this novel pathway in sepsis pathogenesis, and the identification of macrophages as the population responsible may enable their therapeutic depletion in the clinic.

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The *Plasmodium* apicoplast is essential for the construction of glycophosphatidylinositol anchors needed for egress and invasion

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The apicoplast is a relict plastid of cyanobacterial origin found in most apicomplexan parasites, including *P. falciparum*. The apicoplast can be selectively targeted by antibiotic- and herbicide-like drugs and treatment of asexual *P. falciparum* with such inhibitors causes a peculiar 'delayed death' phenotype, where treated parasites arrest in the subsequent intraerythrocytic development cycle. Despite the widespread use of such drugs as malaria prophylactics (e.g. doxycycline), the molecular basis of delayed death has not been thoroughly described. Understanding these mechanisms will assist in informing appropriate clinical usage of apicoplast inhibitors. The apicoplast is responsible for the biosynthesis of isoprenoid precursors, which have at least three downstream fates in the *Plasmodium* parasite: protein prenylation, ubiquinone and dolichols. The effect of protein prenylation and ubiquinone loss in the parasite has been previously described, but the effect of dolichol loss has not been characterised. Dolichols are required for the biosynthesis of glycosylphosphatidylinositol (GPI) anchors, the predominant type of protein glycosylation present in *P. falciparum*. Many GPI-anchored proteins are thought to be essential for both parasite egress and reinvasion. We performed immunofluorescence assays and electron microscopy on *P. falciparum* with inhibited apicoplasts but with exogenous rescue of their prenylation depletion. In these parasites, GPI-anchored proteins become mis-localised from their normal membrane association. These GPI-anchorless parasites also exhibited an egress defect and are unable to undergo proper segmentation and rupture the parasitophorous vacuole. Through flow cytometry-based invasion assays, we found that these GPI-anchorless parasites are also substantially less invasive. Therefore, our data indicates that apicoplast inhibitors cause a defect in GPI anchor biosynthesis that is important for both egress and reinvasion of asexual-stage *P. falciparum*.

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Immuno-epigenomic analysis identifies attenuated interferon responses in naïve CD4 T cells of adolescents with peanut and multi-food allergy

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Background

IgE-mediated food allergies have been linked to suboptimal naïve CD4 T (nCD4T) cell activation in infancy, underpinned by epigenetic and transcriptomic variation. Similar attenuated nCD4T cell activation in adolescents with food allergy have also been reported, but these are yet to be linked to specific epigenetic or transcriptional changes.

Methods

We generated genome-wide DNA methylation data in purified nCD4 T cells at quiescence and following activation in a cohort of adolescents (aged 10-14) with peanut/multi-food allergy (FA, n=29), and age-matched non-food allergic controls (NA, n=18). Additionally, we assessed transcriptome-wide gene expression and cytokine production in these cells following activation.

Results

We found widespread changes in DNA methylation in both NA and FA nCD4T cells in response to activation, associated with the T cell receptor signalling pathway. Adolescents with FA exhibit unique DNA methylation signatures at quiescence and post-activation at key genes involved in Th1/Th2 differentiation (*RUNX3, RXRA, NFKB1A, IL4R)*, including a differentially methylated region (DMR) at the *TNFRSF6B* promoter, linked to Th1 proliferation and associated with various immune disorders (rheumatoid arthritis, psoriasis and systemic lupus erythematosus). Combined analysis of DNA methylation, transcriptomic data and cytokine output in the same samples identified an attenuated interferon response in nCD4T cells from FA individuals following activation, with decreased expression of several interferon genes, including *IFN-γ* and a DMR at key downstream gene *BST2*.

Conclusion

We find that attenuated nCD4T cell responses from adolescents with food allergy are associated with specific epigenetic variation, including disruption of interferon responses at multi-omic levels, indicating dysregulation of key immune pathways that may contribute to a persistent FA phenotype.

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Structural Basis of Coronavirus E protein interactions with human PALS1 PDZ domain

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SARS-CoV-2 infection leads to coronavirus disease 2019 (COVID-19), which is associated with severe and life-threatening pneumonia and respiratory failure. However, the molecular basis of these symptoms remains unclear. SARS-CoV-1 E protein has previously been shown to interfere with the control of cell polarity in human epithelial cells by binding to the PDZ domain of PALS1, a key component of the Crumbs polarity complex. We now show that the C-terminal PDZ binding motifs of SARS-CoV-1 and SARS-CoV-2 bind the PALS1 PDZ domain with 28.4 and 22.8 mM affinity, whereas the related sequence from MERS-CoV did not show any binding. We then determined the crystal structures of PALS1 PDZ domain bound to both SARS-CoV-1 and SARS-CoV-2 E protein PDZ binding motifs. Our findings

establish the structural basis for SARS-CoV-1/2 mediated subversion of Crumbs polarity signalling, and serve as a platform for the development of small molecule inhibitors to disrupt SARS-CoV-1/2 mediated disruption of polarity signalling in epithelial cells.

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Pseudomonas aeruginosa releases outer membrane vesicles containing plasmid DNA that can be transferred to recipient bacteria.

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Bacterial acquisition of antimicrobial resistance (AMR) genes via horizontal gene transfer (HGT) has contributed to the rise of antibiotic resistance globally. Recently, outer membrane vesicles (OMVs), released by all Gram-negative bacteria, have been described as a novel mechanism of HGT. OMVs package and protect DNA and can transfer DNA to recipient bacteria, however this has only been observed for a limited number of bacterial species. In this study, we aimed to examine OMVs produced by the opportunistic pathogen *Pseudomonas aeruginosa* to determine their ability to package, protect, and transfer DNA, and whether bacterial growth conditions, such as planktonic or biofilm growth conditions altered DNA packaging within *P. aeruginosa* OMVs.

To do this, *P. aeruginosa* OMVs were isolated from planktonic cultures and were found to package and protect DNA, including plasmid DNA encoding for an AMR gene. DNase treatment of planktonic derived OMVs revealed that a small proportion of DNA was contained within OMVs which was protected from DNase degradation. Planktonic derived *P. aeruginosa* OMVs were able to transfer plasmid DNA encoding for an AMR gene to recipient *P. aeruginosa* at a greater efficiency than transformation with plasmid DNA alone. In comparison, OMVs isolated from biofilm cultures of *P. aeruginosa* contained more copies of plasmid DNA that was protected from DNase treatment, compared to planktonic derived OMVs. Furthermore, *P. aeruginosa* biofilm derived OMVs were more efficient at transferring plasmid DNA to recipient *P. aeruginosa* compared to planktonic derived *P. aeruginosa* OMVs. We are currently testing the transformation efficiency of biofilm derived *P. aeruginosa* OMVs when added to recipient *P. aeruginosa* biofilms, to determine the natural occurrence of OMV-mediated HGT.

Collectively, these studies will advance our limited knowledge regarding DNA packaged within OMVs and the contribution of OMVs to the transfer of AMR genes to facilitate the spread of antibiotic resistance.

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Identifying novel commensal virulence strategies and their driver during preterm neonatal sepsis

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There are >26,000 Australian infants born preterm (<37 weeks GA) each year who account for well over two-thirds of neonatal deaths and one-third of childhood disabilities. Neonatal late-onset sepsis (LOS; onset between 3-28 days postnatally) is a major contributor to this burden, resulting in both immediate and long-term sequelae. *Staphylococcus epidermidis (S. epidermidis)* is the predominant sepsis pathogen in preterm infants, accounting for >60% of cases in Australia. *S. epidermidis* constitutes a major component of the ubiquitous commensal skin and mucous membrane microbiota of all humans, and consistent with commensalism, rarely causes invasive infections in adults or even term infants. It remains unclear, which pathogenic determinants *S. epidermidis* employs to become invasive pathogens in immunocompromised hosts, such as preterm infants.

For my Ph.D. project, I aim to directly spy on sepsis-causing pathogens during the invasion of the preterm infant host. To achieve this, I am developing a dual RNA-sequencing (dual RNA-seq) protocol and pipeline to simultaneously analyze the gene expression changes occurring in the blood cells of the neonatal host and infecting pathogen during an episode of sepsis.

Currently, I am optimizing a clinically compatible RNA extraction protocol for the sensitive detection of low-abundance bacterial transcripts from human whole blood using a laboratory model of sepsis. I will use this model to define a set of stereotypic and species-specific virulence and host defense genes upregulated during host-pathogen interactions in a pilot cohort of healthy preterm infants, term infants, and adult hosts challenged with the two important sepsis-causing pathogens, *S. epidermidis* and *Staphylococcus aureus* (*S. aureus*). I will then validate my findings by applying dual RNA-seq to a small set of clinical sepsis samples to understand what makes the preterm infant so susceptible to commensal sepsis and set a crucial first step to find new ways to treat and prevent serious infections experienced by the most vulnerable population of infants.

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Identification of SARS-CoV-2 induced senescence reveals a new therapeutic target for COVID-19

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SARS-CoV-2 is the virus responsible for the COVID-19 disease pandemic. The elderly and individuals with comorbities are high risk groups for severe disease symptoms and mortality - but many of their cellular determinants for poor prognosis are yet to be identified. The high magnitude of inflammatory imbalances, inadequate immune response and long-covid aetiology in COVID-19 infections also remains unknown. One significant commonality between each high-risk group is an accumulation of senescent cells. These are cells that have

been subjected to cellular stress and or/injury resulting in a loss of proliferative capacity, resistance to apoptosis, and increase in metabolic and secretory activity.

Current literature suggests there are several similarities between the biological and signalling properties of senescence and COVID-19 manifestations. These include higher proportions of pro-inflammatory molecules, changes in tissue morphology, increase in cellular metabolism, decreased immune capacity and higher occurrence of ARDS. This research studied these similarities in order to determine whether or not cellular senescence plays a role in COVID-19 disease severity and long-covid aetiology.

A number of immortalised and primary human cell models were infected with live SARS-CoV-2 virus, before screening for senescence induction through the use of confocal immunofluorescent imaging, qRTPCR and senescence associated Beta-Galactosidase staining. It was evident that the infected models had a substantially higher count of senescence in contrast to the mock non-infected cell models.

The results showed that infection with SARS-CoV-2 can induce senescence in human cells. This may explain the reduction of immune capacity seen in severe COVID-19 infections, as well as the increased production of pro-inflammatory molecules that induce to the cytokine storm. It also provided an insight into long-covid aetiology; as senescence can cause extended disease morbidity, an increased persistence of pathogens and accumulation of proinflammatory molecules - leading to a higher prevalence of tissue and organ damage.

Fortunately, there are pharmaceuticals known as senolytics available that can remove senescent cell accumulation, and have been shown to reduce COVID-19 severity in human cell lines and animal models. This will hopefully advance the screening of senolytics into human trials and help us to reduce long-term symptoms in otherwise recovered COVID-19 patients.

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Non-canonical ubiquitin-mediated regulation of the dendritic cell receptor Clec9A and the modulation of immune responses

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Dendritic cells (DC) use a variety of cell surface receptors to monitor the environment for potential dangers, including cells that have died of non-homeostatic causes (eg. infected cells), to induce appropriate immune responses. Clec9A is a DC-specific Damage-Associated Molecular Pattern receptor, that facilitates the processing of dead cell-derived antigen (Ag) for cross-presentation thereby controlling immune responses to infected and damaged cells. A major focus of our research is identification of the molecular mechanisms that underpin Clec9A function and the control of immune responses, and on the development of Clec9A-targeting approaches for enhancing immune responses.

We recently identified a novel regulator of Clec9A and Ag cross-presentation, the E3 Ubiquitin ligase RNF41. We discovered RNF41 directly ubiquitinates the extracellular domains of Clec9A, to regulate receptor fate and antigen processing. At steady state, RNF41 ubiquitination of Clec9A facilitates novel interactions to regulate Clec9A levels. However, after dead cell uptake, these interactions are altered to favour antigen presentation. We define a non-canonical ubiquitination-mediated mechanism for the regulation of Clec9A, reflecting the unique properties of Clec9A as a receptor specialized for delivery of antigens for cross-presentation. These findings provide important insights into antigen cross-presentation and have implications for the development of approaches to modulate immune responses.

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Lipid droplets act as platforms for innate immune signalling proteins

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Lipid Droplets (LDs) were initially considered simply as a cellular energy source but are now recognised as critical organelles in signalling events, transient protein sequestration and inter-organelle interactions. Recently, our lab has demonstrated that LDs are upregulated during viral infection, and that this upregulation contributes to an enhanced interferon response from the infected cell indicating for the first time that the LD contributes to an effective immune response, however the mechanism of this is unknown.

Here, we describe for the first time that there are several critical key antiviral signalling molecules that localise to the LD during this response. We have optimised techniques to isolate pure lipid droplets from primary immortalised astrocyte cells before and following activation of viral RNA signalling pathways. Proteomic analysis has revealed there was 92 significantly upregulated proteins on LDs following stimulation with 13% of the significantly enriched proteins being associated with the interferon response. Of these, MX1 and ISG15 were significantly upregulated on LD fractions at both 8 and 24hrs following RNA viral mimic stimulation, whereas we see proteins such as viperin, RIG-I and STAT1 exclusively upregulated at 24hrs. Many significantly upregulated proteins are thought to be cytoplasmic, therefore, to confirm the localisation of these signalling proteins to the LD, a technique was designed to perform fluorescent confocal microscopy on isolated fluorescently stained lipid droplets probing for the identified immune proteins; and this, along with western blotting, has confirmed the localisation of these proteins to LDs.

Here, we demonstrate that there are important antiviral immune signalling proteins that localise to the LD following viral mimic stimulation, indicating that the LD can act as a signalling platform for signalosome formation to aid host immunity. The mechanism by which these proteins localise to the LD and the function of this is still being explored by our laboratory.

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Molecular basis underpinning the citrullinated self-antigen-mediated T cell immunity

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T cell-mediated adaptive immune response is critical to discriminate the myriad of self-peptides from non-self-peptides presented by HLA molecule. Despite the key role of the HLA in protective immunity, HLA class II molecules are frequently correlated with aberrant T cell immunity, including autoimmunity and other immune-mediated inflammatory diseases. For example, HLA-DR4 allomorphs bearing the Shared Susceptibility Epitope (SE) are associated with increased susceptibility to rheumatoid arthritis (RA). The breaking of T cell tolerance can be attributed to several mechanisms, including unusual TCR-HLA docking topologies, altered peptide-binding to HLA molecules, molecular mimicry, and neoepitope generation. For example, certain post-translational modifications (PTM), such as the deamidation of glutamine, peptide trans-splicing, and citrullination, generate neo-epitopes with improved binding to a given HLA molecule. However, the interplay between the HLA molecule, post-translationally modified epitope and the responding T cell repertoire remain unclear.

To understand the molecular basis of the citrullinated self-epitope, HLA-DR4, and CD4 T cell, we synthesized citrullinated fibrinogen peptide, which is found abundantly in joint synovium of RA patient, and investigated structurally their impact on HLA-DR4 recognition. Using HLA-DR4 transgenic mice as a tool, we immunized the mice with single (Fibb-74cit69-81) and double citrullinated (Fibb-72,74cit69-81) fibrinogen b-peptide. Our result led to a population of HLA-DR4Fib b-74cit69-81 tetramer^{+} T cells that exhibited biased T cell receptor (TCR) b-chain usage, which was attributable to selective clonal expansion from the pre-immune repertoire. Crystal structures of pre- and post-immune TCRs showed that the SE of HLA-DR4 represented a main TCR contact zone. Immunisation with a double-citrullinated epitope (Fibb-72,74cit69-81) altered the responding HLA-DR4 tetramer+ T cell repertoire, which was due to the P2-citrulline residue interacting with the TCR itself. Together, our findings showed that citrullinated epitope revealed dual functionality, in both HLA-DR4 presentation and a direct TCR recognition determinant. In addition, analogous biased TCR b-chain usage towards the Fibb-74cit69-81 peptide was observed in healthy HLA-DR4^{+} individuals and HLA-DR4^{+} RA patients, thereby suggesting a link to human RA.

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Identifying the mechanisms of action of novel invasion-blocking compounds in *Plasmodium falciparum*

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Malaria is a devastating acute febrile disease caused by *Plasmodium* parasites that are spread to people through the bites of infected female *Anopheles* mosquitoes. Malaria clinical symptoms (e.g. fever, chills, nausea and vomiting) manifest during the parasite blood stage when parasites invade human red blood cells (RBCs), proliferate and reinfect RBCs. In 2020, nearly half of the world's population was at risk of malaria and 627,000 malaria deaths were reported.[1] *Plasmodium falciparum* is the deadliest species, accounting for almost all malaria deaths in 2021.[1] In recent years, parasites' emerging resistance against all frontline antimalarials contribute to the slow decrease in malaria cases and threatens the progress of malaria eradication. This stresses the importance of new drugs blocking novel parasite mechanisms, specifically parasite invasion, an extracellular process critical for parasite proliferation. To accelerate the discovery of new antimalarials, Medicines for Malaria Venture released two open-access compound libraries, the Malaria and Pathogen Box. Our group's recent screening of the Pathogen Box for RBC invasion inhibitors unveiled the invasion-blocking compound, MMV687794.[2] Whole-genome sequencing and variant analyses of MMV687794-resistant parasites indicated mutations in an α/β-hydrolase, possibly responsible for RBC lipid remodelling for downstream parasite processes (e.g. lipid metabolism and/or signal transduction) essential for efficient invasion. These mutations have been introduced into wild-type parasites using CRISPR/Cas9 to establish that the mutations alone confer resistance to MMV687794. Likewise, an epitope tag and riboswitch are introduced into the gene, allowing for further functional biochemical and phenotypic analyses of the α/β-hydrolase via protein knockdown and localisation. If MMV687794 is shown to target the α/β-hydrolase specifically, protein crystallography of the compound-enzyme complex is achievable, which may lead to improved rational drug design and increase our understanding of parasite invasion machinery. Indirect immunofluorescence microscopy thus far suggests that the α/β-hydrolase is localised to the rhoptry, supporting the enzyme's crucial role in invasion.

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The transmembrane adaptor SCIMP recruits Syk for TLR activation

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Spleen tyrosine kinase (Syk) is a non-receptor protein tyrosine kinase, which is known to be involved in adaptive and innate immune signalling. A prototypical Syk recruitment mechanism to cell surface has been well characterised in ITAM-based immunoreceptor signalling pathways, which includes the T cell receptors, B cell receptor and Fc receptors. In these cases, Syk is activated by binding of its two SH2 domains to appropriately spaced phosphorylated tyrosine motifs. However, TLR4 has no conserved ITAM motif, therefore the underlying mechanism for Syk activation and recruitment to TLR4 is not known. One possibility is that the Syk-TLR association is indirect, relying on a scaffolding protein. In our study, we identified the transmembrane adaptor SCIMP, a member of the pTRAP family, plays such a role in bridging Syk to TLR4 for regulating TLR4 phosphorylation and generating pro-inflammatory responses. Mechanistically, we showed that two phosphorylated tyrosines (Y96 and Y120) of SCIMP serve as binding sites for N-SH2 and C-SH2 domains of Syk, thus tethers to Syk. Activated Syk subsequently functions to phosphorylate both TLR4 and SCIMP. In addition, phosphorylation of each signalling molecule by Syk also stabilised the association of the TLR4-SCIMP-Syk ternary complex. These results provide evidence that Syk activity and scaffolding role of SCIMP are both indispensable for TLR signal transduction. Therefore, we identified SCIMP as a novel Syk scaffold, which helps to propagate TLR4 signal transduction and inflammation.

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Infection-induced WNT responses in macrophages are regulated by innate immune receptor activation

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Publish consent withheld

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Molecular insights into the HLA-B35 molecules' classification associated with HIV control

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Human leukocyte antigen (HLA) class I molecules have been shown to strongly influence the immune response to HIV infection and acquired immunodeficiency syndrome (AIDS) progression. Polymorphisms within the HLA-B35 molecules divide the family into 2 groups Px and Py. The Px group is associated with deleterious effects and an accelerated disease progression in HIV^+ patients, while the Py group is not. The classification is based on the preferential binding of a Tyrosine at the C-terminal part of the peptide in the Py group, and a non-Tyrosine residue in the Px group. However, there is lack of knowledge on the molecular differences between the two groups. Here, we have investigated three HLA-B35 molecules, namely HLA-B*35:01 (Py), HLA-B*35:03 (Px), and HLA-B*35:05 (unclassified). We selected an HIV-derived peptide, NY9, and demonstrated that it can trigger a polyfunctional $CD8^+$ T cell response in HLA-B*35:01^+/HIV^+ patients. We also provided the first αβTCR repertoire analysis of the NY9-specific T cells. We determined that in complex with the NY9 peptide, the Py molecule was more stable than the Px or the unclassified molecule. We solved the structures of the three HLA molecules in complex with the NY9 peptide, and structural similarities with HLA-B*35:01 would classify the HLA-B*35:05 within the Py group.

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Screening the MMV Malaria Box for inhibitors of *Plasmodium falciparum* protein export

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Malaria parasites need to traffic proteins to various cellular regions such as organelles, membranes and even onto the cell exterior. Blood-stage *Plasmodium* parasites have established an additional destination for their proteins which is out into the cytoplasmic compartment of their host red blood cell (RBC). *P. falciparum* parasites invest almost 10% of their proteome into modifying their RBC to acquire plasma nutrients, avoid host immunity and likely to perform a myriad of yet-undefined functions. The *Plasmodium* Translocon of Exported Proteins (PTEX) is an essential protein complex which resides in the parasitophorous vacuole membrane enveloping the intracellular parasites.

PTEX acts as a conduit through which exported proteins are transported into the RBC cytoplasm. Due to the essential nature of PTEX and protein trafficking more generally, this pathway could be developed as an excellent drug target.

The Medicines for Malaria Venture (MMV) Malaria Box is a library of 400 compounds with anti-malarial activity. We screened the MMV Malaria Box for inhibitors of *P. falciparum* protein secretion and export. For this screen parasites were transfected with a bioluminescent Nanoluciferase fusion protein containing an export sequence, allowing trafficking of the protein into the host RBC. After drug treatment, differential fractionation of the cells allowed measurement of the fraction of bioluminescent protein trafficked into the parasite, parasitophorous vacuole and RBC compartments. Using this screen, several compounds were shortlisted for their ability to inhibit protein export to the host RBC. In this work we used a variety of assays to further characterise the activity of these shortlisted export inhibitors and to examine their effect on parasite activity. Further, we aim to discover the targets and mechanism of action of these compounds in hopes of developing them into future antimalarials.

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Profiling circulating immunoglobulins and cytokines in Acute Rheumatic Fever Patients

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Acute Rheumatic Fever (ARF) is a severe autoinflammatory disease that can develop after a Streptococcus A (StrepA) infection in susceptible children. ARF is a stark example of health inequality in New Zealand, with Māori and Pacific children 20-40 times more likely to experience ARF. Disease pathogenesis remains poorly understood, and this contributes to the lack of specific therapies and treatments.

In a previous systems immunology analysis, we observed that immunoglobulin subclass 3 (IgG3) and complement component 4 (C4) were significantly elevated above the clinical reference ranges in >90% of ARF patients, and that IgG1, IgG3, IgA and C4 were able to distinguish ARF patients from matched healthy controls.[1]

In this study we have extended these analyses to include circulating cytokines in an expanded cohort comprised of 60 ARF cases and 60 age and ethnically matched controls drawn from recently completed large-scale studies of ARF and StrepA infections in New Zealand.[2,3] Circulating concentrations of 20 cytokines, as well as the four previously identified immune features were quantified using bead-based multiplex assays. The results corroborate earlier findings with IgG1, IgG3, IgA and C4 significantly elevated in ARF patients compared to matched healthy and acute StrepA pharyngitis controls. Furthermore, significant elevation of IL-6, IL-7, TNF-a and APRIL were observed in ARF patients, while IP-10 elevation was associated with StrepA pharyngitis. While IL-6 and TNF-a have previously been associated with rheumatic fever and rheumatic heart disease, the identification of lymphocyte growths factors IL-7 and APRIL provides new insight into ARF immunopathogenesis. Correlations and cluster analysis highlight the interplay between immune features in this expanded disease signature, which may help identify targets for immune-modulating drugs or immune-based therapies for ARF in the longer term.

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Transcriptional and epigenetic regulation of CCL17 and CCL22 by GM-CSF and IL4

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The CCR4 receptor is expressed by Th17 and regulatory T cells, and an imbalance between these two T cell subpopulations is thought to drive autoimmunity and its associated chronic inflammation. CCL17 and CCL22 are the functional ligands of the CCR4 receptor. These chemokines share a nucleotide homology of 32% and are found in close proximity to each other on both the human and mouse chromosomes. Despite their similarity, they are variably expressed in rheumatoid arthritis (RA), where CCL17 is highly upregulated in the synovial fluid of patients and CCL22 is detected at very low levels. This expression suggests that these chemokines are regulated differently.

GM-CSF and its receptor are currently being targeted in clinical trials for RA. GM-CSF is highly upregulated in the synovial fluid of RA patients, and it has also been shown to upregulate CCL17 expression. IL4 is another cytokine that upregulates CCL17 expression, but it is detected at very low levels in RA patients. These cytokines have contrasting roles in inflammation but they both upregulate CCL17 production by promoting JMJD3 demethylase activity and IRF4 expression.

We report here that GM-CSF and IL4 upregulates CCL22 expression in human monocytes, human macrophages, and mouse macrophages. Its upregulation is variably dependent on JMJD3 and IRF4 in these cell types, which suggests distinct signalling pathways in different cell types. Moreover, GM-CSF and IL4 independently activate the transcription factors STAT5 and STAT6, respectively, and their activation is crucial for CCL17 and CCL22 expression in all three cell types. This variable regulation of these seemingly similar chemokines sheds light on the nuances of cell types and their role in autoimmune diseases. As therapies begin targeting more specific, downstream mediators, delineating the signalling pathways activated by key inflammatory cytokines, and discerning differences between immune cell types, will increase the efficacy of future therapies.

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Determining the effect of HBV splice variant 9 (Sp9) on wildtype HBV replication

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Introduction: Over 296 million people are currently living with chronic hepatitis B which can lead to the development of liver cirrhosis and hepatocellular carcinoma (HCC). Prior to nuclear export, the pregenomic RNA (pgRNA) can be spliced by the host cell spliceosome to form shorter RNA sequences known as splice variants. Splice variants retain the encapsidation signal and so are packaged into core particles, reverse transcribed into shorter DNA genomes and secreted from the cell as defective viral particles. 17 splice variants have been characterised thus far, and whilst the role of splice variants currently remains unknown, an increased proportion of splice variants in patient sera has been associated with the development of HCC. Furthermore, different splice variants have been shown to have different effects on wildtype HBV replication. Due to the deletions in the open reading frames, splice variants can encode novel fusion proteins, and some novel fusion proteins have been found to play a role in their respective splice variant's effect on wildtype HBV replication. It remains unknown how the second most common splice variant, Sp9 and its novel fusion proteins, affects wildtype HBV replication.

Methods: A greater than genome length (1.3mer) Sp9 clone was co-transfected with a replication competent wildtype HBV clone and the replication phenotype of wildtype HBV was analysed five days post-transfection. Sp9 novel fusion protein overexpression plasmids and mutant Sp9 clones that knocked out Sp9's novel fusion proteins were also co-transfected with WT HBV and the replication phenotype of wildtype HBV was analysed.

Results: Co-transfection of Sp9 with WT HBV markedly reduced HBV DNA, pgRNA and S mRNA production, as well as intracellular HBV core and S protein production. Intracellular and secreted HBV E and S antigen levels were also markedly reduced by Sp9. Cytotoxicity assays confirmed that Sp9 is not cytotoxic to the cells. Co-transfection of WT HBV with Sp9 novel protein knockout clones partially restored HBV replication.

Conclusion: The role of HBV splice variants in HBV replication and pathogenesis remains unclear. This study shows that Sp9 significantly reduces HBV replication, which may be due to the novel fusion proteins expressed by Sp9.

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Regulation of natural killer cell function in *Salmonella* Typhimurium infection

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Immune "checkpoint" inhibitor antibodies have revolutionized cancer therapy by reactivating tumour-killing immune cells. More recently, immune checkpoint therapy is emerging for its potential in restoring immunity against infectious agents. Checkpoint inhibitors primarily block inhibitory pathways in tumour-resident T cells, however interest in other effector populations, such as natural killer (NK) cells, is also growing. NK cells are key to cancer immunosurveillance, particularly in settings of metastasis, yet their potential for controlling bacterial infection is less clear. NK cells are dependent on the cytokine interleukin (IL)-15 for their survival, development, and function, but how this cytokine is regulated remains enigmatic. IL-15 signalling naturally employs negative feedback mechanisms to keep NK cell activation and function in check. One such mechanism is cytokine-inducible SH2-containing protein (CIS), which is induced by IL-15 and was described as a potent intracellular NK cell checkpoint that suppresses IL-15 receptor signalling. In addition, we have previously discovered that tumours can neutralize NK cell metabolism, proliferation, and activation by TGF-β immunosuppressive pathways by inducing plasticity of NK cells and differentiation into innate lymphoid cell (ILC)1-like subsets, which have a reduced capacity for tumour killing. Here, we investigate whether enhancement of NK cell function by targeting these regulatory mechanisms can improve anti-bacterial immunity, using Salmonella Typhimurium as a model. We identified for the first time NK cell to ILC1-like plasticity within a bacterial infection model, however the precise driver of plasticity in this model remains elusive. We further describe that CIS-deficient mice displayed enhanced pro-inflammatory function and dramatically enhanced anti-bacterial immunity. However, a CIS and TGF-β signalling-double-deficient NK cell mouse model did not synergize for increased resistance to infection.

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The Persistence of Neutralising Antibodies up to 11 months after SARS-CoV-2 Infection in the Southern Region of New Zealand

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Circulating antibodies are important markers of prior infection and protective immunity. Questions remain with respect to the durability and functionality of SARS-CoV-2 antibodies. We comprehensively analysed the antibody dynamics of 189 PCR-confirmed COVID-19 cases up to 8 months post-infection in New Zealand following the first nation-wide lockdown in 2020. We found that neutralising antibodies to the ancestral strain, were relatively stable over the study period. Due to the emergence of SARS-CoV-2 variants of concern (VoC) we subsequently extended the analysis of neutralising antibodies in 78 of these COVID-19 cases from the Southern region up to 11 months post-infection against the alpha, beta and delta VoC. There was no known community transmission in the region over the study period due to New Zealand's elimination status at the time, nor had any participants received a COVID-19 vaccine. In the absence of re-exposure,

antibody reactivity to the viral spike protein, as well as neutralising antibodies to both the ancestral strain and the delta variant remained relatively stable between 8 and 11 months post-infection. This suggests long-lived antibody responses can be generated from a single natural infection event. However, given the risks of serious disease associated with SARS-CoV-2 infection and the recent emergence of the omicron variant, vaccination remains strongly recommended. Assessement of omicron neutralisaing antibodies in the cohort are underway and will be presented.

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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 patients with sepsis spend in intensive care units, but they do not lead to a reduction in overall mortality. Recently, the 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 whole-genome CRISPR screening in mice, we identified a triggering receptor expressed on myeloid cells (TREM) family receptor, TREML4, as a key regulator of inflammation and immune cell death in sepsis. Genetic ablation of Treml4 in mice demonstrated that TREML4 regulates a host of cellular responses, particularly of innate immune cells, during polymicrobial sepsis, leading to an overall increase in survival rate, both during the acute and chronic phases of the disease.

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T follicular helper cell responses during uncomplicated falciparum and vivax malaria

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Malaria is a significant cause of mortality and morbidity globally. *Plasmodium falciparum* and *P.* vivax are the parasite species responsible for the majority of malaria cases. Protective immunity to both *P. falciparum* and *P. vivax* is mediated by antibodies. Induction of protective antibodies is driven by CD4 T follicular helper cells which activate B cells during infection. To date, there is only a single study of Tfh cells during infection for *P.vivax* and *P. falciparum*, but no study has directly investigated the influence of parasite each species on Tfh cell responses during malaria in humans.

We assessed Tfh cell responses in adults with either uncomplicated *Plasmodium falciparum* (n=8) or *Plasmodium vivax* (n=8) malaria compared to healthy uninfected endemic controls (n=5). Tfh cell responses were measured ex vivo by flow cytometry and functional capacity measured by intracellular staining of cytokines produced following PMA/Ionomycin stimulation.

We observed significant increase in Tfh cells numbers and activation of Tfh cells across species. Both Th1 and Th2 – Tfh cell subsets are activated by both species. However, there are no differences in the Tfh subset distribution and cytokine profiles between species.

Our data shows that Tfh cells are activated in *P falciparum* and *P.vivax* infections but the parasite species does not influence their activation and function during infection. These findings provide insights into the influence of malaria parasite species on Tfh cell responses and warrants further assessment for strategies targeting Tfh cell responses to improve vaccine efficacy.

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A blunted GPR183/oxysterol axis during dysglycemia results in delayed recruitment of macrophages to the lung during *M. tuberculosis* infection

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We previously reported that the oxidised cholesterol-sensing receptor GPR183 is significantly downregulated in blood from tuberculosis (TB) patients with diabetes compared to TB patients without co-morbidities and that lower GPR183 expression in blood is associated with more severe pulmonary TB on chest-x-ray consistent with observations in dysglycemic mice.

To further elucidate the role of this receptor and its endogenous high affinity agonist 7α,25-di hydroxycholesterol (7α,25-OHC) in the lung, we studied high fat diet (HFD)-induced dysglycemic mice infected with *M.tuberculosis* .We found that the 7α,25-OHC-producing enzymes cholesterol 25-hydroxylase (CH25H) and cytochrome P450 family 7 subfamily member B1 (CYP7B1) were highly upregulated upon *M. tuberculosis* infection in the lungs of normoglycemic mice, and this was associated with increased expression of GPR183 indicative of effective recruitment of GPR183-expressing immune cells to the site of infection. We demonstrated that CYP7B1 was predominantly expressed by macrophages in the centre of TB granulomas. Expression of CYP7B1 was significantly blunted in lungs from HFD-fed dysglycemic animals and this coincided with delayed recruitment of macrophages to the lung during early infection and more

severe lung pathology. GPR183 deficient mice similarly had reduced macrophage recruitment during early infection demonstrating a requirement of the GPR183/oxysterol axis for macrophage infiltration into the lung in TB.

Together our data demonstrate that oxidised cholesterols and GPR183 play an important role in positioning macrophages to the site of *M. tuberculosis* infection and that this is impaired by HFD-induced dysglycemia, adding a mechanistic explanation to the poorer TB outcomes in patients with diabetes.

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Spike specific T cell mediated immunity in vaccinated and COVID-19 recovered individuals

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the ongoing coronavirus disease 2019 (COVID-19) pandemic. With the increasingly high uptake of the vaccines available, efforts are being made worldwide to understand and compare the immune response to SARS-CoV-2 in vaccinated and COVID-19 recovered individuals including the impact of T cell immunity.

We assessed the *ex-vivo* T cell response to Spike-derived peptides in HLA-A*02:01^{+} vaccinated and COVID-19 recovered individuals and identified novel epitopes, as well as the difference in response between the two cohorts of donors. *In vitro* peptide stimulation revealed T cell-mediated cross-reactivity with variant of concern (VOC) derived peptides, as well as the TCR repertoire of selected spike epitopes. In addition, we have also characterised HLA-A*02:01 presentation of spike-derived epitopes and their recognition by T cell receptors at the atomic level, their affinity for VOC epitope and their stability.

Our study contributes to a deeper understanding of the T cell mediated immunity from SARS-CoV-2 infection and COVID-19 vaccines, their key differences, that could be exploited to design universal vaccines against VOC.

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Modulation of inflammatory cytokine production through cGMP and Interleukin-1 receptor associated kinase-3 (IRAK3)

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IRAK3 is a critical negative regulator of innate immunity, and involved in many diseases such as sepsis and asthma. Thus, IRAK3 is potentially a diagnostic biomarker or immunotherapy target. IRAK3 down-regulates inflammatory responses and is required for endotoxin tolerance where the production of inflammatory cytokines is diminished upon constant exposure or re-challenge of endotoxins. However, mechanisms of IRAK3 actions are not fully understood, and IRAK3 actions in inflammation are dependent on cell type, type of stimuli and stimulation duration. Recent studies showed IRAK3 contains a guanylate cyclase (GC) centre that can generate cyclic guanosine monophosphate (cGMP) (1). Mutations in the catalytic site of the GC centre modify IRAK3 function in transiently transfected HEK293 cells (1). cGMP is a second messenger regulating multiple physiological processes including inflammation. Since effects of low levels of cGMP on inflammation are unknown, we undertook dose-response studies on monocytic cell lines (THP-1 and THP-1 BLUE) during endotoxin challenge measuring inflammatory responses. Sub-nanomolar concentrations (0.1 nM) of membrane permeable 8-Br-cGMP reduced LPS-induced NF-κB activity and cytokine (IL-6 and TNF-α) production. Pharmacologically upregulated cellular cGMP level reduced cytokine production. Downregulation of cellular cGMP increased the level of cytokines, while membrane permeable cGMP at 0.1 nM inhibited this effect. Using CRISPR/Cas9 we generated IRAK3 knock-down THP-1 cell lines to investigate if IRAK3 was necessary for cGMP effects on inflammation. Unlike the wildtype cells, 8-Br-cGMP at 0.1 nM to 100 nM did not suppress inflammatory responses in IRAK3-knockdown cells. Complementation of the IRAK3-knockdown cells with wildtype IRAK3, but not mutant IRAK3, suppressed cytokine production. Taken together, low levels of cGMP form a critical component of IRAK3 action and that this indeed may be via cGMP enriched nanodomain formed by IRAK3 itself.

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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. T cells are divided into the αβ or γδ lineages. Progenitors that enter the thymus remain multipotent, with the potential to differentiate into a range of cell types. T lineage identity is then fixed early in T cell development. Later, γδ T cells branch off, while αβ development continues onto additional stages. 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 lineage. 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 (scRNA-seq) was employed to re-assemble *de novo* a model of the early stages in T cell development based on the transcriptional profiles of individual cells. >20,000 CD4-CD8- double negative (DN) and γδ thymocytes were analysed using Chromium 10x scRNA-seq over three runs. Hierarchical clustering revealed that early thymocyte populations are much more complex than the standard view of T cell development. Assembly of these thymocyte populations into developmental trajectories based on gene expression suggested that the decision to differentiate into αβ versus γδ T cells occurs at a

much earlier stage than the current model and that distinct DN1 and DN2 sub-populations are specific to either the αβ or γδ developmental pathways. To confirm that specific sub-populations are restricted to either αβ or γδ lineages, they were sorted and analysed for lineage outcomes in OP9-DL1 cultures. We show that lineage is already restricted in DN1 thymocytes, which is considered the earliest stages of T cell development. Thus, γδ developmental appears to occur in a parallel with αβ development and not a branch as previously thought.

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Investigating Memory CD8+ T cell Heterogeneity in Tissues and Tumours

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Tissue resident memory (T_{RM}) cells permanently reside within barrier tissues where they provide protection against a plethora of invading pathogens and cells of tumorigenic origin. T_{RM} cells represent a unique memory population, sharing a core residency signature across various tissues distinct from that of circulating memory T cells. Tumour-infiltrating lymphocytes (TIL) share aspects of this T_{RM} cell signature, with the presence of TIL-T_{RM} associated with improved survival in cancer patients. It is well established that the circulating memory T cell pool is highly heterogeneous, yet T_{RM} cells have been typically viewed as a relatively homogenous population identified by the canonical $CD8^+$ T_{RM} cell markers CD103 and/or CD69. However, recent findings have challenged this notion, showing that $CD8^+$ T_{RM} cells within a given tissue can have diverse functions, express different transcription factors, and display altered cytokine profiles. Our understanding of the extent of T_{RM} cell diversity, and the implications of this heterogeneity for immune protection is only starting to be uncovered. Furthermore, the factors that control T_{RM} cell heterogeneity are not known. Using viral infection and tumour models, we aim to uncover what factors govern T_{RM} cell diversity within tissues and the tumour microenvironment. Such discoveries will be key in the development of novel immunotherapeutic strategies designed to target T_{RM} cell populations.

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Antibody profiles 6 years following reduced-dose quadrivalent HPV vaccine schedules in adolescent Fijian girls

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Persistent infection with human papillomaviruses (HPV) can cause cervical cancer, which is the fourth most common cancer in women worldwide. Most cases occur in low- and middle-income countries (LMIC) where resource constraints pose significant barriers to prevention. Prophylactic HPV vaccines have demonstrated strong protection against persistent HPV infection, cervical pre-cancerous lesions and cancer. These vaccines are currently recommended as 2-dose HPV vaccine schedule separated by 6 months in boys and girls under the age of 15, although emerging data suggest that a single dose of the HPV vaccines offers similarly strong protective antibody responses. At present, very little is known about the characteristics of the antibody response following reduced-dose HPV vaccine schedules. In this study, we examined the antibody profiles of adolescent Fijian girls who previously received 1 or 2 doses of 4vHPV (Gardasil®, Merck Inc.) 6 years earlier, compared to those who received 3 doses. A prospective cohort study was undertaken in 200 Fijian girls (aged 15-19 years at the commencement of the study) who previously received 0, 1, 2 or 3 doses of 4vHPV in 2008-2009. Blood was taken before and 28 days following a booster dose of 2vHPV (Cervarix®, GSK). HPV16/18-specific antibody subclasses (total IgG, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2 and IgM) in serum were measured using a HPV multiplex immunoassay on a subset of the study samples, with equal numbers of participants in each dose group (N=80, 20/group). After 6 years, there were no significant differences in antibody profiles between the 2- and 3-dose groups. HPV16/18-specific geometric mean FI were significantly lower for the 1-dose group compared to the 2-dose group in terms of IgG (HPV16; p=0.0006, HPV18; p=0.0071 respectively), IgG1 (HPV16; p=0.0246), and IgA1 (HPV16; p=0.0042). No significant differences were found between these groups for IgM, IgA2, IgG2, IgG3 and IgG4. Post-2vHPV, HPV16/18-specific antibody profiles were similar between groups who previously received 1, 2 or 3 doses of 4vHPV. These data suggest that 1-dose vaccine schedules may induce functional antibody responses similar to 2 or 3 doses, and provide immunological evidence supporting 1-dose HPV vaccine schedules in LMICs.

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Enhanced nitric oxide production by macrophages treated with SPSB-iNOS inhibitors conjugated to cell-penetrating peptides

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Nitric oxide (NO) is a key effector molecule of the innate immune response, and plays a crucial role in macrophage killing of infective agents.[1] NO is produced by inducible NO synthase (iNOS) in macrophages in response to foreign stimuli. The lifespan of iNOS is regulated by proteasomal degradation, which is mediated by binding to SPRY domain containing SOCS box proteins (SPSB).[2] Our earlier studies revealed that the DINNN sequence at the N-terminus of iNOS is the key motif for SPSB binding.[2] Disruption of the iNOS-SPSB interaction resulted in enhanced lifespan of iNOS, increased NO production and pathogen killing.[2] A series of linear (7-mer, 13-mer) and cyclic (CP0-3) peptide inhibitors of the iNOS-SPSB interaction have been designed based on the DINNN motif and refined for increased binding affinity, stability and drug likeness.[3-5]

In this study we have conjugated the inhibitors with cell-penetrating peptides (CPPs) and demonstrated that binding to SPSB is not compromised by this conjugation. We have assessed the successful uptake of fluorophore tagged inhibitor-CPP conjugates by RAW 264.7 and immortalised bone marrow derived macrophage (iBMDM) cell lines. We have designed and optimised an assay to evaluate the potential of CPP-cargo for enhance NO production and found that these inhibitors elevated NO level. We have also shown that these inhibitors are not toxic to macrophages. The findings of this study will be useful in further optimising the design of SPSB inhibitor-CPP conjugates.

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Benchmarking and developing human pluripotent models of macrophage biology

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Human pluripotent stem cell (hPSC)-derived macrophages offer new opportunities to understand the role of development or tissue context in innate immune cell function. Immune responsiveness to pathogenic challenge is known to be impacted by a macrophage's history of prior exposure, as well as ontogeny and tissue context. Therefore, we explore the factors of *in vitro* derivation likely to influence macrophage phenotype and function, and highlight the gaps in, not only our understanding of macrophage development and function, but also in hPSC models that aim to mimic their *in vivo* counterparts. We developed a transcriptomic atlas to assess impact of ontogeny, experimental treatment and tissue residency on molecular phenotype. We revealed gaps in hPSC-macrophages, revealing several differences between hPSC-macrophages, monocyte-derived macrophages and primary cells. These differences include poor maturation in hPSC-macrophages in the absence of priming signals such as IFN, or repeated exposure to LPS. We demonstrate the requirement for priming in hPSC-macrophages and discover the importance of re-stimulation events in shaping macrophage activation. We further assess phenotypic heterogeneity in both monocyte- and hPSC-macrophages using single-cell sequencing. We demonstrate synchronised population responses to LPS activation and further provide evidence for priming in shaping macrophage responsiveness. These findings highlight that macrophages are shaped by prior activating, or priming, signals which can be recapitulated in the laboratory. Outcomes of this work are expected to improve routine macrophage derivation from hPSC sources, as exploitation of priming provides future opportunities to shape the quality of acute or long-term macrophage responsiveness for diverse applications.

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Decoding the Immune Response: Next Generation Sequencing of the Immune Repertoire

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The study of complex immunological diseases and tumor microenvironments has advanced through recent developments in sequencing of the immune repertoire. Using this approach, the interrogation of disease progression is facilitated through analysis of millions of V(D)J combinations from B cell antibodies (BCRs) and T cell receptors (TCRs). One major challenge of immune repertoire sequencing is to accurately capture the structural and sequence complexities of antibodies and TCR genes during both library preparation and bioinformatic analysis**.** Here, we present a method for accurate sequencing of full-length immune gene repertoires of B cells and T cells.

RNA was extracted from tissues and peripheral blood mononuclear cells (PBMCs) and used for reverse transcription, during which unique molecular identifiers (UMIs) were added to discretely barcode each mRNA molecule. BCR- and TCR-specific PCR primers were used to enrich full-length BCR and TCR sequences. We have implemented a data analysis pipeline to assemble the full length BCR/TCR transcripts and to collapse PCR copies of each mRNA fragment into a single consensus sequence using UMIs. UMI incorporation enables the absolute quantification of input RNA molecules and accurate ranking of antibody/TCR clone abundance. Furthermore, this method facilitates detection of distinct and shared clones in tissue and blood samples, allowing identification of disease-specific clones to evaluate immunotherapy effects. Our method accurately and sensitively detects target TCR clones down to 0.01%, enabling minimal residual disease (MRD) assessment.

Our immune repertoire sequencing approach allows accurate clonal determination for both BCR and TCR. This technique is applicable for a variety of applications including design of antibody chains for *in vitro* synthesis, investigation of T cell infiltration of tumor microenvironments, and monitoring of minimal residual disease in cancer patients.

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Investigating the epigenetic and transcriptional regulation of cytokine production by *Plasmodium falciparum*-exposed monocytes

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Malaria is a *Plasmodium falciparum*-caused febrile blood borne disease that drives excessive production of proinflammatory cytokines and promotes the development of severe malaria symptoms. To understand this excessive cytokine production, the epigenetic and transcriptional pathways regulating cytokine production is investigated, as this may reveal novel therapeutic targets to treat malaria.

In this study, the transcriptional NF-kB-JMJD3 pathway is investigated in the production of cytokines TNF, IL-1β, IL-6 and IL-10; as well as elucidating malaria-induced histone modifications in monocytes. Naïve monocytes were treated with lysed *P.falciparum*-infected red

blood cells (iRBC) for up to 4 hours. Cytokine gene and protein expression were measured by qPCR and ELISA, respectively, while the activity of transcription factors (e.g. NF-kB phosphorylation) and histone modifications (e.g. histone 3 lysine 27 trimethylation (H3K27me3)) were determined by Western blotting. Enriched histone extracts from iRBC-treated monocytes were screened using a Histone 3 modification multiplex assay to detect differently regulated histone modifications.

Results showed that iRBC-treated monocytes produced elevated cytokine levels, but without altering NF-kB phosphorylation or H3K27me3 levels. However, 2-fold and 1.2-fold increases in novel H3K4me1 and H3K4me3 modifications, respectively, were identified in iRBC-treated monocytes through the multiplex histone 3 assay. Pharmacological inhibition of H3K4-methyltransferase activity with WDR5-0103 did not affect global H3K4 methylation levels, but significantly reduced secreted levels of TNF and IL-6 y iRBC-treated monocytes.

In conclusion, these results collectively indicate that malaria-induced cytokine production in monocytes is not regulated through the NF-kB-JMJD3 pathway, rather through the H3K4 modifications. Further investigation into the location of the H3K4me1 and H3K4me3 modifications along the TNF and IL-6 gene locus is warranted. This may further support a case for targeting such modifications as potential anti-malarial therapeutics.

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Novel immunotherapeutic strategies for bronchopulmonary dysplasia

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The postnatal maturation of the immune system is poorly understood; as is its impact on illnesses afflicting preterm infants, such as bronchopulmonary dysplasia (BPD), a chronic lung disease. BPD affects up to 65% of very preterm infants and is characterized by inflammation and arrested development of alveoli and pulmonary blood vessels, leading to compromised lung function. The dysangiogenesis causes marked reduction in cross-sectional area, abnormal pulmonary vascular tone and elevated pressure in the pulmonary vasculature, resulting in pulmonary hypertension secondary to BPD (BPD-PH). BPD-PH occurs in up to 39% of severe BPD cases and represents its most significant complication. If not halted, BPD-PH can progress to right heart failure and up to 50% mortality. Currently, safe and effective treatments for BPD/BPD-PH are not available.

Investigating 51 preterm infants (blood obtained longitudinally at birth, day 1, weeks 1&2, and 36 weeks gestational age), 20 healthy term infants (birth, 3-16 weeks) and 5 healthy adults, we observed strong associations between type 2-polarization in circulating CD4+T cells and BPD (odds ratio up to 24). Unexpectedly, maternal magnesium sulfate therapy and delayed hepatitis B vaccination were associated with weaker type 2-polarization (e.g., up to 91% fewer type 2-polarized cells with hepatitis B-vaccination on d4-6 versus soon after birth), potentially conferring protection against BPD/BPD-PH. In a murine model of neonatal BPD/BPD-PH, blocking the type 2-mediators STAT6 or IL-4, IL-5 and/or IL-13 ameliorated lung inflammation and protected alveolar and vascular integrity: BPD-triggered increases in IL-1β and IL-13, and losses of pulmonary capillaries were prevented.

Our work advances knowledge on developmental immunology and its impact on early life diseases such as BPD. We identify maternal magnesium sulfate therapy, delayed hepatitis B vaccination and inhibition of type 2 mediators as promising therapeutic avenues that may lead to effective treatments for BPD/BPD-PH, relieving inflammation-driven suffering in our youngest patients.

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Probing the molecular basis of curbing TLR4-driven inflammatory responses

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Publish consent withheld

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Development of a high throughput ex-vivo screening assay for peptide-based T cell cancer vaccines

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Introduction: Cytotoxic CD8+ T lymphocyte (CTL) cells are central in mediating anti-tumour immunity. Hence, CTLs are a major target for cancer immunotherapy, such as peptide-based T cell vaccines. The primary mechanism of CTL anti-tumour response is through cell-mediated cytotoxicity. Therefore, we developed an *ex-vivo* assay to evaluate the ability of novel therapeutics to induce CTL cytolytic activity on numerous target cell lines. We analysed the cytotoxic killing response of *in-vivo* generated antigen-specific CTLs, on fluorescently labelled target cells *ex-vivo*. Using this direct *ex*-vivo assay, we will be able to screen and identify potent and widely applicable vaccine formulations.

Method: Mice were immunised with a vaccine formulation (antigen + adjuvant) to generate *in-vivo* vaccine antigen-specific CTLs. On day 7, CTLs were harvested from lymph nodes and isolated via AutoMACS separation. Subsequently, CTLs were incubated (18 hours) *ex-vivo* with fluorescently labelled target (hi-FITC) and control (lo-FITC) cell lines at various effector: target cell ratios (0:1, 1.5:1, 3:1, 6:1, 12:1, 25:1, 50:1, 100:1). Cell samples were analysed via flow cytometry, and the CTL specific lysis was calculated based on the frequencies of the target and control cell lines.

Results: The level of target cell specific killing was found to correlate with an increasing effector: target cells ratio with observable killing occurring at *6:1 ratio* and reaching a maximum CTL lysis of *81.67% at 100:1 ratio*. Additionally, non-specific CTL response was absent.

Conclusion: We have developed an *ex-vivo* assay that allows analysis of CTL response on target cells. This assay will be instrumental in translation for high throughput screening of cancer cell lines. In addition, it will significantly reduce animal use in research, as opposed to conventional *in-vivo* testing. It will also overcome the limitation of testing a single vaccine candidate in a single animal model. Thus, this method will allow rapid identification of lead vaccine formulations against cancer targets.

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A bi-specific VHH antibody targeting the CD1d-Natural Killer T cell axis that displays potent anti-tumour immunity.

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Antibody-mediated modulation of major histocompatibility complex (MHC) molecules, or MHC class I-like molecules, could constitute an effective immunotherapeutic approach. We describe how single-domain antibodies (VHH), specific for the human MHC class I-like molecule CD1d, can modulate the function of CD1d-restricted T cells and how one VHH (1D12) specifically induced strong type I natural killer T (NKT) cell activation. The crystal structure of the VHH1D12-CD1d(α-GalCer)-NKT T-cell receptor (TCR) complex revealed that VHH1D12 simultaneously contacted CD1d and the type I NKT TCR, thereby stabilizing this interaction through intrinsic bispecificity. This led to greatly enhanced type I NKT cell-mediated antitumor activity in in vitro, including multiple myeloma and acute myeloid leukemia patient-derived bone marrow samples, and in vivo models. Our findings underscore the versatility of VHH molecules in targeting composite epitopes, in this case consisting of a complexed monomorphic antigen-presenting molecule and an invariant TCR, and represent a generalizable antitumor approach.

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Role of PHIST protein in knob formation

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A key pathophysiology of malaria is the ability of infected erythrocytes to adhere to the venous endothelium of the host. An erythrocyte infected by *Plasmodium falciparum* develops visible protrusions on the erythrocyte membrane as the parasite develops; these structures are called “knobs”. The knob structure consists of a spiral protein scaffold of unknown composition that is closely associated with the RBC membrane skeleton. The spiral structure is supported by the knob-associated histidine-rich protein (KAHRP) which forms a ring around the base of the structure. Despite the importance of this structure to the presentation of the major virulence antigen, *Pf*EMP1 and severe disease state, little is known about its protein composition, or the proteins required for its assembly. To investigate the composition of the knobs we performed immunoprecipitation experiments, using a cell line expressing GFP tagged KAHRP. These experiments identified several candidate genes from the PHISTb family of exported proteins including the previously uncharacterised protein PF3D7_0532300. In our work, we show that PF3D7_0532300 localises to the RBC membrane skeleton. Gene knockout experiments show that deletion of this protein leads to large extended, lobed structures and smaller vesicle structures at the RBC membrane. Examination of these abnormal structures using super-resolution microscopy show a reorganisation of RBC membrane skeleton components suggesting a possible role for PF3D7_0532300 in facilitating and organising the knob structure and its formation.

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Novel immunomodulation of the non-classical major histocompatibility complex class I-related (MR1) protein and mucosal associated invariant T (MAIT) cells by human herpesviruses

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The antigen-presenting molecule MR1 presents microbial metabolite ligands from vitamin B2 biosynthesis to Mucosal Associated Invariant T cells (MAIT cells). While bacteria and fungi drive the MR1 biosynthesis pathway, viruses do not synthesise vitamin B metabolites and thus had not previously been implicated in MR1 expression or its antigen presentation. We have taken a multi-faceted approach to explore the relationship between herpesvirus infections and MR1 and/or MAIT cell responses.

We demonstrate that several human herpesviruses inhibit MR1 cell surface upregulation, including a potent inhibition by herpes simplex virus type 1 (HSV-1), varicella zoster virus (VZV) and human cytomegalovirus (HCMV). However, these viruses appear to employ distinct mechanisms to modulate MR1. For example, whilst HSV-1 and VZV profoundly impair total MR1 protein expression, MR1 that had already trafficked to the cell surface before infection was protected from virus-mediated targeting, yet HCMV efficiently targeted both intracellular and pre-existing surface MR1. We also show that a consequence of MR1 targeting is an impaired capacity of the MAIT TCR and/or primary MAIT cells to recognise MR1-restricted ligands during infection of target cells. Thus, virus-mediated targeting of MR1 defines an immunomodulatory strategy that functionally disrupts the MR1-MAIT cell axis.

In our related studies profiling immune reconstitution in haematopoietic stem cell transplant (HSCT) patients with the complication of HCMV reactivated infection, we identify MAIT cell levels at the initial detection of HCMV reactivation as distinguishing patients who subsequently developed low-level versus high-level HCMV reactivation. This data in the HSCT setting highlights MAIT cell levels at the first detection of reactivation as a prognostic marker that may guide clinical decisions regarding pre-emptive therapy. In summary, we provide the first identification of virus-mediated modulation of the MR1 antigen presentation pathway as well as identify impacts on MAIT cells in a clinically relevant setting of virus infection.

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A tree-based software for high-resolution analysis of metagenomic samples

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Rapid advancement of metagenomic sequencing technologies has accelerated the generation of vast biological datasets, revealing complex communities of interacting microbes performing vital roles in many ecosystems. High-throughput shotgun sequencing can be used to analyse these microbial ecosystems, generating whole-community information through reading short sequences of DNA. It has been the task of reference-based metagenomic analysis pipelines to assign these short reads of DNA to taxonomic clades and generate a taxonomic profile of the community. However, the discovery of taxonomically similar isolates with large phenotypic differences challenges the use of such taxonomic profiles, as it demonstrates the existence of crucial functional characteristics that likely cannot be captured through taxonomic clades. exPAM is a software package designed to address this challenge, integrating vast collections of high-quality reference genomes with sequence-based distance trees to establish, with increased resolution, the read abundance and phylogenetic prevalence of metagenomic datasets. The exPAM approach has a capacity to generate isolate-level profiles of these samples, enabling

more thoroughly resolved downstream functional analyses that are crucial to uncovering key biological mechanisms within these communities. Alongside this development of metagenomic profiling methods, the emergence of cutting-edge gastrointestinal microbiota culturing techniques has effected surging discovery of novel microbial isolates. To aid the discovery of microbial species, exPAM alerts the user to putative novel sequences from the sample, alongside an estimation of their phylogenetic neighbourhood. This knowledge enables researchers to identify promising targets for further experimentation and deepen our understanding of these complex biological communities.

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3'-UTR length changes form a novel layer of regulation through non-ISGs in the macrophage response to IFNb

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Interferon signaling is one of the most important mechanisms shaping innate immune responses and needs to be tightly regulated to successfully fight infections and modulate immune responses while avoiding toxicity.
Type I interferons (IFNs) have been shown to induce multiple transcriptional, translational and metabolic changes. The global response of murine and human macrophages to IFNb stimulation was characterized previously using multi- omics strategies, which gave insight into a complex regulatory network of transcripts, proteins and metabolites that results in global reprogramming of the cell.

Post-transcriptional gene regulation is an important component of this network and is centered around 3'-untranslated regions (3'-UTRs), regions heavily targeted by miRNAs and harboring binding sites for many RNA-binding proteins. Poly-A-tail sequencing (PAT-seq) experiments revealed that many transcripts expressed shortened 3'-UTRs in response to IFNb, a result of changed alternative polyadenylation (APA) patterns. APA and changed 3'-UTR lengths are emerging fields of broad importance in physiological and pathological processes that are only starting to be explored. Differences in APA patterns and their regulation have not previously been studied in context of IFN.

Recent publications have described a scaffold-like role for 3'-UTRs that facilitates the formation of different protein complexes depending on 3'-UTR length, which can affect localization and function. This unique regulatory mechanism was investigated for two IFNβ-regulated transcripts with shortened 3'-UTRs, *EIF4EBP2* and *MAVS*. 3'-UTR-dependent protein-protein interactions were identified by mass spectrometry using tagged overexpression constructs encoding the different transcript isoforms.

This study describes a new aspect of interferon signaling and a novel layer of regulation through genes that are not part of the typical and well-characterized interferon transcriptional response. It shows how differential expression of distinct 3'-UTR transcript isoforms influences macrophage innate immune responses.

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Limited recognition of highly conserved regions of SARS-CoV-2

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Understanding the immune response to severe acute respiratory syndrome coronavirus (SARS-CoV-2) is critical to overcome the current coronavirus disease (COVID-19) pandemic. Efforts are being made to understand the potential cross-protective immunity of memory T cells, induced by prior encounters with seasonal coronaviruses, in providing protection against severe COVID-19. In this study we assessed T-cell responses directed against highly conserved regions of SARS-CoV-2. Epitope mapping revealed 16 $CD8^+$ T-cell epitopes across the nucleocapsid (N), spike (S) and ORF3a proteins of SARS-CoV-2 and five $CD8^+$ T-cell epitopes encoded within the highly conserved regions of the ORF1ab polyprotein of SARS-CoV-2. Comparative sequence analysis showed high conservation of SARS-CoV-2 ORF1ab T-cell epitopes in seasonal coronaviruses. Paradoxically, the immune responses directed against the conserved ORF1ab epitopes were infrequent and subdominant in both convalescent and unexposed participants. This subdominant immune response was consistent with a low abundance of ORF1ab encoded proteins in SARS-CoV-2 infected cells. Overall, these observations suggest that while cross-reactive $CD8^+$ T cells likely exist in unexposed individuals, they are not common and therefore are unlikely to play a significant role in providing broad pre-existing immunity in the community.

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Purine and carbohydrate availability drive *Enterococcus faecalis* fitness in wound infections

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Enterococcus faecalis is commonly isolated from different wound types. However, despite its prevalence, the pathogenic mechanisms of *E. faecalis* in wound infections remains poorly understood. We adopted an *in vivo E. faecalis* transposon sequencing approach to identify fitness factors that were crucial for initial colonization and persistence of *E. faecalis* during wound infections in a mouse model. We demonstrated that purine biosynthetic genes and a phosphotransferase system (PTS) were important for *E. faecalis* colonization and persistence, respectively. Purine metabolite levels were found to be lower in the wound site during initial colonization compared to the persistence phase, highlighting the importance of *de novo* purine biosynthesis during initial colonization. Furthermore, the PTS identified was characterized to be involved in the transport of mannose and galactose. Preliminary carbohydrate quantifications of the wound site using ELISA showed that mannose was higher during the persistence phase of infection compared to initial colonization, suggesting availability of various carbohydrates changes as the infection progresses, potentially impacting *E. faecalis* wound pathogenesis. Future work includes quantification of galactose and glucose (the main energy source) of *E. faecalis*-infected wound site to understand the carbohydrate profile as infection progresses. Also, to gain insight into how a mannose/galactose PTS contributes to the reduced fitness observed during the persistence phase, we performed an *in vitro* transcriptomic analysis using the mannose/galactose PTS gene deletion mutant (ΔOG1RF_10021). When mannose was the sole carbohydrate source, shikimate and purine biosynthetic genes in the ΔOG1RF_10021 mutant were downregulated compared to the isogenic wild-type strain, indicating that mannose/galactose transport is interconnected with shikimate and purine biosynthesis. Together, our results demonstrate that dynamic microenvironment changes at the wound site affects pathogenic requirements and mechanisms of *E. faecalis* during infection and raise the possibility of inhibiting *E. faecalis* mannose/galactose transport to control wound infections.

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Assessment of IgG3 as a serological exposure marker for *Plasmodium vivax* malaria in moderate endemic areas

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A more sensitive surveillance tool is needed to accelerate the elimination of *Plasmodium vivax*. *P. vivax* causes low parasitemia and is also found in other organs, apart from in blood circulation, which may not be detected using microscopy, PCR, or RDT. To address this challenge, our laboratory has developed an 8-antigen panel that can detect total IgGs as serological markers of *P. vivax* exposure within the prior 9 months in low endemic areas. In higher endemic areas, total IgG is more long-lived, resulting in poorer performance of this panel. In this study, we aimed to adapt our serological marker tool by applying a more short-lived antibody biomarker. Using a multiplex assay, we first measured antibody kinetics of total IgG, IgG1, IgG3, IgM and C1q-fixing antibodies against 29 *P. vivax* antigens over 36 weeks following asymptomatic *P. vivax* infection in PNG children (n=33). IgG3 and C1q-fixing antibodies declined faster to background level than total IgG, IgG1 and IgM. We then assessed IgG3 performance in classifying recent exposure in a cohort of Peruvian individuals (n=590). IgG3 had sensitivity and specificity of 70%, while total IgG was a better marker, with sensitivity and specificity of 80%. We further explored factors that impact the acquisition and decay of IgG3 in this cohort. IgG3 was associated with age, living in a higher endemic area, and having three or more blood stage *P. vivax* infections within the last 13 months. This result showed that higher exposure is required to attain IgG3 against the 29 *P. vivax* proteins used, so further study is still needed before implementing IgG3 for a serological marker tool.

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The impact of hypoxia on Zika virus replication is cell type specific

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Zika Virus (ZIKV) caused global concern when outbreaks in South America were linked to dramatic increases in microcephaly and other neurological complications in babies born to infected mothers. This was due to infection of the foetus via the placenta, particularly following maternal infection in the first trimester, while in contrast term placenta appears refractory to infection.

Virus replication is highly dependent on the host cellular environment with virus-host factor interactions significantly impacting outcomes of virus infection. It is thus unsurprising that oxygen tension, and subsequent changes to the cellular environment, can also impact replication of many viruses in cell culture models. Different tissue microenvironments within the body have varying oxygen tensions and

current evidence suggests most viruses replicate most efficiently at an oxygen tension reflecting that in the tissue they most readily infect. Low oxygen tension is a hallmark of the early placenta and subsequent stabilisation of hypoxia inducible factors (HIFs) are key to normal placental development. The early placenta is a target of ZIKV infection, however this virus has a broad tissue tropism leading us to investigate the effect of oxygen tension on ZIKV replication.

Experiments in HTR8 cells, a human first trimester placental trophoblast cell line, suggest that hypoxia may restrict ZIKV infection, while in Huh7 cells, a human hepatocyte cell line, hypoxia appeared to enhance ZIKV replication. Although contradictory to the HTR8 results, these Huh7 results concur with previous studies using Huh7 cells to investigate hypoxia and Dengue Virus infection, a related flavivirus. Ongoing experiments are investigating the role HIF plays in ZIKV infection, and the potential impact hypoxia has on other flaviviruses in a cell type dependent manner. Investigating the diverse effects hypoxia has on viral replication will increase understanding of cell specific pathways important in regulating both hypoxic responses and virus replication.

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Identifying inhibitors of plasmid conjugation to reduce the spread of antibiotic resistance genes

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Antibiotic resistance is a global health crisis accelerated by conjugative plasmids. Incompatibility P plasmids (IncP-1) are major contributors to antibiotic resistance because they are conjugative, broad host range and are stably maintained in diverse organisms. RP1 (IncP-1 plasmid) and R91 (IncP-10 plasmid) were identified in two *Pseudomonas aeruginosa* strains in a Birmingham hospital. R91 reduces the conjugal transfer of RP1, a phenomenon known as fertility inhibition. Understanding the mechanism of fertility inhibition could help to reduce conjugation to combat antibiotic resistance. We sequenced and characterised R91-5 (a derepressed mutant of R91) and used transposon mutagenesis to identify the three genes (*fipB1, fipB2* and *fipB3*) responsible for fertility inhibition of RP1. We show through genetic manipulation and studying protein interactions that the RP1 coupling protein, TraG, is targeted by the fertility inhibition genes. This improved understanding of the fertility inhibition of IncP-1 plasmids and will help to us slow the spread of antibiotic resistance.

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Lyn inhibits PI3Kγ activity in TLR activated macrophages.

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Macrophages are armed with Toll-like receptors (TLRs) for detecting and eliminating danger signals. Activation of TLRs results in classical activation (M1 polarization) of macrophages and triggers pro-inflammatory responses, then also resolves inflammation after clearing the danger. Class IB phosphatidylinositol 3-kinase (PI3K), PI3Kγ, has emerged as a key determinant of macrophage programming and polarization. Our lab has previously defined how in M1 cells, activated by TLRs, the LRP1-Rab8a-PI3Kγ complex cross-talking with TLRs, acts to modulate Akt/mTOR signaling and cytokine outputs that are biased towards an anti-inflammatory axis to help suppress inflammation. PI3Ks have closely aligned functions with members of the Src family kinases (SFKs). Lyn is one such SFK, which has known and varied roles in modulating macrophage programming and inflammatory status. In this study, through GST pull-down assays, mass spectrometry analysis and co-immunoprecipitation assays, we have revealed a novel interaction between Lyn and PI3Kγ in TLR pathways. Using Lyn KO BMMs and PI3Kγ specific inhibitor, we showed Lyn negatively regulates PI3Kγ/Akt signaling and promotes expression of pro-inflammatory mediators. Furthermore, we have also pinpointed regulatory SH3 domain of Lyn to be responsible for interaction with the catalytic subunit of PI3Kγ. Live cell imaging revealed macropinosomes as the location for Lyn/PI3Kγ interaction. These results reveal new complexities and insights for modulating Akt signaling and macrophage programming through the involvement of Lyn and PI3Kγ.

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Investigating the molecular mechanisms of STING responses

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Pattern recognition receptors (PRRs) play a vital role in the detection of pathogens and danger signals within the innate immune system. Cyclic GMP-AMP synthase (cGAS) acts as PRR that detects cytosolic dsDNA, a common hallmark of infection. Additionally, cGAS can also recognise host dsDNA that leaks from the mitochondria or nuclei. Detection of dsDNA by cGAS induces the production of the novel cyclic dinucleotide (CDN) 2'3'-cGAMP. 2'3'-cGAMP binds and activates the endoplasmic reticulum resident protein Stimulator of Interferon Genes (STING). Following activation, STING induces a potent type I interferon (type I IFN) response which stimulates transcription of interferon stimulated genes (ISGs). ISGs encode antiviral proteins that facilitates elimination of infected cells. Moreover, the cGAS-STING pathway also activates other non-IFN cellular responses via the transcription factor NF-κB and MAPKs to induce production of proinflammatory cytokines. Recent studies have established the importance of non-IFN STING responses in the context of immunity and disease. From murine models, the pathogenesis of the STING-mediated autoinflammatory disease, appears strongly dependent upon non-IFN response, such as NF-κB activation, rather than IRF3 activation. Similarly, non-IFN STING responses have been found to be critical for protection against HSV-1 viral infection and anti-tumour immunity. This illustrates the importance of studying the mechanism that mediate STINGs non-IFN responses, which can provide the groundwork for new therapeutics development.

Upon activation STING recruits the downstream kinase TANK-binding kinase 1 (TBK1) to induce the production of type I IFNs. Recently, TBK1 and its homologue IκB kinase epsilon (IKKε) have recently been shown to induce the canonical activation of NF-κB in response to STING activation. However, the mechanisms through which TBK1 and IKKε are activated and how they mediate STING responses remain

poorly understood. My PhD project will focus on characterising the molecular mechanisms involved in the recruitment and activation of TBK1 and IKKε, and how they regulate downstream non-IFN STING responses.

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LRRC15 suppresses SARS-CoV-2 infection and controls collagen production

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Although ACE2 is the primary receptor for SARS-CoV-2 infection, a systematic assessment of factors controlling SARS-CoV-2 host interactions has not been described. Here we used whole genome CRISPR activation to identify host factors controlling SARS-CoV-2 Spike binding. The top hit was a Toll-like receptor-related cell surface receptor called leucine-rich repeat-containing protein 15 (LRRC15). LRRC15 expression was sufficient to promote SARS-CoV-2 Spike binding where it forms a cell surface complex with LRRC15 but does not support infection. Instead, LRRC15 functioned as a negative receptor suppressing both pseudotyped and live SARS-CoV-2 infection. LRRC15 is expressed in collagen-producing lung myofibroblasts where it can sequester virus and reduce infection in trans. Mechanistically LRRC15 is regulated by TGF-β, where moderate LRRC15 expression drives collagen production but high levels suppress it, revealing a novel lung fibrosis feedback circuit. Overall, LRRC15 is a master regulator of SARS-CoV-2, suppressing infection and controlling collagen production associated with “long-haul” COVID-19.

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The role of STAT5 in CCL17-mediated inflammation

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Chronic inflammation is an underlying issue seen in multiple debilitating diseases such as rheumatoid arthritis. Granulocyte macrophage-colony stimulating factor (GM-CSF) is a key cytokine that contributes to the development and sustainment of inflammation, in part, through the upregulation of C-C motif chemokine ligand 17 (CCL17). The binding of GM-CSF to its receptor activates JAK2 and STAT5 phosphorylation. This prompts the upregulation of JMJD3, IRF4 and subsequently CCL17 which is secreted out of the cell thus exacerbating the inflammatory microenvironment.

Glucocorticoids (GCs) are first line treatments used broadly in numerous inflammatory diseases. Dexamethasone (Dex) is a commonly prescribed GC, however long-term use and high dosage is associated adverse side effects. The relationship between dexamethasone and its ability to alleviate chronic inflammation is still not completely understood, highlighting the need to explore its inhibitory mechanisms and pathways.

We report here that in GM-CSF-mediated CCL17 inflammation, Dex has the ability to intervene with the STAT5 phosphorylation event in human monocytes and mouse macrophages. This interaction is seen to drastically suppress the expression of downstream regulatory proteins, including interferon regulatory factor 4 (IRF4), thus preventing the over secretion of CCL17. These findings provide evidence into how Dex mechanistically alleviates chronic inflammation. As a result, this knowledge can form the basis of new drug development that target more specific aspects of the signalling cascade, that either reduce or completely prevent the side effects that are commonly associated with long term glucocorticoid use.

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Salmonella Typhimurium induces cIAP1 degradation to promote death in macrophages

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Programmed cell death processes are a key mechanism by which the human innate immune system restricts the growth and dissemination of gastrointestinal pathogens such as *Salmonella*. Non-typhoidal serovars infect the small intestine and underlying innate immune cells to cause gastroenteritis with symptoms of diarrhoea, nausea, vomiting, and fever, and can induce systemic infection if left unchecked in immunocompromised individuals. By activating cell death mechanisms such as pyroptosis, apoptosis and necroptosis, infected macrophages may halt intracellular bacterial replication, and enable the extracellular release of inflammatory cytokines and danger signals. However, *Salmonellae* interrupt these innate immune processes by using two specialised Type III Secretion Systems (T3SSs) to introduce bacterial effector proteins directly into the host cell cytosol; thus manipulating the cellular environment to promote bacterial survival. As such, characterisation of novel effector protein functions is crucial to understanding the success of these pathogens.

Our research discovered that 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. We observed strong association between cIAP1 loss and increased cellular cytotoxicity, with corresponding caspase -8 and -3 activation. Depletion of cIAP1 was associated with functional *Salmonella* Pathogenicity Island 1 (SPI-1) T3SS effector translocation, and was not prevented by pan-caspase, proteasomal or lysosomal inhibitors. Anti-cIAP1 immunoblot detected a low molecular weight peptide following *S.* Typhimurium infection, suggesting that SPI-1 effector/s may cleave cIAP1 during infection. Current work combines several molecular and *in vitro* techniques to explore the cIAP cleavage mechanism and determine the responsible SPI-1 effector protein, with transfection screens suggesting several key candidates. Future work will assess the involvement of cIAP proteins in overall susceptibility

to *Salmonella* infection *in vivo*. This finding suggests a new role for *Salmonella* effector proteins in activating, rather than preventing, host cell death, which we hypothesise may promote dissemination of the bacteria.

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Increased breadth of Group A *Streptococcus* antibody responses in children with acute rheumatic fever compared to precursor pharyngitis and skin infections

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Group A *Streptococcus* (GAS) causes superficial pharyngitis and skin infections as well as serious autoimmune sequelae such as acute rheumatic fever (ARF). ARF can progress to chronic rheumatic heart disease that is associated with significant morbidity and mortality in Māori and Pacific children in New Zealand and Aboriginal children in Australia. Candidate vaccines that can prevent GAS infections are being developed but have not yet reached licensure, and the limited understanding of ARF pathogenesis presents a major hurdle for the field. Immune priming by repeated GAS infections is thought to trigger ARF, and there is growing evidence for the role of skin infections in this process. The aim of this study was to utilise our recently developed 8-plex immunoassay to characterise antibody responses in sera from New Zealand children with a range of clinical GAS disease; ARF (n=80), GAS-positive pharyngitis (n=117), GAS-positive skin infection (n=51) and closely matched healthy controls (n=134). The 8-plex assay comprises antigens used in clinical serology for the diagnosis of ARF (SLO, DNase B and SpnA), and five conserved putative GAS vaccine antigens (Spy0843, SCPA, SpyCEP, SpyAD and the Group A carbohydrate). Serological responses differed by ethnicity, with Māori and Pacific children having significantly higher GAS antibodies than other ethnic groups at baseline and following infection. Antibody levels slowly waned over time, though the rate of decay appeared to be quicker for SpnA and Spy0843 highlighting differences in kinetics between antigens. Most notably, the magnitude and breadth of antibodies in ARF was very high, giving rise to a distinct serological profile. An average of 6.5 antigen-specific reactivities per individual was observed in ARF, compared to 3.9 in skin infections and 3.1 in pharyngitis. This suggests the ARF profile is the result of repeated precursor pharyngitis and skin infections that progressively boost antibody breadth and magnitude.

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Anti-viral defences in the male reproductive tract: Expression and localisation of the novel type I interferon epsilon

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Interferon epsilon (Ifnε), a novel type-I interferon constitutively expressed in female reproductive epithelia, protects female mice against sexually transmitted viruses. Production, cellular localization, and functions of Ifnε in the male reproductive tract have not been studied previously. Since many viruses, including HIV, HSV2, HepB, mumps, Zika, and SARS disrupt male reproductive function, investigation of this potent anti-viral agent in the male was warranted.

Expression and localization of Ifnε in the male reproductive tract was studied by indirect immunofluorescence and qRT-PCR in 25 and 56 day old wild-type mice relative to *Ifnε*$^{-/-}$ mice (n = 8/genotype). Wild-type testes between days 5 and 180 were examined to determine *Ifnε* expression during postnatal development. Cellular sites of production were determined in individual testicular cell types isolated from 44 day old mice using Percoll gradient centrifugation, centrifugal elutriation and lectin-adherence.

Ifnε was differentially expressed within the male reproductive tract. Expression was very high in the testis, absent in the epididymis, and low in the vas deferens at both day 25 (prior to the completion of sperm production), and day 56 (mature spermatogenesis). Testicular *Ifnε* first appeared between day 20 and 25, and was expressed in meiotic and post meiotic germ cells, and in interstitial cells, particularly macrophages. Interferon-stimulated genes (ISG), such as *Isg15*, *Irf7,* and *Oasl2*, displayed low expression in the testis, and gradually increased towards the cauda epididymis and vas deferens, in parallel with other type-I interferons, such as *Ifnb1*. Unlike in the female, *Ifnε* deficiency did not reduce ISG expression within the male reproductive tract.

Constitutive expression of Ifnε in the mature testis, the region most prone to viral infections within the male reproductive tract, suggests a crucial role for Ifnε in testicular innate immunoregulation and protection, whereas other type-I interferons may play a more significant protective role in the distal epididymis.

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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 using T cell receptors (TCRs) of limited diversity, and exhibit rapid, 'innate-like' functions. In particular, MAIT cells recognise microbial riboflavin metabolites via their invariant *Trav1-Traj33*$^{+}$ TCRα chain and potently secrete IL-17 and IFN-γ upon activation. Notably, MAIT cells comprise 0.1-10% of circulating T cells in humans, the cause of this wide variance in frequency being poorly understood. Recent evidence has suggested that MAIT, NKT, and γδT cells may be regulated by similar environmental or genetic factors and may reside within a shared niche.

We showed that NKT cell-deficient mice have increased MAIT cells and demonstrated that this increase was due to the loss of NKT cells rather than their restricting element, CD1d. MAIT cells were also markedly increased in γδT cell-deficient mice and expand further in NKT/γδT cell double-deficient mice. Expanded MAIT cells phenotypically and functionally resembled their wildtype (WT) counterparts. As MAIT cells were increased in the thymus, we analyzed *Trav1-Traj33* transcripts within developing thymocytes and showed that

overall *Trav1-Traj33* transcript levels were increased 2-fold within γδT- and NKT/γδT-deficient thymocytes relative to WT controls. As γδT-deficient mice harbor a modified TCRδ locus, our findings imply that TCRα rearrangement may be altered in these mice, potentially manifesting in greater rearrangement of distal *Trav* gene segments like *Trav1* and increased intrathymic generation of MAIT cells. However, increases in peripheral MAIT cells exceeded increases in the thymus, indicating that MAIT cells may compete with peripheral NKT and γδT cells for similar homeostatic factors and expand in their absence. Accordingly, we show that adoptively transferred MAIT cells underwent more proliferation within NKT/γδT-deficient hosts relative to WT controls.

Together, our findings highlight a shared niche in which MAIT, NKT, and γδT cells co-exist and compete for common homeostatic factors. Importantly, these findings provide insights into factors regulating MAIT cell levels and cautions the interpretation of studies on NKT and γδT cells using NKT- or γδT-deficient mice, respectively, due to previously unappreciated increases in MAIT cell levels and potential alterations in TCRα chain rearrangement in γδT-deficient mice.

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The mechanisms of *P. aeruginosa* OMV biogenesis may alter their cargo composition and subsequent biological functions.

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Outer membrane vesicles are small nanoparticles produced by Gram-negative bacteria as part of their natural growth. There are currently two well understood mechanisms of OMV biogenesis, the budding of OMVs from the cell membrane during normal bacterial growth and the formation of OMVs during endolysin mediated explosive cell lysis. OMVs are released by bacteria to contribute to bacterial functions and can play a role in bacterial communication and survival. Specifically, OMVs can package antimicrobial compounds to be delivered to competing bacteria. However, it is currently unknown whether the mechanism of biogenesis can determine the packaging of cargo into OMVs and their biological functions. Therefore, in this study we examined whether the production of OMVs by budding or by explosive cell lysis could determine their composition and their subsequent antimicrobial activity.

OMVs were isolated from three *Pseudomonas aeruginosa* strains that produced OMVs either naturally, by budding only or predominately by explosive cell lysis. We compared the production and cargo composition of OMVs produced by all three *P. aeruginosa* strains and then determined their antimicrobial activity. We found that OMVs produced by explosive cell lysis could significantly inhibit *P. aeruginosa* growth whilst OMVs produced by budding could not inhibit *P. aeruginosa* growth. However, OMVs could significantly inhibit the growth of *Staphylococcus aureus* irrespective of their mechanism of biogenesis. We are currently investigating the proteome of *P. aeruginosa* OMVs to understand if there are any differences in the packaging of proteins into OMVs based on their mechanism of biogenesis and to specifically determine the antimicrobial compounds that are packaged into *P. aeruginosa* OMVs.

Overall, these results suggest that only *P. aeruginosa* OMVs produced by explosive cell lysis can inhibit the growth of their parent bacterium whilst the mechanism of OMV biogenesis does not determine their ability to inhibit *S. aureus* growth. Therefore, our data provides insight into how the mechanism of OMV biogenesis can regulate their cargo composition and advance our understanding of how OMV cargo dictates their subsequent biological functions.

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High prevalence of Capsid-antibody-complexes (CACs) in sera of chronic hepatitis B patients and its association with chronic liver disease

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Hepatitis B Virus (HBV) infects a quarter of a billion people worldwide and is responsible for a series of liver diseases from hepatitis, cirrhosis to hepatocellular carcinoma. The current dogma holds that there are two major viral/subviral particles, i.e., virions (Dane particle) and surface antigen particles (spherical and filamentous) which is currently under challenge by observations of a variety of incomplete viral particles. We recently proposed the existence of Capsid-Antibody-Complexes (CACs) which results from the release of naked capsids from hepatocytes and binding of specific antibodies in circulation. CACs contains immature viral DNA and a significant amount of HBV RNA (PMID: 30282709). In this study, we further investigate the prevalence of such particles in chronic carriers or hepatitis patients. We first developed a microplate-based semi-quantitative assay using C1q to capture immune complexes and capsid-horseradish peroxidase to detect anti-core antibody within them. The specificity of this method was confirmed by a series of samples of non-HBV aetiologies (Hepatitis C, Nonviral hepatitis, Systemic Lupus Erythematosus etc). In HBV infected individuals, a high prevalence of CACs (77%, n=220) was observed indicating the near-universal existence of this particle type. Quantitative PCR measurement of HBV DNA and RNA in C1q-captured immune complexes showed significant enrichment of viral nucleic acids which coincides with the molecular composition of CACs. Intriguingly, the level of CACs strongly correlates with ALT in both HBeAg positive and negative patients. (ALT e+ r=0.62, P<0.0001, e- r=0.66, P<0.0001) suggesting its role in immunopathology of chronic hepatitis B. Indeed, preliminary data showed significant higher CACs level in patients with histological evidence of hepatic inflammation and complement-deposition. In conclusion, the high prevalence of Capsid-antibody-complexes in chronic hepatitis and their association with liver necroinflammation suggests the underestimated roles of virus-specific immune complexes in HBV-induced liver pathology.

Culturing human respiratory bacteria to reveal spatial changes and implications in health and disease

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More than 100 trillion microbes inhabit the human body (Ursell et al, 2012). This vast assemblage of organisms, which is termed the microbiome, form distinct microbial communities that are now known to have an important role in maintaining health and modulating states of disease (Wypch et al, 2019**;** Pattaroni et al, 2018; Budden et al, 2017). In a healthy state, humans are widely assumed to be born sterile (de Goffau at el, 2019). Microbial colonisation usually commences from birth (Wypch et al, 2019) and matures in parallel with the neonatal immune system and other organs (Wypch et al, 2019). Lung microbiome research is limited by the invasiveness of lung sampling and reliance on sequence-analysis approaches. Consequently, the majority of lung bacteria remain uncultured, despite evidence they play an essential role in human health and disease (Lloyd-Price et al, 2017).

We aim to culture individual bacterial isolates from the nasopharynx and lung of neonates, infants and children and compare bacterial load and diversity between these anatomical sites. Non-bronchoscopic bronchoalveolar lavages and nasopharyngeal swabs were obtained from seven intubated neonates at Monash Children's Hospital. Patients without a primary lung disease were included as controls (n=1). Samples were pooled in pairs for processing and respiratory bacteria was cultured on seven different media types. Thereafter, samples were sent for metagenomic sequencing to evaluate community diversity and relative species abundance. 990 isolates representing 21 distinct bacterial species were cultured. 16S rRNA gene sequencing revealed these 21 species grew on brain-infused heart, anaerobic, chocolate and Wilkin's Chalgren media only. The ability to now culture distinct respiratory bacteria has provided an initial insight into bacterial load and diversity, thereby enabling functional validation of the respiratory bacteria to reveal their importance in health/disease.

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