Young Investigator Symposium

Walter and Eliza Hall
Institute of Medical Research
Thursday 10 October 2019

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Welcome to the 2019 VIIN Young Investigator Symposium

Dear Colleagues and Friends,

On behalf of the members of the Victorian Infection and Immunity Network's Executive Committee, we welcome you to the 2019 Young Investigator Symposium.

In particular, we welcome our keynote speakers, Prof Eric Reynolds and Dr Sarah Dunstan of the University of Melbourne.

This is the 12th year that the VIIN has convened a symposium for young investigators. We have been fortunate to hold the Young Investigator Symposium at the Walter and Eliza Hall Institute for many of these years and thank the Walter and Eliza Hall Institute and Prof Doug Hilton for continuing to support and enable this important and valuable event for the infection and immunity community.

We are indebted to a great many other people and organisations who have been vital to bringing the 2019 meeting about. These include:

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

Catarina Almeida, *University of Melbourne*
Rebecca Ambrose, *Hudson Institute of Medical Research*
Michelle Audsley, *Monash University*
Tiffany Bouchery, *Monash University*
Nicole Campbell, *Hudson Institute of Medical Research*
Daniel Dlugolenski, *Deakin University*
Andrew Fleetwood, *University of Melbourne*
Carlo Giannangelo, *Monash Institute of Pharmaceutical Sciences*
Carmen Lao, *Victorian Infection and Immunity Network*
Rhea Longley, *Walter and Eliza Hall Institute*
Emma McHugh, *University of Melbourne*
Sarah McLean, *Swinburne University*
Hamish McWilliam, *University of Melbourne*
Ronan Mellin, *Walter and Eliza Hall Institute*
Nicole Messina, *Murdoch Children's Research Institute*
Faye Morris, *Monash University*
Ursula Norman, *Monash University*
Jason Paxman, *La Trobe University*
Linda Reiling, *Burnet Institute*
Rebecca Smith, *Victorian Infection and Immunity Network*
Leon Tribolet, *CSIRO*

- Session chairs and judges for oral presentations and posters. Altogether, there are more than 50 of you, which is a reflection of the increasing popularity of this event each year. We are grateful to each and every one of you for your time and expertise.
- Jodie Hemingway, Jaclyn Hoystead and Carolyn MacDonald from the Walter and Eliza Hall Institute's Events Management and Communications and Marketing teams, as well as the AV, photography, and house-keeping teams. Thank you also to Mary and Steve the Caterers.
- The sponsors and advertisers for this symposium. Your support is more and more important to making this event a success and we thank you for being here with us today.
- The 15 universities, departments, medical research institutes and government agencies that support the VIIN annually through financial contributions. Without your support, our activities would cease.

Finally, thank you to everyone who is here as a delegate or to present a talk or poster. This meeting is for you. We always appreciate your participation.

Sincerely,

Prof Paul Hertzog and Prof Heidi Drummer, Co-Convenors of VIIN

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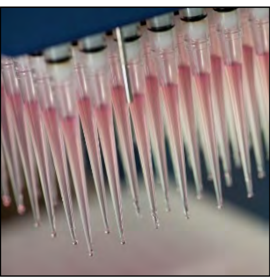
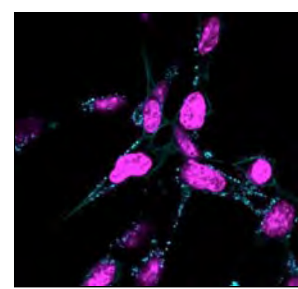
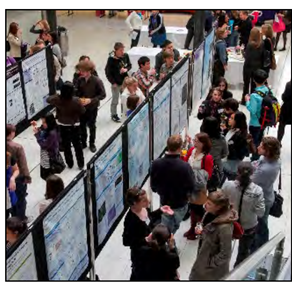
Evening poster session	
Lunchtime networking session & Keynote Speaker Sponsor	
Morning and afternoon tea	
Registration session	

Oral Presentation prizes	
Science Bites Prizes	
Poster prizes	
Best question prizes	**People's Choice Awards**

For links to sponsor websites:
https://www.viin.org.au/event/viin-young-investigator-symposium-2019

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

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

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

VIIN Contributors include:

For enquiries contact: info@viin.org.au

The Hartland Oration at Lorne

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

In honour of Liz's contribution to VIIN, the network is delighted to have established the Hartland Oration. This oration will be delivered at the Lorne Infection and Immunity Conference from 2018 onwards. The Hartland Orator will be selected at this VIIN Young Investigator Symposium and will be the post-doctoral researcher giving the best 10 minute oral presentation. This outstanding young researcher will receive free registration to the 2020 Lorne Infection and Immunity Conference and $500 toward their accommodation, meals and transport.

Additional prizes at the VIIN Young Investigator Symposium

As in years past, prizes will also be awarded for:

- Best questions: a cash prize. Be sure to engage in question time after the talks and identify yourself by name and institute to win a Best Question prize!
- Poster prizes for best student and best post-doc
- Science Bites prizes for best student and best post-doc (3 minute oral presentations)
- Best VIIN Young Investigator Symposium PhD talk (10 minute oral presentation)
- People's Choice Awards (drop your voting slips into the boxes at the registration desk before 5.50pm)

LORNE INFECTION & IMMUNITY CONFERENCE 19—21 FEB 2020

10 YEARS ANNIVERSARY

ORAL ABSTRACTS & CDA AWARDS DUE: FRIDAY 25 OCTOBER 2019 **EARLY-BIRD REGISTRATION CLOSES:** FRIDAY 29 NOVEMBER 2019 **POSTER ABSTRACTS CLOSE:** TUESDAY 14 JANUARY 2020

INTERNATIONAL SPEAKERS

Sara Cherry *University of Pennsylvania, USA*
Genetic and mechanistic studies of viral-host interactions

Dana Philpott *University of Toronto, Canada*
NOD proteins in bacterial infection

Gong Cheng *Tsinghua University, China*
Immunopathogenesis of mosquito-borne flaviviral diseases

Mike Blackman *The Francis Crick Institute, UK*
Host-pathogen interactions toward malaria vaccines and drugs

Franca Ronchese *Malaghan Institute, NZ*
Th2 signatures and allergic inflammation

Arturo Zychlinsky *Max Planck Institute for Infection Biology, Germany*
Chromatin and immunity

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for Student Presenters (short talk, oral presentation or poster)

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NATIONAL SPEAKERS

Laura Mackay *Peter Doherty Institute, VIC*
Memory T cell responses

Dena Lyras *Monash University, VIC*
Functional biology of bacterial pathogens

Ian Henderson *Institute for Molecular Bioscience, QLD*
Genetic, biochemical and structural studies of bacterial cell membranes

James Fraser *University of Queensland, QLD*
Sex virulence and evolution in pathogenic fungi

Malcolm McConville *University of Melbourne, VIC*
Metabolomics and cell biology of protozoa

Anna Coussens *Walter and Eliza Hall Institute, VIC*
Heterogeneity of tuberculosis and HIV pathogenesis

Rowena Bull *University of New South Wales, NSW*
Host evolutionary dynamics of RNA viruses

Kate Schroder *Institute for Molecular Bioscience, QLD*
Inflammasome function during infection and inflammation

Georgina Hold *University of New South Wales, NSW*
Gastrointestinal microbiota in human health and disease

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The Annual Meeting of the

VIIN Young Investigator Symposium Program 2019

Walter and Eliza Hall Institute of Medical Research, Parkville
Davis Auditorium

08:30 - 09:00	Registration
09:00	**Welcome and Acknowledgement of Country** **Catarina Almeida, University of Melbourne, Department of Microbiology and Immunology, on behalf of the Organising Committee**
09:10 - 10:00	**SESSION 1: Oral presentations I, Davis Auditorium** **Chairs: Jessica Borger, Monash University, Department of Immunology and Pathology and Annabell Bachem, University of Melbourne, Department of Microbiology and Immunology**
09:10 - 09:20	The human γδ T-cell receptor (TCR) is poised to communicate *Plasmodium falciparum* malaria infection **Anouk von Borstel, Monash University, Biomedicine Discovery Institute, Department of Biochemistry and Molecular Biology (PD)**
09:20 - 09:30	Characterisation of the zinc substrate binding protein AdcA from *Streptococcus pneumoniae* **Aimee Tan, University of Melbourne, Department of Microbiology and Immunology (PD)**
09:30 - 09:40	Genetic investigation of a novel flagellotropic bacteriophage in *Bacillus cereus* **Steven Batinovic, La Trobe University, Department of Physiology, Anatomy & Microbiology (PD)**
09:40 - 09:50	Identification of distinct neutrophil populations in the blood of G-CSF treated donors **Katherine Martin, Walter & Eliza Hall Institute of Medical Research, Inflammation Division (PD)**
09:50 – 10:00	Guided missile strikes: Targeting mechanisms by novel bacteriophages **Rhys Dunstan, Monash University, Biomedicine Discovery Institute, Infection & Immunity Program, Department of Microbiology (PD)**
10:00-10:30	**Morning Tea - Tapestry Lounge**
10:30 - 11:00	**SESSION 2: Keynote Session I, Davis Auditorium** **Chair: Jason Paxman, La Trobe University, La Trobe Institute for Molecular Science, Department of Biochemistry and Genetics**
10:30 - 11:00	Targeting subgingival polymicrobial dysbiosis by vaccination to prevent the progression of periodontitis **First Keynote Speaker: Eric Reynolds, University of Melbourne, Melbourne Dental School and Oral Health CRC**
11:00 - 11:30	**SESSION 3: Oral presentations II, Davis Auditorium** **Chairs: Michelle Audsley, Monash University, Department of Biochemistry and Molecular Biology**
11:00 - 11:10	Assaying translation in apicomplexan parasites: a doxycycline case study **Emily Crisafulli, University of Melbourne, Department of Biochemistry and Molecular Biology, Bio21 Institute Molecular Science and Biotechnology Institute (PG)**

<--Click on the green box to go to abstracts

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11:10 - 11:20	A map of CXCR3 ligands reveals distinct intranodal niches for CD8 effector and stem-like memory cells **Brigette Duckworth, Walter and Eliza Hall Institute of Medical Research, Immunology Division (PG)**
11:20 - 11:30	Intracellular lipid droplet accumulation occurs early following viral infection and is required for an efficient interferon response **Ebony Monson, La Trobe University, Department of Physiology, Anatomy and Microbiology (PG)**
11:30-12:00	**SESSION 4: Science Bites I** **Chairs: Simone Park, University of Melbourne, Department of Microbiology and Immunology and Tianyue Zhao, Monash University, Department of Microbiology**
11:30 - 11:33	The epigenetic regulation of B cells mediated by Polycomb Repressive Complexes during acute and chronic infections **Andrea Di Pietro, Monash University, Biomedicine Discovery Institute, Department of Biochemistry and Molecular Biology (PD)**
11:33 - 11:36	What's in a swab? Majority of healthy individuals have detectable intraoral human herpes virus **Tami Yap, University of Melbourne, Melbourne Dental School (PD)**
11:36 - 11:39	Structural and functional investigation of CTD of CzcD transporters; members of the cation diffusion facilitator family of metal efflux proteins **Saumya Udagedara, La Trobe University, Department of Biochemistry and Genetics (PD)**
11:39 - 11:42	*WITHDRAWN*
11:42 - 11:45	Characterisation of the Arg-GlcNAc glycosyltransferase effectors from *Salmonella enterica* **Jiyao Gan, Hudson Institute of Medical Research, Centre for innate immunity and infectious Diseases (PG)**
11:45 - 11:48	Lethality due to DPP9 deficiency is rescued by deletion of the NLRP1 inflammasome **Cassandra Harapas, Walter and Eliza Hall Institute of Medical Research, Inflammation Divison (PG)**
11:48 -11:51	Granulocyte Colony Stimulating Factor Promotes Lung Damage in Neonatal Mice with Bronchopulmonary Dysplasia **Lakshanie Wickramasinghe, Monash University, Central Clinical School, Department of Immunology and Pathology (PG)**
11:51 - 11:54	Identifying the targets of functional antibodies against a major malaria antigen **Daniel Marshall, Burnet Institute, Immunology (Hons)**
11:54 - 11:57	Non-specific IgA inhibits HIV broadly neutralising antibody (bNAb) Fc functions **Samantha Davis, University of Melbourne, Department of Microbiology and Immunology (PG)**
11:57 - 12:00	Sortase A Inhibition as a route to treat antibiotic resistance in *Staphylococcus aureus* **Stephanie Louch, Monash University, Biomedicine Discovery Institute, Department of Biochemistry and Molecular Biology (Masters)**
12:10-12:50	**Poster Session 1 and Lunch - Tapestry Lounge and Seminar Room 2**

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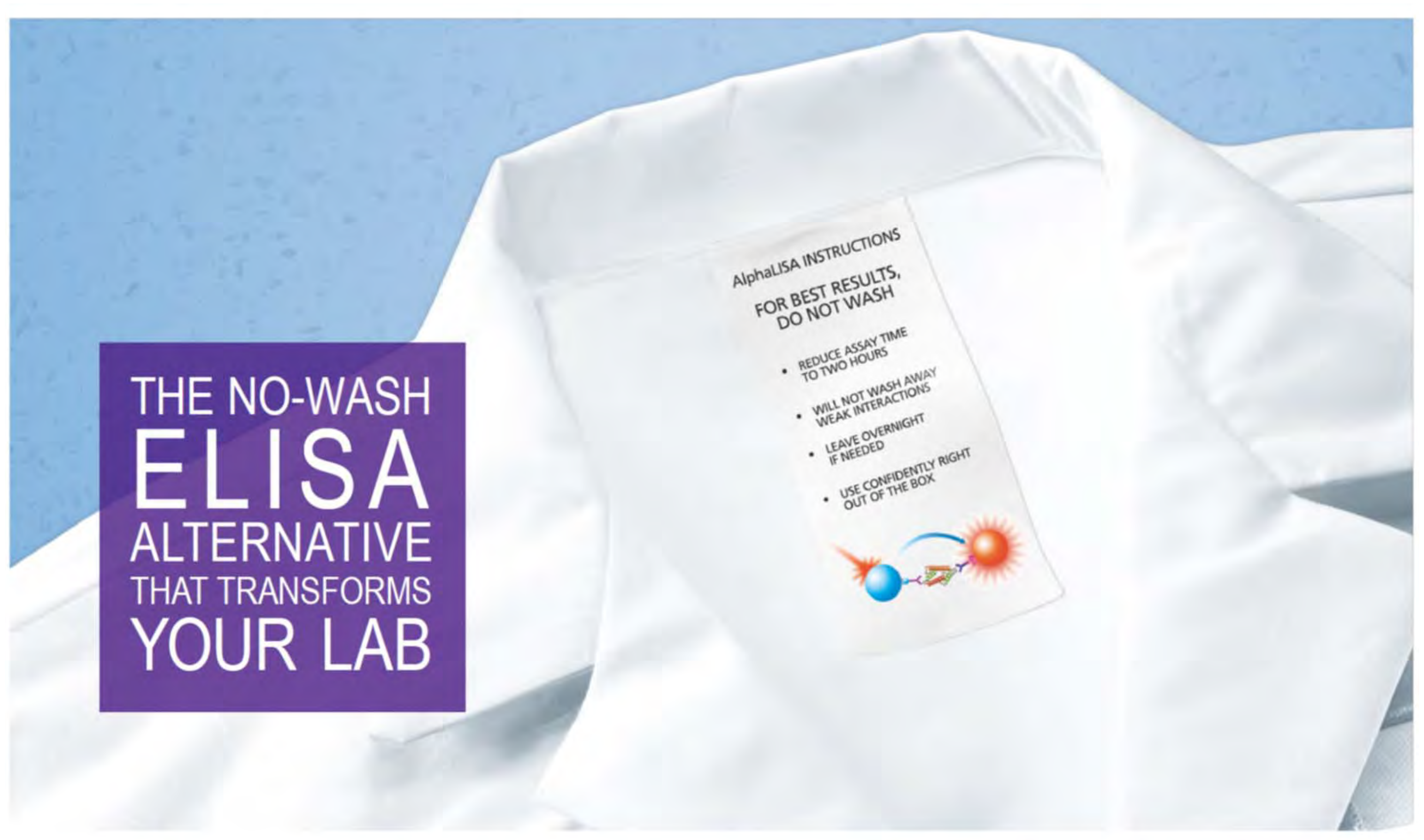
13:00-13:30	**SESSION 5: Keynote Session II, Davis Auditorium** **Chair: Rhea Longley, Walter and Eliza Hall Institute, Population Health and Immunity Division**
13:00 - 13:30	Genomics to tame tuberculosis and typhoid **Second Keynote Speaker: Sarah Dunstan, University of Melbourne, Peter Doherty Institute**
13:30-14:30	**SESSION 6: Oral presentations III, Davis Auditorium** **Chairs: Catarina Almeida, University of Melbourne, Department of Microbiology and Immunology and Linda Reiling, Burnet Institute**
13:30 - 13:40	Mechanism of daptomycin resistance in *Staphylococcus aureus* mediated by mutations in *mprF* **Jhih-Hang Jiang, Monash University, Biomedicine Discovery Institute, Department of Microbiology (PD)**
13:40 - 13:50	Structural studies of human antibody responses against leading malaria vaccine antigen PfCSP **Stephen Scally, Walter and Eliza Hall Institute of Medical Research Infectious Diseases and Immune Defence Division (PD)**
13:50 - 14:00	Rabies against the machine: using artificial intelligence to identify extracellular biomarkers of lyssavirus infection **Ryan Farr, CSIRO, Australian Animal Health Laboratory (PD)**
14:00 - 14:10	Mimicking NETs with self-assembling nanofibres **Jennifer Payne, Monash University, Biomedicine Discovery Institute, Department of Biochemistry and Molecular Biology (PD)**
14:10 - 14:20	Genome-wide molecular screen reveals novel HCMV genes essential for viral egress **Svenja Fritzlar, Monash University, Biomedicine Discovery Institute, Department of Microbiology (PD)**
14:20 - 14:30	The gut microbiome: A source of antimicrobial resistance for pathogens **Emily Gulliver, Hudson Institute of Medical Research, Centre for innate immunity and infectious diseases (PD)**
14:30-15:00	**SESSION 7: Science Bites II, Davis Auditorium** **Chairs: Rebecca Ambrose, Hudson Institute of Medical Research and Tiffany Bouchery, Monash University, Department of Immunology and Pathology**
14:30 - 14:33	From phenotypic screens to mode of action: a metabolomics approach to guide the development of novel anti-trypanosomal drugs **Anubhav Srivastava, Monash Institute of Pharmaceutical Sciences, Faculty of Pharmacy and Pharmaceutical Sciences (PD)**
14:33 - 14:36	Sphingosine-1-phosphate receptor 5 (S1P5) critically regulates tissue-resident memory T cell formation **Maximilien Evrard, University of Melbourne, Department of Microbiology and Immunology (PD)**
14:36 - 14:39	Effects of APOA1, ABCA1 and LCAT Mutations on Monocyte Activation in Humans – a Double Edged Sword **Siroon Bekkering, Murdoch Children's Research Institute, Infection & Immunity theme, Department of Inflammatory Origins (PD)**
14:39 - 14:42	Clinical predictors of severe RSV infection in children <2 years at The Royal Children's Hospital, Melbourne **Danielle Wurzel, University of Melbourne, Department of Paediatrics (PD)**
14:42 - 14:45	Can shark antibodies be used as an alternate against bacterial proteins in the establishment of urinary tract infections?



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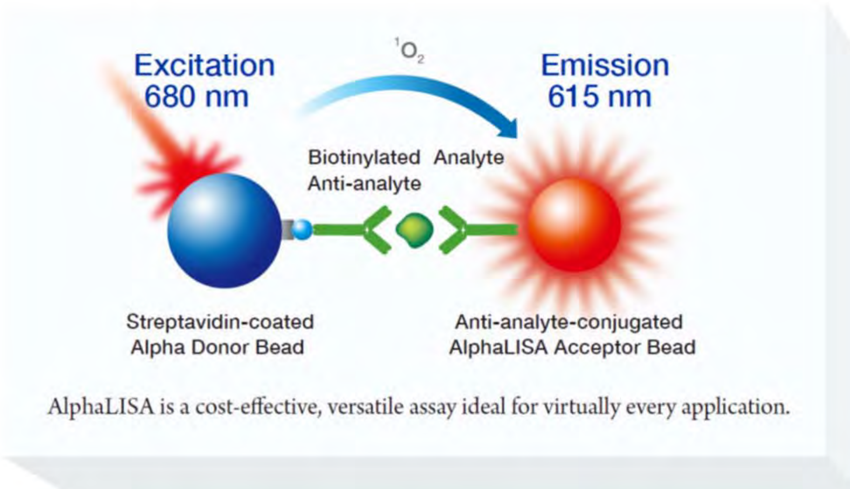

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	Gabriela Constanza Martinez Ortiz, La Trobe University, La Trobe Institute for Molecular Science, Department of Biochemistry and Genetics (PG)
14:45 - 14:48	CD1a-restricted T cells: A unique population of lipid reactive T cells **Catriona Nguyen-Robertson, University of Melbourne, Department of Microbiology and Immunology (PG)**
14:48 - 14:51	Host exosome biogenesis blueprints construction of the Human Cytomegalovirus virion **Declan Turner, Monash University, Biomedicine Discovery Institute, Department of Microbiology (PG)**
14:51 - 14:54	Immune and Inflammatory Mechanisms of Crohn's Disease **April Raftery, Monash University, Department of Immunology and Pathology (PG)**
14:54 - 14:57	Endogenous annexin-A1 attenuates intestinal microbiome dysbiosis in a mouse model of diabetes **Shan Huang, La Trobe University, Department of Physiology, Anatomy & Microbiology (PG)**
14:57 - 15:00	Functional and structural studies of the glycoprotein E2 of a rodent hepacivirus. Exploring a novel model system to inform Hepatitis C vaccine development **Felicia Schlotthauer, Burnet Institute, Life Science (PG)**
15:00 - 15:30	Afternoon Tea, Tapestry Lounge **Chat with Keynote Speaker**
15:30 - 16:30	**SESSION 8: Oral Presentations IV** **Chairs: Carlo Giannangelo, Monash Institute of Pharmaceutical Science and Daniel Dlugolenski, Deakin University**
15:30 - 15:40	Sex dependent differentiation of regulatory T cells in the visceral adipose tissue **Santiago Valle Torres, University of Melbourne, Department of Microbiology & Immunology (PG)**
15:40 - 15:50	The role of necroptosis in bacterial gut infection **Vik Ven Eng, Hudson Institute of Medical Research, Centre for Innate Immunity and Infectious Diseases (PG)**
15:50 - 16:00	Screening the medicines for malaria venture pathogen box for invasion and egress inhibitors of the blood stage of *Plasmodium falciparum* **Madeline Dans, Burnet Institute, Life Sciences (PG)**
16:00 - 16:10	Effective, low cost preservation of human stools for gut microbiome investigation in a helminth endemic region **Katharina Stracke, Walter and Eliza Hall Institute of Medical Research, Population Health and Immunity Division (PG)**
16:10 - 16:20	Covalent binding of complement C3 to MHC II mediated trogocytosis between conventional dendritic cells and MZ B cells in the spleen **Patrick Schriek, University of Melbourne, Department of Biochemistry and Molecular Biology (PG)**
16:20 - 16:30	Therapeutically targeting Myc in gastric cancer **Riley Morrow, Olivia Newton-John Cancer Research Institute, Cancer & Inflammation Laboratory (PG)**
16:30 - 17:00	**SESSION 9: Science Bites III, Davis Auditorium** **Chairs: Sarah McLean, Swinburne University, Department of Chemistry and Biotechnology and Evelyn Tsantikos, Monash University, Department of Immunology and Pathology**
16:30 - 16:33	IL-23 co-stimulation drives antigen-specific MAIT cell activation and enables vaccination against bacterial infection

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	Huimeng Wang, University of Melbourne, Department of Microbiology and Immunology (PD)
16:33 - 16:36	Inflammatory biomarkers in patients with end-stage renal disease co-morbid with latent tuberculosis **Milla McLean, Peter Doherty Institute, Melbourne University, Department of Microbiology and Immunology (RA)**
16:36 - 16:39	Defining naturally acquired antibody longevity to *Plasmodium vivax* antigens in western Thailand **Zoe Shih-Jung Liu, Walter and Eliza Hall Institute of Medical Research, Population Health and Immunity Division (PG)**
16:39 - 16:42	Dissecting the role of topical antibiotic use in the emergence of multidrug-resistant *Staphylococcus aureus* **Yi Nong, University of Melbourne, Department of Microbiology & Immunology (PG)**
16:42 - 16:45	Finding the Achilles Heel in *Pseudomonas aeruginosa* **Rachael Impey, La Trobe University, Institute for Molecular Science (PG)**
16:45 - 16:48	Investigating metabolic pathways in *Coxiella burnetii*, the causative agent of the zoonotic disease Q fever, and their role in pathogenesis **Janine Hofmann, University of Melbourne, Asia-Pacific Centre for Animal Health, Melbourne Veterinary School (PG)**
16:48 - 16:51	Investigating transcriptional correlates of naturally acquired immunity to Malaria **Stephanie Studniberg, Walter and Eliza Hall Institute of Medical Research, Infectious Diseases and Immune Defence Division (PG)**
16:51 - 16:54	SopF, a phosphoinositide binding effector, promotes the stability of the nascent *Salmonella*-containing vacuole **Nicole Lau, University of Melbourne, Department of Microbiology & Immunology, Peter Doherty Institute for Infection and Immunity Institute (PG)**
17:10 - 17:50	**POSTER SESSION II, Food and Drinks - Tapestry Lounge and Seminar Room 2**
18:15	**ORAL/POSTER PRIZE PRESENTATION**

PG = Postgraduate Student, **PD** = Postdoctoral Researcher, **RA** = Research Assistant

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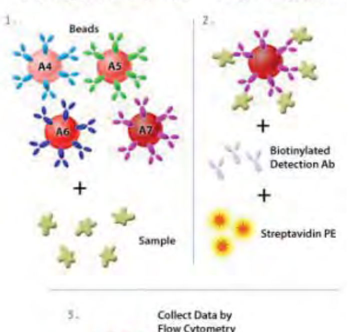

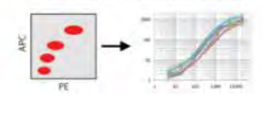

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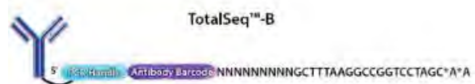

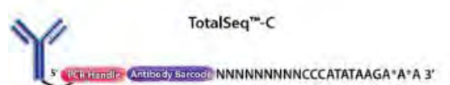

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VIIN Young Investigator Symposium Poster Program 2019

Walter and Eliza Hall Institute of Medical Research, Parkville

12:10-12:50	**Poster Session I and Lunch - Tapestry Lounge and Seminar Room 2**
1	Understanding the mechanisms involved in the induction of hyper inflammation during severe influenza virus infection **Abdullah Bawazeer, Hudson Institute of Medical Research, Centre for Innate Immunity and Infectious Diseases (PG)**
2	Evaluation of synergistic meropenem-ciprofloxacin combination dosage regimens for critically-ill patients with altered pharmacokinetics via mechanism-based modelling and dynamic hollow fibre infection model **Akosua Agyeman, Monash University, Centre for Medicine Use and Safety (PG)**
3	A Role for Fibrillarin in Paramyxovirus Replication **Marina Alexander, CSIRO, Australian Animal Health Laboratory (PD)**
4	*Acinetobacter baumannii* employs multiple pathways for zinc and cadmium efflux **Saleh Alquethamy, University of Melbourne, The Peter Doherty Institute for Infection and Immunity (PG)**
5	Langerhans cells drive chronic proliferative dermatitis phenotype in SHARPIN deficient mice **Holly Anderton, Walter and Eliza Hall Institute for Medical Research, Cell Signalling and Cell Death Division (PD)**
6	The effect of an indoor residual spraying intervention on the reservoir of asymptomatic *Plasmodium falciparum* infections in Bongo District, Ghana **Dionne Argyropoulos, University of Melbourne, Bio21 Molecular Science and Biotechnology Institute, School of BioSciences (PG)**
7	Dendritic cells paralysis post systemic inflammation: the effect of microenvironment **Mitra Ashayeri-Panah, University of Melbourne, Peter Doherty Institute of Infection and Immunity (PG)**
8	Transcriptomic analysis reveals perturbations of cellular signalling networks in human lung epithelial cells due to polymyxin treatment **Mohammad Azad, Monash University, Biomedicine Discovery Institute, Department of Microbiology (PD)**
9	Expression of immunoinhibitory molecules PD-L1 and PD-L2 by dendritic cells **Annabelle Blum, University of Melbourne, Department of Biochemistry and Molecular Biology (PG)**
10	*Plasmodium vivax* invasion: structural scaffolds and human monoclonal antibodies **Li Jin Chan, Walter and Eliza Hall Institute for Medical Research, Infectious Diseases and Immune Defence Division (PG)**
11	The local microenvironment drives the identity of tissue-resident memory T cells **Susan Christo, University of Melbourne, Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity (PD)**
12	Targeting host cell death pathways to promote clearance of *Leishmania donovani* **Michelle Clark, Walter and Eliza Hall Institute for Medical Research, Infectious Diseases and Immune Defence Division (PG)**
13	The prevalence and risk factors for obstetric complications at childbirth and in the postpartum period for women in East New Britain, Papua New Guinea **Hannah Clark, University of Melbourne, School of Population and Global Health and Burnet Institute, Maternal and Child Health Program (UG)**
14	The physiological role of RNAse Zc3h12c in lymphoid tissues architecture and inflammatory responses **Elise Clayer, University of Melbourne, Department of Medical Biology (PG)**
15	Viperin Enhances the dsDNA mediated Type-I Interferon Response to Clear HBV and HSV-1 Infections **Keaton Crosse, La Trobe University, Department of Physiology, Anatomy and Microbiology (PG)**
16	Exploring environmental health practitioners' perceptions of barriers and enablers to preventing cryptosporidiosis in aquatic facilities

	Lauren Cullinan, Swinburne University of Technology, Department of Chemistry and Biotechnology (PG)
17	Host mobilisation of zinc following *Streptococcus pneumoniae* infection **Bliss Cunningham, University of Melbourne, Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity (PD)**
18	Novel fluorescent TNF reporter mouse strains for characterisation of TNF expression **Destiny Dalseno, Walter and Eliza Hall Institute for Medical Research, Inflammation Division (PG)**
19	Distinct immune responses elicited from cervicovaginal epithelial cells by lactic acid and short chain fatty acids associated with optimal and non-optimal vaginal microbiota **David Delgado-Diaz, Burnet Institute, Life Science discipline (PG)**
20	Full genome sequencing and phylogenetic analysis of Australian Canine/feline –like G3P[3], G3P[8] and G3P[9] rotaviruses from children **Elena Demosthenous, Monash University, Biomedicine Discovery Institute, Department of Microbiology (UG)**
21	Genetic and phenotypic characteristics of hyper-virulent community-acquired *Acinetobacter baumannii* **Carina Dexter, Monash University, Biomedicine Discovery Institute, Department of Microbiology (PD)**
22	Different *Wolbachia* strains maintain diverse pathogen blocking phenotypes in an inbred laboratory *Ae. aegypti* colony across different dengue stereotypes **Johanna Duyvestyn, Monash University, Institute of Vector-Bourne Diseases (RA)**
23	Predominantly antibody-deficient patients with non-infectious complications have reduced naive B cells, Treg, Th17 and Tfh17 **Emily Edwards, Monash University, Department of Immunology and Pathology (PD)**
24	Womens' knowledge of newborn danger signs during pregnancy, childbirth and the postpartum period: findings from a cohort study in rural Papua New Guinea **Donya Eghrari, University of Melbourne, Department of Global and Population Health (PG)**
25	The *Coxiella burnetii* effector protein MceB targets the host cell mitochondrion during infection **Laura Fielden, University of Melbourne, Department of Biochemistry and Molecular Biology (PG)**
26	Cigarette smoke-induced depletion of alveolar macrophages contributes to delayed clearance of *L. pneumophila* **Markus Fleischmann, University of Melbourne, Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute (PG)**
27	Organ-specific expression of fatty acid binding proteins in tissue-resident lymphocyte **Raissa Fonseca, University of Melbourne, Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity (PD)**
28	Targeting Mind Bomb-2 and MIP-1, two New Regulators of Tumor cell Survival during TRAIL Signaling **Anna Gabrielyan, Walter and Eliza Hall Institute for Medical Research, Inflammation Division (PG)**
29	Characterisation of the *Haemophilus influenzae* PsaA ortholog, HIPsaA **Katherine Ganio, University of Melbourne, Department of Microbiology and Immunology (PD)**
30	Understanding the effect of naturally occurring human MLKL polymorphisms on necroptosis and disease **Sarah Garnish, Walter and Eliza Hall Institute for Medical Research, Inflammation Division (PG)**
31	Discovering the mechanism behind Ceftazidime-avibactam killing of multidrug resistant *K. pneumonia*: an untargeted metabolomics study **Drishti Ghelani, University of Melbourne, Department of Pharmacology and Therapeutics (UG)**
32	Genomic analysis and exploration of putative drug resistant loci in Malaria parasites **Jiru Han, Walter and Eliza Hall Institute for Medical Research, Population Health and Immunity Division (PG)**
33	Detection and immunophenotyping of antigen-specific memory B cells to measure influenza vaccination responses

	Gemma Hartley, Monash University, Department of Immunology and Pathology (UG)
34	Bioinformatic analysis of eosinophil peroxidase and its implications for model and target species **Caitlin Jenvey, La Trobe University, Agribio Centre for Agribioscience, Department of Animal, Plant and Soil Sciences (PD)**
35	Production and immunogenicity testing of high-stability soluble HIV-1 glycoprotein vaccine candidates based on a transmitted-founder isolate **Brianna Jesaveluk, Burnet Institute, Life Sciences (RA)**
36	Bacterial membrane vesicles from the human microbiome contain antibiotic resistance genes **Ella Johnston, La Trobe University, Department of Physiology Anatomy & Microbiology (PG)**
37	Investigating the role of *Plasmodium falciparum* exported proteins that bind the new permeability pathway complex protein RhopH2 **Thorey Jonsdottir, Burnet Institute, Life Sciences (PG)**
38	Manipulation of host mitochondrial protein import by *Legionella pneumophila* **Yilin Kang, University of Melbourne, Department of Microbiology and Immunology (PD)**
39	Protein O-fucosyltransferase 2-mediated O-glycosylation of MIC2 is dispensable for *Toxoplasma gondii* tachyzoite infection **Sachin Khurana, Walter and Eliza Hall Institute for Medical Research, University of Melbourne, Department of Medical Biology (PG)**
40	Cell death modulation by *Coxiella burnetii* effectors **Robson Kriiger Loterio, University of Melbourne, Department of Microbiology and Immunology (PG)**
41	Identification and characterization of novel hepatocyte effector proteins of the human malaria parasite *Plasmodium falciparum* **Jelte Krol, Walter and Eliza Hall Institute for Medical Research, Infectious Disease and Immune Defence Division (PG)**
42	Identifying genetic variant is key to treating chronic inflammatory lung disease **Maverick Lau, Monash University, Department of Immunology and Pathology (PD)**
43	Exploring the functional interactions between *Coxiella burnetii* Dot/Icm effectors **Yi Wei Lee, University of Melbourne, Department of Microbiology and Immunology (PG)**
44	The application of whole genome sequencing in the prediction of phenotypic antimicrobial resistance in non-typhoidal *Salmonella* circulating Australia **Cheryll Sia, University of Melbourne, Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity (PG)**
45	Repurposing the antifungal Tavaborole as a drug candidate against visceral leishmaniasis **Reetika Manhas, Jawaharlal Nehru University, School of Life Sciences (PD)**
17:10-17:50	**Poster Session II and Drinks, Tapestry Lounge and Seminar Room 1**
18:15	**Oral / Poster / Science Bites and Best Question Prize Presentations**
46	*Acinetobacter baumannii* PAAR proteins: A tale of functional redundancy **Jessica Lewis, Monash University, Biomedicine Discovery Institute, Department of Microbiology (PG)**
47	Host-pathogen interaction between *Legionella* and *Acanthamoeba* **PengFei Li, University of Melbourne, Department of Microbiology and Immunology (PG)**
48	Naturally Acquired Human Antibodies to *Plasmodium falciparum* Transmission-Stage Antigen Pfs230 **Ashley Lisboa-Pinto, Burnet Institute, Life Sciences (RA)**
49	Oligomerization-driven MLKL ubiquitylation during necroptosis **Zikou Liu, Walter and Eliza Hall Institute for Medical Research, Inflammation Division (PG)**
50	Development and optimisation of a silkworm (*Bombyx mori*) infection model for bacteriophage therapy trials

	Stephanie Lynch, La Trobe University, Department of Physiology, Anatomy and Microbiology (PG)
51	The neonatal Fc receptor regulates the presentation of antigens targeted to the dendritic cell receptor DEC205 **Christophe Macri, University of Melbourne, Bio21 Molecular Science & Biotechnology Institute**
52	Zinc homeostasis in *Klebsiella pneumoniae* **Eve Maunders, University of Melbourne, Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity (RA)**
53	Defining the differences in functional antibody response between NAI and vaccine-induced immunity to human **Jordyn McDonough, Burnet Institute, (UG)**
54	*Plasmodium falciparum* inhibits the formation of neutrophil extracellular traps (NETs) **Agersew Mengist, University of Melbourne, Department of Medicine (PG)**
55	Cbl-b regulates the activation threshold of Natural Killer cells **Lizeth Meza Guzman, Walter and Eliza Hall Institute for Medical Research, Inflammation Division (PG)**
56	Dynamics of polymorphism in a leading *Plasmodium falciparum* vaccine candidate - Reticulocyte Binding Protein Homologue 5 (RH5) **Myo Naung, Walter and Eliza Hall Institute for Medical Research, University of Melbourne (PG)**
57	Insights into mechanisms of action of interleukin-1 receptor associated kinase 3 (IRAK 3) **Trang Nguyen, La Trobe University, La Trobe Institute for Molecular Sciences (PG)**
58	Exposure to *Plasmodium* sp. blood-stage impairs liver Trm-mediated immunity **Maria Nogueira de Menezes, University of Melbourne, Department of Microbiology and Immunology (PD)**
59	Characterization of the putative cysteine protease effectors, OspD2 and OspD3, from *Shigella* species **Yogeswari Chandran, University of Melbourne, Peter Doherty Institute and Hudson Institute of Medical Research (PG)**
60	The role of ADAMTS15 versicanase in the immune response to influenza A virus infection **Jess Pedrina, Deakin University, School of Medicine (PG)**
61	Investigating secreted autotransporter toxins from pathogenic *Escherichia coli* strains **Akila Pilapitiya, La Trobe University, Department of Biochemistry and Genetics (PG)**
62	Dissection of the assembly and function of the *Plasmodium* export machinery **Ethan Pitman, Deakin University, School of Medicine (PG)**
63	Characterisation of bacterial effector kinases from enteropathogenic *Escherichia coli* **Georgina Pollock, Hudson Institute of Medical Research, Centre for Innate Immunity and Infectious Diseases (PD)**
64	A rapid colourimetric method to assess complement activation on *Plasmodium falciparum*-infected erythrocytes using 2,7-diaminofluorene **Dilini Rathnayake, University of Melbourne, Department of Medicine, Peter Doherty Institute for Infection and Immunity (PG)**
65	The health of mothers and newborns in Papua New Guinea: A narrative review **Gianna Robbers, Burnet Institute, Maternal and Child Health Program (RA)**
66	Disulphide bond dependent bacterial pathogenesis **Carlos Fernando Santos Martin, La Trobe University, Department of Biochemistry and Genetics (PG)**
67	The catcher in the RIG-I: treatment of respiratory infections by RIG-I agonists **Lara Schwab, University of Melbourne, Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity (PG)**
68	IFNγ controls macrophage TLR responses by activating cell death **Daniel Simpson, Walter and Eliza Hall Institute for Medical Research, Inflammation Division (PG)**
69	Global 3'-utr length changes mediated by interferon beta in murine and human macrophages **Sarah Straub, Hudson Institute of Medical Research, Centre for Innate Immunity and Infectious Diseases (PG)**
70	Developing a core outcome set to measure the effects of pain management during labour and childbirth

	Annie Tan, University of Melbourne, School of Population and Global Health (PG)
71	Antiviral activities of interferon-inducible GTPases **Melkamu Bezie Tessema, University of Melbourne, Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity (PG)**
72	Effects of obesity on vascular and circulating immune cells in mice **Vivian Tran, La Trobe University, Department of Physiology, Anatomy and Microbiology (PG)**
73	Dissecting the origin of epitopes targeted by abacavir-specific T-cells **Johanna Tuomisto, Monash University, Biomedicine Discovery Institute, Department of Biochemistry and Molecular Biology (PG)**
74	Mutations in the guanylate cyclase centre and surrounding residues of IRAK3 can modulate NF-κB signalling **Ilona Turek, La Trobe University, Department of Pharmacy and Biomedical Sciences (PD)**
75	Characterising a novel anti-inflammatory activity of Genestein **Tomalika Ullah, Hudson Institute of Medical Research, Centre for Innate Immunity and Infectious Diseases (PG)**
76	Immunomodulatory effect of Renin-angiotensin inhibitors on T-lymphocytes in mice with Colorectal Liver Metastases **Dora Lucia Vallejo Ardila, University of Melbourne, Austin Health, Department of Surgery (PG)**
77	Identifying Antibody to VAR2CSA in Pregnant Women **Putri Warta, University of Melbourne, Department of Medicine, Peter Doherty Institute for Infection and Immunity (PG)**
78	Elucidation of novel phosphorylation sites in rabies virus P protein. **Ericka Watts, Monash University, Biomedicine Discovery Institute, Department of Microbiology (PG)**
79	*Salmonella* Typhimurium induces cIAP degradation in macrophages in a SPI-1 dependent manner **Madeleine Wemyss, Hudson Institute of Medical Research, Centre for Innate Immunity and Infectious Diseases (PG)**
80	Large genomic deletions in *Legionella pneumophila* identify new genes influencing intracellular replication and lung infection **Raissa Wibawa, Hudson Institute of Medical Research and University of Melbourne, Department of Microbiology and Immunology (PG)**
81	The role of Flt3 in dendritic cells **Kayla Wilson, University of Melbourne, Department of Biochemistry and Molecular Biology (PG)**
82	Sitagliptin enhances anti-tumour immunity and delays tumour progression in a syngeneic model of ovarian cancer **Amy Wilson, Monash University, Department of Immunology and Pathology (PG)**
83	Lytic form of cell death as a novel route for Migration inhibitory factor (MIF) release **Shahrzad Zamani, Monash University, School of Clinical Sciences at Monash Health (PG)**
84	The antimicrobial effect of *Pseudomonas aeruginosa* outer membrane vesicles. **Lauren Zavan, La Trobe University, Department of Physiology, Anatomy and Microbiology (PG)**
85	Decipher the mechanisms of polymyxin resistance in *Acinetobacter baumannii* by genome-scale metabolic modelling **Jinxin Zhao, Monash University, Department of Microbiology**
86	Elucidating the Zn(II)-binding mechanism of *Streptococcus pneumoniae* AdcAII **Marina Zupan, University of Melbourne, Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity (PG)**
18:50	**Oral / Poster / Science Bites and Best Question Prize Presentations**

PG = Postgraduate Student, **PD** = Postdoctoral Researcher, **RA** = Research Assistant, **UG** = Undergraduate Student

SESSION 1
Oral Presentations I

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The human γδ T-cell receptor (TCR) is poised to communicate *Plasmodium falciparum* malaria infection

Anouk von Borstel[1*], Peter Crompton[2], Jamie Rossjohn[1] and Martin S. Davey[1]

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* = presenting author

The malaria parasite *Plasmodium falciparum* induces severe infection and is responsible for ±600,000 annual deaths. Elevated γδ T cell levels have been linked to clinical protection, but their role in sensing *P. falciparum* infection is unclear. γδ T cells are a poorly understood T lymphocyte population, classically known for their rapid innate-like functions. Recent advances in T cell receptor (TCR) repertoire sequencing have shown that human γδ T cells can follow a divergent TCR-dependent adaptive biology.
We have employed novel multiparameter flow cytometry and γδTCR repertoire sequencing to understand the response of the γδTCR repertoire to repeated *P. falciparum* exposure in humans. Our analysis showed a dramatic increase in adaptive γδ T cells after acute *P. falciparum* infection. These adaptive γδ T cells transitioned from a $CD27^+$ naïve-like population towards a distinct effector subset, expressing cytotoxic mediators (i.e. granzyme B and perforin), homing receptors (e.g. CX_3CR1) and transcription factors (i.e. Hobit and Eomes) associated with clonal expansion. Although it has been shown that the γδTCR repertoire is stable over time in healthy individuals, γδTCR repertoire sequencing in seasonally *P. falciparum* infected individuals revealed selective expansion of individual γδTCRs in response to *P. falciparum* exposure. Within these clonally expanded γδ T cells we observed subsequent activation upon re-infection with *P. falciparum* as detected by an increased frequency of $CD27^-CD38^+$ γδ T cells. Together, these data indicate that the γδ T cell population mounts an adaptive immune response to *P. falciparum* infection *in vivo*.
Our data furthermore suggest the existence of malaria-associated antigens that are recognized by the γδTCR. Vaccination strategies to modulate the γδ T cell response may provide important new avenues to control *P. falciparum* infection.

Characterisation of the zinc substrate binding protein AdcA from *Streptococcus pneumoniae*

Aimee Tan[1*], Stephanie L. Neville[1], Katherine Ganio[1], Zhenyao Luo[2], Jacqueline R. Morey[3], Bostjan Kobe[2] and Christopher A. McDevitt[1]

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* = presenting author

Streptococcus pneumoniae (the pneumococcus) is the world's foremost bacterial pathogen. Despite extensive vaccine regimens, *S. pneumoniae* remains the leading cause of bacterial pneumonia, accounting for 15% of all childhood disease mortalities and a global economic health burden of more than US$4 billion annually. Essential to the survival of the pneumococcus is the acquisition of nutrients, such as metals ions, from the host. The first-row transition metal ion zinc is also essential to the virulence and pathogenicity of the pneumococcus and this necessitates that the pneumococcus scavenge this element.

S. pneumoniae acquires zinc using the ATP-binding cassette transporter, AdcCB, and two zinc-specific cluster A-I solute binding proteins (SBPs), AdcA and AdcAII. Although AdcA and AdcAII contribute to zinc recruitment, both SBPs are required for full virulence. Here, we characterised the structural and functional properties of AdcA. The SBP has a two-domain structure joined via a flexible linker region, comprising an N-terminal cluster A-I SBP domain and a C-terminal domain, which is homologous to periplasmic zinc-chaperone protein ZinT. Using mutant variants of AdcA and assessing the impact on growth phenotypes and cellular metal accumulation, we show that the N-terminal domain is necessary and sufficient for zinc uptake by *S. pneumoniae*. Analysis of the zinc-binding site residues revealed that although the Histidine 63 was not essential for metal binding, it was crucial for zinc import. This observation suggests that this residue may contribute to allosteric interaction with the AdcCB transporter or metal ion release. Collectively, these findings provide crucial new insights into the structural and functional properties of AdcA, information that provides a robust foundation for future antimicrobial design.

Genetic investigation of a novel flagellotropic bacteriophage in *Bacillus cereus*

Steven Batinovic[1*], James Wilson[1], Daniel Rice[1], Vaheesan Rajabal[1] and Steve Petrovski[1]

[1]*Department of Physiology, Anatomy & Microbiology, La Trobe University, Bundoora, 3086, Australia*

* = presenting author

The rise of antibiotic resistance in bacteria represents a large threat to human health and consequently new therapies are needed. Bacteriophages (phages) are viruses that infect and kill bacteria. The use of phage therapy, either as a complement or as an alternative to antibiotics, provides an attractive means to treat infection and curb the rise of antibiotic resistance. In this work we focus on the isolation and investigation of phages targeting the foodborne and clinically-relevant spore-forming gram positive bacterium, *Bacillus cereus*. Genomic and ultrastructural investigation of a novel siphoviridae phage targeting *B. cereus* revealed a 107 kb genome and a highly elaborate distal tail structure. To discern the role of this unique tail structure in phage-host interactions, we generated host mutants that were resistant to infection by the phage. Using whole-genome sequencing with Illumina short-read and Oxford Nanopore long-read sequencing, all resistant host strains were found to contain SNPs or genome rearrangements within a ~40 kb region of the chromosome harboring genes required for motility, the *fla-che* operon. To confirm the role of flagellar-mediated motility in phage infection, we designed and implemented the first CRISPR-Cas9 system in *B. cereus* to demonstrate that the phage is in fact a flagellotropic, that is, it utilises the host flagellum as the site of attachment. Specific deletion of the flagellin genes in the host *B. cereus* strain was shown to be protective against phage infection. We are currently examining how flagellar motility and rotation influences phage attachment and injection into the host. Ultimately, the isolation of phages to clinically relevant pathogens and understanding their infectivity will lead to effective treatment options in an era of rising antimicrobial resistance.

Identification of distinct neutrophil populations in the blood of G-CSF treated donors.

Katherine Martin[1]*, Daniela Zalcenstein[1], Peter Hickey[1], Tracey Baldwin, Nick Wilson[2] and Ian Wicks[1,2]

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Neutrophils are the most abundant leukocyte in the blood and typically the first mobilized to a site of injury or infection where they employ a number of strategies to provide critical first-line defence. While once considered to be a homogeneous population of mature cells, there is a growing appreciation that neutrophils can have different phenotypes and functions depending on the context. Despite the acceptance that neutrophils are heterogenous, few studies have explored how these subsets are different or how they contribute to the inflammatory process. Here, we use single-cell RNA sequencing (scRNA-seq) as an unbiased approach to identify and characterise discrete neutrophil populations in the blood of healthy controls (HC) and individuals treated with G-CSF.

Method: Neutrophils were isolated from the blood of HC and donors who were pre-treated with a single dose of G-CSF. Single cell transcriptome libraries were generated by adapting the CelsSeq2 protocol. scPipe was used to perform read alignment and UMI-aware gene count summarization against the GENCODE v28 gene annotations. The resulting gene count matrix was analysed using R/Bioconductor software to identify clusters of transcriptionally similar cells.

Results: We generated scRNAseq data from neutrophils isolated from 4 HC and 6 G-CSF treated individuals and identified at least 9 distinct clusters of cells, four of which were present exclusively in the blood after G-CSF treatment. Based on the expression of known cell surface markers, these four clusters unique to G-CSF donors were largely composed of immature forms of neutrophils and around 50% of the neutrophils in each of these four clusters were characterised as low density. The clusters present in only G donors contained at least 313 uniquely upregulated genes which were predominantly involved in cell cycle and cellular metabolism pathways. Based on results obtained from the scRNAseq analysis, this project will determine how the unique clusters of neutrophils are functionally distinct, and how they might contribute to the inflammatory process.

Conclusion: Here we identify four distinct neutrophil subsets present exclusively in individuals treated with G-CSF. Based on their distinct gene expression signature, these may represent phenotypically and functionally distinct neutrophil subpopulations.

Guided missile strikes: Targeting mechanisms by novel bacteriophages.

Rhys A. Dunstan[1*], Rebecca Bamert[1], Francesca Short[2], Derek Pickard[2], Matthew Belousoff[1], Von Torres[1], David Goulding[2], Gordon Dougan[2] and Trevor Lithgow[1]

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Bacteriophages, or phages, are the most varied and abundant biological entities on the planet and differ substantially in the bacterial hosts they infect. In order to infect its host a phage must first recognize a receptor on the surface of the bacterial cell. Tailed phages use a broad range of receptor binding proteins, such as tail fibres and tail spikes, to target their cognate receptor. These receptors may include lipopolysaccharide (LPS), capsular polysaccharide (CPS), outer membrane proteins (OMPs) such as porins or other more elaborate proteinaceous structures such as fimbriae and flagella. We have isolated several novel phages that target *Klebsiella pneumoniae*, a pathogen that has become a major healthcare burden, particularly in the hospital setting. The genomes of these phages have been sequenced and their structural morphology characterized by electron microscopy (EM). Transposon Directed Insertion Sequencing (TraDIS) was used to identify bacterial mutants that were resistant to each individual phage detailing the host genes required for phage infection. These genes included the porin *ompK36* and several other genes involved in the biosynthesis or secretion of CPS. We have also identified novel capsule depolymerases produced by these phages which act to degrade the bacterial capsule. Using *in vitro* assays, we have characterized the host range, activity and stability of one of these enzymes and have determined its structure using Cryo-EM. This work provides a greater understanding of the targeting mechanisms used by *Klebsiella*-targeting phages and provides a framework for their potential applications as therapeutics or use in the biotechnology industry.

SESSION 2
Keynote speaker

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Victorian Infection and Immunity Network

Young Investigator Symposium

10 October 2019

Laureate Professor Eric C. Reynolds AO FICD FTSE FRACDS

Centre for Oral Health Research, The University of Melbourne

Targeting subgingival polymicrobial dysbiosis by vaccination to prevent the progression of periodontitis

Periodontitis is a chronic inflammatory disease that destroys the tooth supporting tissues, including the alveolar bone. It can affect 1 in 3 adults, with 1 in 10 adults displaying severe forms of disease. We have demonstrated in a longitudinal clinical trial of periodontitis patients that the level of two species *Porphyromonas gingivalis* and *Treponema denticola* in subgingival plaque can predict periodontitis progression. Using an animal model of disease *P. gingivalis* has been demonstrated to be a keystone pathogen in initiating dysbiosis and bone loss. Targeting the pathogen by vaccination in the animal model can prevent dysbiosis and disease progression. We have now obtained funding through the Medical Research Commercialisation Fund and from CSL, our commercial partner, to test the vaccine in Phase I/II human clinical trials.

Professor Reynolds was a Post Doc at the WEHI with Alan Harris before joining the Melbourne Dental School in the early 80's. At the Dental School he was appointed to the Chair of Dental Science in 1994 and became Head of School in 1998. Professor Reynolds was Head of School from 1998 – 2014 and in 2015 became the Director of the University of Melbourne's Centre for Oral Health Research. The Centre evolved from the successful Oral Health CRC, of which Professor Reynolds was CEO and Director of Research from 2003 – 2018.

SESSION 3
Oral Presentations II

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Assaying translation in apicomplexan parasites: a doxycycline case study

Emily M. Crisafulli[1*], Andrew E. Maclean[2], Kit Kennedy[1], Natalie J. Spillman[3], Leann Tilley[1], Lilach Sheiner[2] and Stuart A. Ralph[1]

[1]Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Australia, [2]Wellcome Centre for Integrative Parasitology, University of Glasgow, Glasgow, UK, [3]School of BioSciences, The University of Melbourne, Parkville, Australia.

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Doxycycline has traditionally been used as a broad-spectrum antibiotic due to its activity as a bacterial translation inhibitor. Notably, doxycycline also has activity against a number of apicomplexan parasites, and is the most commonly used prophylactic for malaria. Its mechanism of action in *Plasmodium*, the parasitic cause of malaria, has not been fully elucidated, however there is substantial evidence that the apicoplast ribosome is the primary drug target. Consistent with this target, doxycycline-treated parasites exhibit a 'delayed death' phenotype, characteristic of inhibitors of apicoplast translation. These parasites successfully survive the first erythrocytic cycle following drug treatment, but die in the subsequent cycle. Interestingly, compared to other delayed death drugs, the difference between concentrations required to elicit a delayed death versus an immediate effect are relatively low for doxycycline. One hypothesis to explain this phenomenon is that doxycycline also targets cytosolic and/or mitochondrial ribosomes. Here, we present a live-cell imaging method that allows us to detect cytosolic, and potentially organellar, translation in *P. falciparum* by fluorescence microscopy. Preliminary data using this assay suggest that doxycycline inhibits cytosolic translation at concentrations that cause immediate parasite death. To complement this data, we demonstrate the novel use of an assay designed to detect genetic knockdown of mitochondrial translation in the related apicomplexan parasite, *Toxoplasma gondii*. We use this assay to assess chemically induced inhibition of mitochondrial translation, demonstrating that doxycycline inhibits mitochondrial translation at concentrations that kill in the first cycle, an effect not seen in parasites treated with other delayed death drugs, even at extremely high concentrations. These data lay the foundation for future study of translation inhibitors in apicomplexan parasites, and elucidate the first cycle target(s) of the most commonly used malaria prophylactic, doxycycline.

A map of CXCR3 ligands reveals distinct intranodal niches for CD8 effector and stem-like memory cells

Brigette Duckworth[1,3*], Fanny Lafouresse[1,3], Verena Wimmer[2,3], Thomas Boudier[2,3], Gabrielle Belz[1,3], Kelly Rogers[2,3] and Joanna Groom[1,3]

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Recent discoveries have expanded our understanding of how T cells are positioned within lymph nodes to enhance responses during infection. Upon activation by dendritic cells (DCs) in the paracortex of a draining lymph node, the chemokine receptor CXCR3 is rapidly upregulated on the surface of antigen-specific T cells. CXCR3 binds two ligands in C57BL/6 mice, CXCL9 and CXCL10, which are produced in the cortical ridge and interfollicular region of draining lymph nodes and provide chemotactic signals to newly activated CXCR3$^+$ T cells. While it is known that CXCR3$^+$ T cell repositioning within these peripheral regions is required to mount optimal immune responses, the cellular partners that regulate CXCR3$^+$ T cell location for this process are poorly understood.
Combining chemokine reporter mice with viral infection, we have characterised the cellular sources of CXCR3 ligands. We show that CXCL9 and CXCL10 chemokines are produced by distinct DC and stromal cell populations. Specifically, we show that CXCL9 is produced by type 1 conventional DCs (cDC1) whereas CXCL10 is produced by type 2 cDCs (cDC2), inflammatory monocytes and stromal cells. We have cleared and imaged intact lymph nodes using light sheet fluorescence microscopy (LSFM) to identify the location of chemokine expressing cells and quantify how unique chemokine expressing compartments influence T cell positioning following infection. We correlate T cells position with the formation of T cell effectors and stem-like memory precursors and dissect how this is influenced by specific CXCR3-mediated interactions. This work highlights the finely regulated choreography of T cell migration following viral infection and provides a platform to tune specific T cell differentiation outcomes.

Intracellular Lipid Droplet Accumulation Occurs Early Following Viral Infection and Is Required for an Efficient Interferon Response

Ebony Monson[1*], Keaton Crosse[1], Weisan Chen[2], Donna Whelan[2] and Karla Helbig[1]

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* = presenting author

Lipid droplets (LDs) are increasingly recognized as critical organelles in signalling events, transient protein sequestration and inter-organelle interactions. As the role of LDs is largely unknown in the robust cellular host response to viral infection, we sort to investigate this.

Both average LD number and size were found to increase by up to 5-fold in response to early infection of Herpes Simplex Virus-1 (HSV-1), Influenza and Zika virus (ZIKV) in a broad range of primary and cultured cell types. LD induction occurred as early as 2 hours post infection, was transient, and returned to basal levels by 72 hours post infection. The peak of LD induction coincided with maximal production of the main antiviral cytokines (type I and III interferon (IFN)), and cells with lowered LD content displayed an impaired IFN response, and increased viral replication. Interestingly, dsRNA driven LD induction occurred in two phases, an initial induction that was type-I IFN independent, and a secondary IFN dependent induction; conversely, dsDNA driven LD induction was exclusively IFN independent. Artificially enhancing LDs in cells prior to infection with both ZIKV and HSV-1 positively augmented the type I and III IFN response leading to a significant reduction in replication and virion production of both viruses, without impeding initial viral entry. Virally mediated LD biogenesis was found to be EGFR dependent, and not reliant on PLA_2, the main host protein involved in homeostatic LD biogenesis, suggesting this induction of LDs is alternate to natural LD biogenesis. Recent proteomic analysis of virally induced LDs has revealed key proteins that are enriched on LDs during an infection, which may underpin the LDs contribution to an efficient interferon response.

Here, we demonstrate for the first time, that LDs play vital roles in facilitating the magnitude of the early innate immune response, in particular the production of IFN following viral infection, and control of viral replication. 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 signalling organelle. Collectively, these results provide the foundations for the development of new antiviral therapeutics.

SESSION 4
Science Bites I

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The epigenetic regulation of B cells mediated by Polycomb Repressive Complexes during acute and chronic infections

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High-affinity antibodies and memory B cells are drivers of effective primary and secondary humoral responses in resolving pathogenic infections. These are mainly formed in the germinal center (GC), where antigen-activated B cells undergo rounds of proliferation and selection to either become antibody-secreting cells or memory B cells to establish long-term protection against recurrent infections.
Our research demonstrated that the transcriptional programs of GC B cells are regulated at different stages by histone modifiers belonging to the Polycomb Repressive Complexes (PRC1 and 2). In particular, the subunit EED/PRC2 was critical for the formation and maintenance of GCs, while BMI1/PRC1 was involved in the differentiation of plasma cells, establishing effective humoral responses towards immunisation and acute viral infection.
Persistent infections result in delayed neutralising antibody responses and the formation of atypical memory populations. We therefore investigated the activity of PRC1 and PRC2 in different murine models of chronic infections, including the Lymphocytic choriomeningitis virus (LCMV).
While the conditional deletion of EED in mature B cells drastically reduced GC B cell frequency in immunisation and acute infections, it had limited effect on polyclonal GCs when mice were infected with persistent pathogens. To investigate whether BMI1 was also dysregulated in chronically infected mice, fluorescence BMI1-reporter mice were used to examine expression in B cell subsets during the response. BMI-1 was upregulated in chronic GCs, whereas little expression was detected in acute GCs. Conditional deletion of BMI-1 in mice challenged with chronic LCMV resulted in decreased plasma cell frequency, however, we observed a quicker weight recovery and a lower viremia than control mice.
Together, revealing fundamental epigenetic differences in chronic B cell responses may open up new therapeutic interventions by using small molecule inhibitors to target dysregulated histone-modifying complexes.

Title: What's in a swab? Majority of healthy individuals have detectable intraoral human herpes virus

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Abstract

Background: Human herpes viruses (HHV) are common viruses associated with persistent oral mucosal ulceration in immunocompromised hosts. Intraoral flocked swabs and saliva present noninvasive sample types for detection using qPCR. Baseline intraoral commensal detection rates using qPCR in immunocompetent individuals with and without mucosal abnormalities has not been comprehensively reported.
Aim: To quantify the intraoral prevalence of HHV using mucosal swabs, saliva samples and qPCR analysis in immunocompetent individuals with and without chronic mucosal abnormalities.
Methodology: Matched saliva and oral swabs were collected from a total of 118 subjects: 70 immunocompetent subjects with no mucosal abnormalities, 22 with mucosal abnormalities and 26 therapeutically immunocompromised individuals. Extracted DNA was analyzed by multiplex qPCR for detection and quantification of Herpes simplex 1 (HSV-1), Herpes simplex 2 (HSV-2), Varicella Zoster Virus (VZV), Epstein Barr Virus (EBV), Cytomegalovirus (CMV) and HHV-6.
Results: At least one HHV was detected in 77.1% of immunocompetent individuals with no mucosal abnormalities, with EBV the most commonly detected at 63.0%. HHV-6 was detected in 23.9%, HSV-1 in 9.8% and CMV in 1.1%. Detection was higher in saliva than in swabs. There was no detection of HSV-2 or VZV. Neither presence of oral mucosal abnormality nor therapeutic immunocompromise was related to increased detection of HHV. Quantified detection levels of HSV-1 DNA was significantly higher than detection levels of EBV, HHV-6 or CMV in both swabs and saliva.
Conclusion: Interpretation of positive HHV detection in therapeutically immunocompromised individuals remains challenging. Commensal detection rates of EBV are high and caution in clinical interpretation of positive detection is warranted. Commensal CMV rates are low and detection by swab and saliva may be clinically relevant. The absence of HSV-2 or VZV suggests detection of these viruses by saliva or swab would be significant. This study presents the first comprehensive study into the commensal detection rates of HHV 1-6 by qPCR in saliva and swabs.

Structural and functional investigation of CTD of CzcD transporters; members of the cation diffusion facilitator family of metal efflux proteins

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Zinc is one of the most abundant transition metals in biology. It is required for numerous vital cellular processes, including as an essential cofactor for metalloenzymes [1]. However, the essential requirement for zinc in cells is offset by an associated toxicity when in excess, partially through competition for protein metal binding sites (a process termed mismetallation). Further, zinc is used as an antibacterial element at the host-pathogen interface as part of the host's innate immune response to infection [2]. Irrespective of the mechanism of the zinc toxicity, bacteria have evolved mechanisms to minimize the detrimental impact of zinc excess. One mechanism involves bacterial metal efflux proteins that show heavy metal resistance, such as those belonging to the Cation Diffusion Facilitator (CDF) family of integral membrane protein transporters [3]. The C-terminal domain (CTD) of these integral membrane proteins plays an important role in the function of the CDF family of proteins. To date, all structurally characterized CTDs of the CDF family have been characterised with a conserved metallochaperone-like αββαβ fold, despite significant sequence diversity within this family of proteins [4].

In this study, we have targeted four CzcD proteins, which are members of the CDF family of proteins from four different bacterial organisms, and solved three crystal structures of CTDs of these CzcD proteins by X-ray crystallography. The structural and biophysical characterization of these proteins have allowed the prediction of the metal binding sites within the CTDs of other CDF proteins for which structural information is unavailable.

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Characterisation of the Arg-GlcNAc glycosyltransferase effectors from *Salmonella enterica*

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

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

Preliminary work from our laboratory revealed that SseK3 modifies several Rab GTPases during *Salmonella* infection of murine macrophages. Here, using co-transfection and immunoprecipitation experiments, we confirmed the modification of Rab1, Rab5 and Rab11 by SseK3. Three arginine residues were confirmed as SseK3 modification sites on Rab1; and the modification exerted on Rab1 by SseK3 was independent of the GTP/GDP – nucleotide binding states of Rab1. Rab1 regulates vesicular protein transport from endoplasmic reticulum to the Golgi apparatus. Importantly, a secreted alkaline phosphatase reporter assay indicated that expression of either SseK3 and SseK2 impeded the secretory pathway in HEK293T cells. When either SseK3 or SseK2 were overexpressed in host cells during infection, a reduction in TNF secretion was observed. The overexpression of SseK3 also resulted in the reduced secretion of GM-CSF, IL-6, IL-1α and IL-10 from infected host cells. This suggests that SseK2 and SseK3 may contribute to *Salmonella* infection by blocking host cell secretory pathways.

Lethality due to DPP9 deficiency is rescued by deletion of the NLRP1 inflammasome

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The inflammasome is a protein complex nucleated by a cytoplasmic innate immune sensor, which facilitates the cleavage of pro-caspase-1 into its active form. Subsequently, caspase-1 cleaves the precursor pro-inflammatory cytokines pro-IL-1β and pro-IL-18, mediating release of their active forms. Several sensor components have been identified that recognise a range of microbial components and cellular stress signals. The exact mechanism of activation of one such pattern recognition receptor NLRP1 has yet to be fully elucidated. Recently, we identified mutations in NLRP1 that cause human autoinflammatory disease associated with over active inflammasome formation. One of these mutations specifically triggers the NLRP1 inflammasome by preventing binding of an inhibitory protein known as Dipeptidyl peptidase 9 (DPP9).

DPP9 is a serine protease which functions to cleave Xaa-Pro dipeptides from the N-terminal of proteins. DPP9 has been linked several cellular pathways, including apoptosis, cell migration and Akt signalling. DPP9 has been implicated in NLRP1 regulation, specifically, treatment with an inhibitor of DPP9, Talabostat, activates the NLRP1 inflammasome resulting in IL-1β release and pyroptosis in human and mouse cells.

Mice harboring a mutation that renders DPP9 catalytically inactivate ($DPP9^{S279A/S279A}$) die within one day of birth. The cause of death is currently unknown. We have found that crossing $DPP9^{S279A/S279A}$ mice to NLRP1 knockout mice ($NLRP1^{-/-}$) rescues lethality. $DPP9^{S279A/S279A}$ $NLRP1^{-/-}$ mice appear runted but otherwise healthy, with both males and females proving fertile This dramatic amelioration of phenotype suggests that the major homeostatic, physiologic role of DPP9 is to prevent NLRP1 inflammasome assembly.

Our results predict that if DPP9 deficiency can be identified in humans, that it should respond to pharmacological blockade of the NLRP1 inflammasome. Although direct NLRP1 inhibitors are not yet described, downstream inhibition of inflammasome derived IL-1β and IL-18 is possible, and genetic crosses to determine the individual contribution of these cytokines to the mouse model of disease are underway.

Our studies help uncover the molecular basis for NLRP1 inflammasome activation, relevant for novel approaches to treat autoinflammatory diseases.

Granulocyte colony stimulating factor promotes lung damage in neonatal mice with Bronchopulmonary Dysplasia

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PUBLISH CONSENT WITHHELD

Identifying the targets of functional antibodies against a major malaria antigen

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Malaria has imposed a significant burden on global health throughout the centuries, and currently remains one of the most potent killers in the realm of infectious disease today. Malaria parasites initially infect a human host in a sporozoite form, in which they migrate to the liver, in the pre-erythrocytic stage of malaria. Recent studies have shown that antibodies can confer protection against the sporozoite stage of infection through IgG-Fc mediated immunity including complement fixation and opsonic phagocytosis. The circumsporozoite protein (CSP) has been known as a major target of protective antibodies. However, details of the target epitopes are poorly understood, and this knowledge is imperative in informing the design of effective malarial vaccines. In order to address this, we have conducted studies to identify the targets of antibodies that are capable of engaging Fcγ receptors (FcγR). We have compared naturally-acquired human antibodies and vaccine induced rabbit polyclonal antibodies with high or low FcγR binding activities using a peptide array representing all the linear epitopes of CSP. Furthermore, we also have characterized a few human monoclonal antibodies to the central repeat region of CSP. Our data suggested that monoclonal antibodies to the central repeat region of CSP may vary in the number of tandem repeats required for antibody binding. These differences may subsequently affect the function of these antibodies in interacting with FcγRs and their ability to promote opsonic phagocytosis by neutrophils. These findings aid in advancing the current understanding of the immune response against the pre-erythrocytic stage of malaria, which potentially aids in designing a vaccine to effectively target the initial stage of malaria.

Non-specific IgA inhibits HIV broadly neutralizing antibody (bNAb) Fc functions

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Considerable evidence supports the role of Fc effector functions against HIV-1, including the moderately protective human HIV RV144 vaccine trial, that associated Fc functions with protection from HIV-1 infection [1]. However, RV144 induced IgA to HIV envelope (gp120) reduced vaccine efficacy and Fc capacity via epitope competition with HIV-specific IgG [2]. Passive transfer of certain broadly neutralizing antibodies (BnAbs) in macaques have shown that both neutralizing and Fc functions are required for protection. Here we endeavour to determine if IgA influences the Fc functions of BnAbs [3].

Pooled HIV-specific IgA (n=10) and pooled HIV negative total IgA (n=6), IgA1, IgA2 and colostrum IgA were used to assess the inhibitory role of IgA on the ability of HIV-specific BnAbs to induce gp120-specific antibody dependent cellular phagocytosis (ADCP). The addition of HIV negative total IgA (median=7.05%, IQR=5.00%, p=0.0500), IgA1 (median=16.91%, IQR=7.63%, p=0.0103), IgA2 (median=16.22%, IQR=2.96%, p=0.0006) and colostrum IgA (median=22.41%, IQR=8.61%, p=0.0004) significantly reduced ADCP compared to responses of various BnAbs and HIV positive IgG alone.

Preliminary findings indicate IgA is capable of reducing IgG mediated ADCP *in vitro*. HIV negative IgA, derived from plasma and colostrum, cannot bind specifically to gp120. Therefore, this inhibitory mechanism is not mediated by IgA epitope competition with IgG. Instead IgA may inhibit BnAb Fc functions in a nonspecific manner, potentially via Fc alpha receptor binding ($Fc\alpha R$) [4]. Future work aims to investigate this mechanism. This data indicates that non-specific IgA can influence BnAb Fc functions and should not be overlooked in passive transfer studies.

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Sortase A Inhibition as a Route to Treat Antibiotic Resistance in *Staphylococcus aureus*

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Staphylococcus aureus readily develops resistance to antibiotics as well as hiding and evading our own immune system. Therefore, new methods to overcome this deadly pathogen are necessary. In this study, we use aryl(β-amino) ethyl ketones (AAEKs) to circumvent antibiotic resistances in *S. aureus* by inhibiting the enzyme sortase. Inhibiting sortase prevents the loading of cell wall-anchored (CWA) proteins onto the cell wall that are key for evasion of the host immune response. *S. aureus* clinical isolates were treated with AAEKs; CWA protein levels, bacterial growth rates, and the immune system response was analysed. Treatment with AAEKs lead to a decrease in CWA protein loading on *S. aureus*, with an accompanying concentration-dependent decrease in bacterial growth rates. Inhibition also altered the human immune response, with neutrophil survival rates increasing significantly on exposure to AAEK treated *S. aureus* as well as alterations in swarming behaviour indicative of a loss of CWA proteins. The action of AAEKs was also increased with the use of vancomycin in whole blood via an additive effect. These results confirm that AAEK treatment alters CWA protein loading, which in turn changes subsequent host cell responses. These findings indicate a possible future use of AAEKs in conjunction with current antibiotics - such as vancomycin - in overcoming *S. aureus* infections.

SESSION 5
Keynote speaker

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Victorian Infection and Immunity Network
Young Investigator Symposium
10 October 2019
Dr Sarah Dunstan
The University of Melbourne

Genomics to tame tuberculosis and typhoid

Dr Sarah Dunstan is a Senior Research Fellow in The Peter Doherty Institute for Infection and Immunity, at The University of Melbourne. Sarah uses genomics to understand host-pathogen interactions of infectious diseases. Sarah completed her PhD at the University of Melbourne in 1998 then undertook a postdoctoral position at Imperial College, London. In 2001, Sarah joined the Oxford University Clinical Research Unit, Vietnam and headed the Human Genetics group. Sarah developed a large program of work on enteric fever, tuberculosis and malaria. In 2013, Sarah returned to the University of Melbourne to continue infectious disease human genomics, and expanded her research to include pathogen genomics.

SESSION 6
Oral Presentations III

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Mechanism of daptomycin resistance in *Staphylococcus aureus* mediated by mutations in *mprF*

Jhih-Hang Jiang[1*], Sigrid Lange[1], Wei Gao[2], Liam Donovan[1], Romain Guérillot[2], Chia Xin Lim[3], Anton Le Brun[4], Bart A. Eijkelkamp[5], Thusitha Rupasinghe[6], Jacqueline Seiffert[1], Benjamin P. Howden[7], Hsin-Hui Shen[8], Anton Y. Peleg[9]

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PUBLISHED CONSENT WITHHELD

Structural studies of human antibody responses against leading malaria vaccine antigen PfCSP

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Malaria is a complex, mosquito-borne disease estimated to cause over 300,000 childhood deaths annually. *Plasmodium falciparum* (*Pf*), the major parasite causative of malaria, accounts for over 85% of malaria mortality, and demonstrates considerable resistance to current drug therapies. It is therefore imperative to develop a vaccine to *Pf* to reduce malaria morbidity and mortality. Reverse vaccinology holds promise to design effective immunogens for the development of malaria vaccines. This concept is based on interrogating the B cell repertoire of infected or vaccinated subjects to identify inhibiting antibodies that will guide immunogen design. The circumsporozoite protein (CSP) is the major surface antigen of Pf sporozoites and a leading malaria vaccine antigen. Here, we structurally and functionally characterized protective and non-protective antibodies to PfCSP from four healthy adults living in the malaria-endemic area of Lambaréné, Gabon, and from eight vaccinated European donors who underwent immunizations with aseptic, purified, cryopreserved Pf sporozoites (PfSPZ Challenge) under chloroquine prophylaxis (PfSPZ-CVac), which resulted in protection against controlled human malaria infection. Our structural delineation of protective and non-protective epitopes highlights key differences of B cell responses during natural exposure and vaccination (1,2). We provide the molecular mechanism underlying clonal selection and affinity maturation of human B cells expressing protective antibodies (3). Finally, we describe at the atomic level the evolution of protective human antibodies against the PfCSP repeat motifs. Collectively, these data provide the blueprints to engineer optimized antigens that can be tested as pre-erythrocytic subunit vaccines.

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Rabies against the machine: using artificial intelligence to identify extracellular biomarkers of lyssavirus infection

Ryan J. Farr[1*], Nathan Godde[1], Chris Cowled[1], Vinod Sundaramoorthy[1], Diane Green[1], Cameron Stewart[1], Andrew Laslett[2], John Bingham[1], Carmel O'Brien[2] and Megan Dearnley[1]

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Rabies (lyssavirus) still has an enormous impact on global mortality, with an estimated 59,000 deaths annually in >150 countries (WHO 2019, https://www.who.int/rabies/epidemiology/en/). This neurotropic virus slowly migrates to the brain via peripheral neurons following exposure to infected saliva, usually through an animal bite. Once there, it causes encephalitis and neurological symptoms, including seizures, confusion, and aggression. Rabies is a clear example of an infection that requires early detection and intervention; it has an unusually long asymptomatic period and once symptoms appear it is almost universally fatal.

MicroRNAs (miRNAs) have recently garnered intense interest as biomarkers of disease as they, 1) respond quickly to physiological stimuli, 2) are easily found within biofluids, either as free molecules, bound to proteins, or encapsulated in exosomes, and 3) display impressive stability *ex vivo*. To investigate whether miRNAs could be used as biomarkers of rabies infection, we developed a human stem cell-derived neuronal model, infected the neurons with different strains of lyssavirus (including Australian bat lyssavirus, a lab-adapted lyssavirus, and two wild-type rabies strains), and then used next-generation sequencing to profile cellular and exosomal miRNAs. Exosomes can cross the blood-brain barrier into peripheral circulation, allowing minimally invasive biomarker detection. We found 24 cellular and 41 exosomal miRNAs that were significantly altered in response to lyssavirus. Using supervised machine learning techniques, these miRNAs could cluster samples based on lyssavirus strain, demonstrating that host miRNA responses are specific to viral subtypes. We then identified the exosomal miRNAs with the highest predictive power to identify rabies infection. A combination of six exosomal miRNAs correctly classified rabies or mock infected neurons with 95% accuracy and a receiver operating characteristic (ROC) area under the curve (AUC) of 0.96. Quantitative PCR is currently being used to validate this model.

By using an unbiased sequencing and computational approach, we have developed an effective model that could form a novel diagnostic platform. This study brings together expertise in stem cell modelling, high containment virology (BSL3), and advanced bioinformatics, and highlights the potential for miRNAs to underpin next-generation molecular diagnostics of infectious disease.

Mimicking NETs with self-assembling nanofibers

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We were inspired by our own defense strategies to tackle antimicrobial resistance. We found our inspiration in the arsenal of weapons used by our innate immune systems first responders, neutrophils. This being the neutrophil extracellular trap (NET) which is deployed by the self-sacrificing neutrophil to entangle the invading microbe in a DNA net. This DNA net is embedded with antimicrobials, which helps ensure the invader cannot escape. We created a proof of concept treatment that mimicked NETs by producing self-assembling nanofibers that are decorated with the glycopeptide antibiotic vancomycin.

Our net-like fibers form by using the propensity of lipidated tri-β-peptides to self-assemble into nanofibers. To decorate these fibers with antibiotics we directly linked a β^3-peptide to vancomycin. Mixing the vancomycin linked with plain β^3-peptides in different ratios resulted in vancomycin being incorporated into fibers. These fibers had different structures that have been visualized using negative stain electron microscopy and atomic force microscopy. These distinct fibers have different antimicrobial activity against antibiotic resistant clinical strains of *Staphylococcus aureus* (Methicillin resistant MRSA or Vancomycin intermediate VISA strains) as measured using a micro broth dilution assay. Creating a fluorescently labelled β^3-peptide allowed us to visualize the fibers wrapping *S. aureus* by stimulated emission depletion microscopy. In addition, surfaces coated with these fibers also reduce the formation of *S. aureus* biofilms.

Inspired by our immune system, we created self-assembling nanofibers from β^3-peptides embedded with vancomycin. These nanofibers entrap and kill antibiotic resistant *S. aureus*.

Genome-wide molecular screen reveals novel HCMV genes essential for viral egress

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PUBLISH CONSENT WITHHELD

The gut microbiome: A source of antimicrobial resistance for pathogens

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

This project is assessing the transfer of AMR from the commensals in the gut microbiota to pathogenic species of bacteria. Initially, commensals from the gut microbiome were screened for resistance to four antibiotics (Tetracycline, Amoxicillin-clavulanic acid, Vancomycin and Metronidazole), and resistant strains were selected to form a panel of 95 AMR strains. Full-length 16S rRNA and whole genome sequencing was performed to derive taxonomic classification and identify and putative AMR genes. To identify novel AMR genes, genomic comparisons will be performed between closely related species with differing antibiotic sensitivities.

Conjugation experiments were performed between the AMR commensals and a number of gastrointestinal pathogens (*E. coli, Enterococcus feacium, Klebsiella pneumoniae* or *Clostridium difficile*), to assess for transfer of AMR from the commensal organisms to the pathogenic strains. By doing this, we aim to identify if any genes are being transferred to enable the spread of resistance, while also identifying species specific barriers to AMR transfer. Additionally, by performing phenotypic analyses, we aim to identify novel AMR genes unable to be identified by typical genotypic approaches. These results should enable more tailored clinical treatment approaches to bacterial infections, while also advancing the development of novel antimicrobials.

SESSION 7
Science Bites II

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From Phenotypic Screens to Mode of Action: A Metabolomics Approach to Guide the Development of Novel Anti-trypanosomal Drugs

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Background

Access to large phenotypic screens has enabled the discovery of novel anti-infective compounds. Translating these compounds into new drugs faces a number of challenges. Finding the mode-of-action (MoA) can help in focussing efforts to develop the most promising leads.

Method

Using a medium-throughput method, we performed untargeted metabolomics analyses of Trypanosoma brucei, a kinetoplastid parasite responsible for neglected tropical diseases nagana and sleeping sickness. This allowed us to investigate the mode of action of 18 potent trypanocidal compounds from the Pathogen Box (available from Medicines for Malaria Venture).

Results

Over 500 metabolite features were identified in this study and mapped to the predicted metabolic network. This approach identified the metabolic pathways targeted by the most potent compounds which spanned nucleotide metabolism, lipid metabolism, co-factor synthesis and redox metabolism. Lysophospholipid metabolism, glutamate metabolism, purine salvage and S-adenosyl-methionine and polyamine pathways were found to be specifically hit. Interestingly, multivariate analyses showed that compounds with similar chemical structures did not always have similar biochemical activities.

Conclusions

This study showed that a simple metabolomics assay can rapidly reveal the MoA of newly discovered anti-infective compounds. This information can be used for prioritising compounds in the optimization pipeline and help in designing combination therapies that target discrete pathways to overcome emerging drug-resistance.

Sphingosine-1-phosphate receptor 5 (S1P5) critically regulates tissue-resident memory T cell formation.

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Tissue-resident memory T (T_{RM}) cells persist at sites of infection where they provide critical local immunity against secondary infection with the same pathogen. Thus enhancing the generation of T_{RM} cells represents an attractive avenue to improve vaccination strategies against infectious agents. Despite this, the pathways that regulate the trafficking and formation of these immune cells are not fully understood. Sphingosine-1-phosphate receptor 5 (S1P5) is a protein receptor involved in cellular migration. However, its role has not been explored in T cells. Our data indicates that S1P5 is differentially regulated during distinct phases of T cell activation, trafficking and differentiation into T_{RM} cells in peripheral organs. S1P5-deficiency increased T_{RM} cell formation while overexpression of this molecule dramatically abolished T_{RM} cell development in tissues including the liver, skin and gut, suggesting that S1P5 can modulate T cell trafficking into tissues and T_{RM} cell differentiation. In addition, we found that S1P5 expression not only affects T_{RM} cell development but was a critical regulator of other tissue-resident lymphocyte populations. Together, our data identifies S1P5 as a novel pathway regulating T cell immunity that could potentially be harnessed for improved vaccine design.

Effects of APOA1, ABCA1 and LCAT Mutations on Monocyte Activation in Humans – a Double Edged Sword

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Plasma high-density lipoprotein cholesterol (HDL-C) levels are inversely associated with cardiovascular risk. Whereas the causal nature of this relationship was challenged by Mendelian randomization studies as well as several outcome trials with HDL-raising drugs, an increasing number of studies revealed effects of HDL on the immune system. *In vivo* data supporting effects on monocytes in patients are lacking. Here, we evaluated whether low HDL-C determined by genetic mutations (*ABCA1, APOA1 and LCAT)* are associated with monocyte activation. We compared the monocyte phenotype from subjects with low-HDL (either ABCA1, apoA-I or LCAT deficient) to normolipidemic healthy controls using flow cytometry, intracellular lipid measurements and functional read-outs such as trans-endothelial migration capacity and cytokine production. Finally, we studied RNA expression of pivotal inflammatory as well as cholesterol homeostasis genes. We found that APOAI and ABCA1 deficiency associate with a pro-inflammatory monocyte phenotype with increased levels of activation markers, increased cytokine production capacity and transendothelial migration capacity compared to controls. In contrast, LCAT deficiency shows an opposite phenotype with reduced transendothelial migration capacity and increased expression of efflux receptors. This is accompanied by a decreased cytokine production capacity and lower activation markers.

In conclusion, different HDL-genotypes are associated with lead opposite monocyte immunophenotypes, resulting in either pro- or anti-atherogenic monocytes. This implies that not all HDL-deficient patients might have the same inflammatory risk and may benefit from optimization of a more personalized treatment strategy.

Clinical predictors of severe RSV infection in children <2 years at The Royal Children's Hospital, Melbourne

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Background: Respiratory Syncytial Virus (RSV) is the leading cause of lower respiratory tract infections in young children worldwide. Risk factors for severe disease vary between populations and settings and inform the effective targeting of preventative interventions e.g. vaccines. This study aims to identify predictors of severe RSV disease in children <2 presenting to The Royal Children's Hospital (RCH), Melbourne, Australia.

Methods: This was a prospective cohort study in children <2 years of age presenting to RCH from May 2017–April 2019. Children who were RSV+ on PCR from naso-pharyngeal specimens collected as part of routine clinical care were eligible for inclusion. Children with nosocomial RSV infection were excluded. Clinical and outcome data were extracted from the hospital electronic medical record. Severe RSV was defined as need for any respiratory intervention.

Results: In total, 742 children were eligible for inclusion in the analyses. Males outnumbered females (n=404; 54%). The median age was 4mo (IQR 1,12) with mode of 1mo. Most were term (n=557/695; 80%). The median gestation of pre-terms was 34wks (IQR 31, 36). A minority had comorbidities (n=127, 17%). Almost half received respiratory intervention (46%); humidified-high-flow-nasal-prong therapy was the most common intervention (n=181; 24%) followed by low-flow oxygen (n=68; 9.2%), non-invasive ventilation (n=64; 8.6%) then invasive ventilation (n=22; 3%). Groups were divided into those who did and did not require respiratory intervention (n=334 and n=403, respectively). Univariate logistic regression revealed younger age (3mo vs 5mo, $p<0.0001$), prematurity (26% vs 12%, $p<0.0001$), cardiac/respiratory comorbidity (12.6% vs 7.7%, $p=0.023$), chromosomal/developmental comorbidity (3% vs 0.7%, $p=0.024$), low birth weight (term <2.5kg) (3.6% vs 1%, $p=0.021$) and parainfluenza virus (PIV) co-infection (6% vs 2%, $p=0.004$) predicted need for respiratory intervention. On multi-variable logistic regression, younger age ($p<0.0001$), prematurity ($p<0.0001$), developmental/chromosomal comorbidity (OR 4.882, 95% CI 1.242,19.19,$p=0.023$) and PIV co-infection (OR 3.69, 95%CI 1.467, 9.281, $p=0.006$) were independent predictors of severity.

Conclusions: Younger age, prematurity and chromosomal comorbidities predict need for respiratory intervention in young children presenting with RSV infection at RCH. The finding of parainfluenza virus co-detection as a predictor of severity is novel and warrants further research.

Can shark antibodies be used as an alternative against bacterial proteins in the establishment of Urinary Tract Infections?

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Urinary tract infections (UTIs) represent one of the most frequent severe bacterial infections [2]. Despite their severity, treatments for these are becoming scarcer due to increasing bacterial antibiotic resistance [3]. Therefore, new approaches to tackle UTIs are urgently required. The main cause of UTIs is Uropathogenic *Escherichia coli* (UPEC), a pathogen that contains a series of virulence factors, which play a key role in the crucial steps of the establishment and persistence of an infection [2]. The biggest family of virulent proteins in UPEC is the Autotransporter proteins (ATs) family, which contains an important group known as the AIDA-I-type adhesins [1, 5]. These surface proteins promote bacterial aggregation and biofilm formation [1, 3, 5]. This study is focused on the most prevalent AT adhesin, Antigen 43 (Ag43) [4]; specifically, this research aims at analysing structure-function relationships, processing and mechanisms of action of Ag43, in order to develop an approach to block its function. The functional domain of an Ag43 homologue, *E. coli* CFT073 Ag43b was expressed and purified and the structure was characterised by X-ray crystallography.
The identification of molecules that block the function of Ag43 has been attempted using single-domain antibodies derived from the variable region (vNAR) of a shark antibody known as the IgNAR. Screening of a vNAR phage-displayed library has resulted in the identification of two binders that specifically recognise Ag43 homologues. Ongoing work with these vNARs will establish their ability to halt the aggregative ability of Ag43.
The outcomes of this project will provide essential data for future studies on the function and inhibition of this key AT to develop future alternative therapeutic approaches to handle UTIs.

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CD1a-restricted T cells: A unique population of lipid reactive T cells

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In contrast to conventional T cells that recognise peptide antigens presented by MHC molecules, other T cells recognise lipid antigens presented by MHC-like CD1 family members, CD1a, CD1b, CD1c and CD1d. Recent studies have suggested that CD1a-restricted T cells comprise a unique T cell population in human blood and may also play a unique functional role in skin. We have produced CD1a tetramers to investigate the phenotype and function of human CD1a-restricted T cells directly *ex vivo*.

Interestingly, we have shown that CD1a-restricted T cells that recognise non-self-lipid antigens, particularly dideoxymycobactin (DDM), a lipid antigen derived from *Mycobacterium tuberculosis*, can also be autoreactive to self-lipids. These T cells that cross-react between multiple lipid antigens (both self and non-self) are unlike conventional T cells that recognise one, unique antigen.

Additionally, we have defined the T cell receptor (TCR) usage of both self- and foreign-lipid-reactive CD1a-restricted T cells, demonstrating that while they exhibit a diverse TCR repertoire, there is some biased usage of certain variable genes. Phenotypic analyses and RNA-sequencing of these cells revealed that are distinct from other unconventional, innate-like T cells, such as natural killer T cells, thereby distinguishing CD1a-restricted T cells as a unique population of unconventional T cells. For example, they do not express innate-like markers such as CD161 and IL-18R. Collectively, these studies represent an important step forward in characterising CD1a-restricted T cells, and further understanding their role in infection and autoimmune responses.

Host exosome biogenesis blueprints construction of the Human Cytomegalovirus virion

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Human Cytomegalovirus (HCMV) extensively re-programs and re-organises the host cell in a myriad of ways to support virion replication and assembly. None is more pronounced than the construction of the cytoplasmic virus factory termed the viral assembly complex (vAC). The vAC is thought to facilitate secondary envelopment of maturing virions, although the molecular mechanisms employed and the host and viral protein determinants remain uncharacterized. To gain novel insights, virions were stringently isolated and sequenced using proteomics, revealing a significant number of host exosome proteins as molecular cargo. Interestingly, exosomes are membranous nanovesicles released from almost all cell types that govern intercellular signalling and communication. Therefore, we hypothesized that this host pathway may be hijacked by HCMV for viral pathogenesis.

Exosomes and virions were isolated from identical cell culture conditions utilising gold standard isolation procedures, then sequenced using state-of-the-art proteomics. Quantitative comparisons between uninfected cell exosomes and virions revealed the vast majority of virion host protein abundance to be exosomal, providing a clear relationship between exosome and virion biogenesis processes. The data was interrogated further for proteins significantly upregulated in the virion compared to exosomes, likely indicating specific functions, and a suite of candidates promoted for functional characterisation. Significant virus inhibition was observed for the majority of targets following siRNA knock-down, with up to 5000-fold reductions for some targets. Mechanistic studies of these targets can pinpoint defects in virus production at various stages along the maturation pathway, including virion envelopment, trafficking and fusion processes, which we show with multiple proof-of-concept examples. Using an unbiased system-wide approach, we propose a model whereby host cell exosome biogenesis machinery provides the molecular scaffold for HCMV virion construction and egress. This model identified a suite of novel determinants essential for virus production and provides a new framework to interpret and study the HCMV egress axis, which represents a unique host-virus interface with substantial potential for potent antiviral therapy.

Immune and Inflammatory Mechanisms of Crohn's Disease

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Crohn's disease is a multifactorial, chronic, relapsing inflammatory bowel disease. Whilst the aetiology is yet to be elucidated, it is commonly hypothesised that dysbiosis of gut microbiota along with intestinal hyperpermeability and inappropriate inflammatory responses to commensal bacteria are a potential mechanism. Current treatments mainly consist of general immunosuppressants and although more targeted therapies are emerging, they are not effective in all patients. Therefore, better understanding of disease mechanisms could identify more effective treatment targets. In this project, the SHIP-1-deficient model of Crohn's disease-like ileitis was used to examine the immune cell makeup and the impact of gut microbiota and intestinal barrier function on disease aetiology and progression. Additionally, the role of the gut-lung axis in driving disease was investigated. Histopathology studies showed that ileitis development in SHIP-1-deficient mice was dependent on environment with mice housed in clean facilities producing the lowest disease penetrance. Mice without ileitis had normal ileal histology and no immune cell infiltration. SHIP-1-deficent mice with ileitis exhibited significant granulocyte infiltration long with disruption of normal villus and crypt architecture and thickening of the muscularis. Severity of ileitis correlated to severity of lung disease; more inflammatory cell infiltration into the gut correlated to increased consolidation and emphysema in the lungs of the same mouse. The bronchoalveolar lavage fluid of SHIP-1-deficient mice with ileitis show evidence of breakdown in pulmonary epithelial integrity with increased erythrocyte infiltration and decreases in IL-22 and IL-23p19, cytokines important in epithelial barrier homeostasis. Dysbiosis was evident in the ilea of SHIP-1-deficient mice with ileitis characterised by a decrease in Akkermansia (Verrucomicrobia phyla) and Sutterella (Proteobacteria phyla), and an increase in Lactobacillus (Firmicutes phyla). This study shows environmental factors influence disease with housing and bacterial colonisation impacting disease onset. Furthermore, intestinal inflammation impacts upon pulmonary inflammation within the same mouse.

Endogenous annexin-A1 attenuates intestinal microbiome dysbiosis in a mouse model of diabetes

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Background: The intestinal microbiota plays an important role in the host immune system, which may contribute to cardiometabolic diseases. Endogenous anti-inflammatory protein, annexin-A1 (*AnxA1*) has been demonstrated to contribute to many inflammatory diseases. However, its impact of microbiota community has not been explored. In this study we investigated the role of endogenous *AnxA1* in regulating diabetes-induced changes in the intestinal microbiota.

Methods: Insulin-resistance was induced in male mice (*AnxA1*$^{+/+}$ and *AnxA1*$^{-/-}$; n=19 and n=21, respectively) with the combination of streptozotocin (55 mg/kg i.p. for 3 consecutive days) with high fat diet (42% energy from fat) or citrate vehicle with normal chow diet and followed for 20-weeks (n=11 and n=7, respectively). At the study end, when all animals were 26 weeks of age, the composition of faecal bacterial from all mice were assessed by automated ribosomal intergenic spacer analysis (ARISA) and quantitative PCR (qPCR). Systemic inflammation was assessed by differential cell count in the whole blood.

Results: Following high fat feeding and STZ-induced hyperglycemia, *AnxA1*$^{+/+}$ and *AnxA1*$^{-/-}$ mice had 2-fold greater blood glucose levels ($p<0.05$) and significantly altered microbial communities ($p<0.05$), with dysbiosis indicated by unfavourably high Firmicutes: Bacteroidetes ratios ($p<0.05$) vs. non-diabetic mice. In non-diabetic *AnxA1*$^{-/-}$ mice, microbial abundance and diversity was significantly changed ($p<0.05$), whereas in diabetic *AnxA1*$^{-/-}$ mice, the diversity of the intestinal microbiota was altered ($p<0.05$). However, no significant difference in the abundance of total bacteria, Firmicutes and Bacteroidetes was observed between these animal groups. Finally, diabetic *AnxA1*$^{-/-}$ mice had significantly elevated neutrophil counts compared to diabetic *AnxA1*$^{+/+}$ mice ($p<0.05$).

Conclusion: These findings provide first evidence that deficiency of *AnxA1* can affect intestinal microbiota microbial community, which may contribute to the chronic low grade inflammation in diabetes.

Functional and structural studies of the glycoprotein E2 of a rodent hepacivirus. Exploring a novel model system to inform Hepatitis C vaccine development.

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Recently a number of hepaciviruses, related to Hepatitis C (HCV), infecting rodents have been identified. Rodent Hepacivirus (RHV) isolated from Norway rats in New York has sparked interest for the development of a surrogate animal model to study HCV vaccinal immunity. RHV shares some key characteristics with HCV and studies have found similarities in the T cell response to virus infection. However, detailed studies comparing the characteristics of the glycoproteins and broadly neutralizing antibody responses are lacking.
We have successfully expressed a soluble version of the RHV E2 protein, allowing us to study its characteristics and comparison with HCV E2. Our data show that RHV E2 is smaller and has considerably less glycosylation sites to HCV, raising the question, whether it uses the rodent homolog receptors for cell entry, or if other receptors play a role. Soluble RHV E2 is a useful tool in analyzing antibody responses to RHV infection and vaccination of rodents. We could already confirm that sera from rats infected with RHV is reactive to soluble RHV E2, indicating a physiological relevant conformation of the protein. Studies of the immune response to vaccination of rats with RHV E2 will allow us to compare the types of immune responses generated by HCV and RHV. This will be important to know to which extend RHV infection in rats can be used as a surrogate model for studies of HCV vaccinal immunity.

SESSION 8
Oral Presentations IV

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Sex dependent differentiation of regulatory T cells in the visceral adipose tissue

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Visceral adipose tissue (VAT) is a metabolically active endocrine organ that plays pivotal roles in the regulation of systemic metabolism. Lean VAT is enriched for type 2 immune cells which includes T helper (Th2) cells, type 2 innate lymphoid cells (ILC2) and anti-inflammatory macrophages. Obesity on the other hand promotes infiltration of inflammatory immune cells, such as CD8 T cells, pro-inflammatory macrophages, and Th1 cells, resulting in type 1 inflammation and ultimately leading to insulin resistance. VAT Treg cells are critical to restrict inflammation and preserve insulin sensitivity and glucose tolerance. Notably, lean VAT is enriched for Treg cells while their population declines during obesity. Our lab previously described that Treg cells specifically require the cytokine IL-33 for their expansion and survival in the VAT. Indeed, VAT Treg cell numbers are substantially reduced in mice deficient for IL-33 or its receptor ST2. Consistent with its central role, administration of IL-33 specifically expanded VAT Treg cells and significantly improved glucose metabolism in obese mice (Vasanthakumar et al. Nat. Immunol. 2015).

We have recently identified pronounced sexual dimorphism in VAT Treg cells. While male VAT was enriched for Treg cells that displayed the typical KLRG1+ST2+ phenotype that we described previously, female VAT contained much fewer Treg cells that did not conform to this phenotype. Further cellular and transcriptional analyses revealed substantial differences between male and female VAT Treg cells and highlighted distinct expression of chemokine receptor CCR2, transcriptional regulators Blimp1 and PPARg, and anti-inflammatory cytokine IL-10. These results identify phenotypically and transcriptionally divergent Treg cell populations that predominate in the VAT of either sex. Notably, this phenotypic dimorphism was not Treg cell intrinsic, but a result of differences in the microenvironments of male and female VAT. Sex hormones imprinted these differences, regulating the differentiation of IL-33 expressing stromal cells in male VAT. These stromal cells supported local Treg cell expansion and the induction of a Blimp1-driven VAT Treg cell transcriptional program in male but not female mice. Thus, our findings reveal a complex sex-dependent feedback circuit that is driven by stroma and Treg cells to limit VAT inflammation and maintain metabolic homeostasis.

The role of necroptosis in bacterial gut infection

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Necroptosis is a programmed inflammatory cell death pathway primarily studied in host defense against viruses. However, recent characterisation of the enteropathogenic *Escherichia coli* (EPEC)-encoded effector EspL, which directly inhibits necroptosis and associated inflammatory pathways by cleaving RHIM domain-containing proteins, RIPK1 and RIPK3, suggests a novel role in bacterial clearance. Preliminary experiments on *Ripk1*$^{-/-}$*Ripk3*$^{-/-}$ deficient mice infected with the EPEC-like mouse pathogen, *Citrobacter rodentium,* demonstrated heightened susceptibility to infection and a significant decrease in serum amyloid A2 (SAA2) within the colon, typically associated with T_H17 responses important for bacterial clearance. As the mechanisms of necroptosis *in vivo* remain unclear, this study sought to clarify the role of RHIM proteins and the underlying pathway mediating exacerbated pathology in infected *Ripk1*$^{-/-}$*Ripk3*$^{-/-}$ deficient mice. Examination of disease in various single and compound knockout mice found that RIPK3 plays a larger role in mediating pathology in gut infection, whereas RIPK1 restricts bacterial systemic dissemination. Additionally, flow cytometry analysis of colonic lamina propria from infected *Ripk1*$^{-/-}$*Ripk3*$^{-/-}$ deficient mice showed a significant reduction in T_H17 cells relative to wildtype controls. This was consistent with qPCR evaluation of inflammatory cytokine levels where a larger fold reduction in *Saa2, Il22* and *Il17a* gene expression was exhibited in mice infected wildtype *C. rodentium* compared to those with an *espL* deletion mutant. Overall, these results show for the first time, a link between RHIM-proteins (innate immunity) and T_H17 responses (adaptive immunity), which will be further investigated to better inform the significance of necroptosis in bacterial pathogenesis and maintenance of gut homeostasis.

Screening the Medicines for Malaria Venture Pathogen Box for invasion and egress inhibitors of the blood stage of *Plasmodium falciparum*

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Plasmodium falciparum causes the most severe form of malaria and with emerging resistance to frontline treatments, there is the need to identify new drug targets in the parasite. One of the most unique processes in the parasite's lifecycle is the invasion and subsequent egress of red blood cells (RBCs). This process is essential for parasite proliferation and survival which makes it an ideal drug candidate. During egress and invasion, many unique parasite ligands, receptors and enzymes are employed that could be druggable targets. To identify potential inhibitors we screened the Medicines for Malaria Venture (MMV) Pathogen Box, a 400 compound library comprised of drugs against neglected tropical diseases, including 125 with antimalarial activity. In the presence of the Pathogen Box compounds, we utilised transgenic parasites exporting the bioluminescent reporter, Nanoluciferase (Nluc), to measure inhibition of parasite egress and invasion. At a concentration of 2 µM, we found 11 compounds that inhibited parasite egress by >40% and 16 compounds that inhibited invasion by >90%. We have further characterised 11 of these inhibitors through biochemical assays and live cell microscopy and have found them to either inhibit egress, directly inhibit parasite invasion or cause general growth defects that manifests as invasion inhibitory effects. We have found the sulfonylpiperazine, MMV020291, to be the most invasion specific inhibitor, blocking successful merozoite internalisation within human RBCs and having no substantial effect on the rest of the cell cycle.

Effective, low-cost preservation of human stools for gut microbiome investigation in a helminth endemic region

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Molecular tools are a cornerstone of diagnostic and epidemiological studies. However, they are no better than the quality of samples they are used on. Studies of gastrointestinal helminth infections and the impacts of infection or diet on gut microbial health require rapid sample processing and/or effective preservation of a range of molecular media, including DNA, RNA and proteins. This preservation must introduce minimal disruption or bias into the data. These challenges are particularly difficult in the field of neglected tropical disease, which overwhelmingly affect impoverished populations in remote, tropical to subtropical, regions globally. Rapid refrigeration or freezing of samples upon collection is often not feasible and costs associated with commercial preservatives are prohibitive. We have investigated three low-cost preservation methods, DESS (DMSO, EDTA, NaCl), 2.5% potassium dichromate and rapid freezing at -80°C for nucleic acid and protein preservation, qPCR-based diagnosis of helminth infection and microbiome characterisation in a cross-sectional study of school-aged children from an endemic region in North-West Thailand. We characterised the gut microbiome using a 16S rRNA amplicon sequencing approach. All three preservatives showed a similar performance in microbiome preservation (no significant differences in alpha diversity measure), whereas DESS provided the best performance for helminth egg preservation. We also tested if faecal samples preserved in DESS can be used for extraction of high-quality RNA and proteins. DESS isolated RNA and proteins were sufficient for subsequent experimental pipelines, however of lower quality and quantity than those extracted from fresh frozen samples. Lastly, we investigated the impacts of helminth infections on the gut microbiome of study participants. Interestingly, we found no significant difference in gut microbiota diversity comparing uninfected versus infected children, however did find alterations in bacterial community composition. Here, we present a cost-effective, non-toxic alternative for stool sample preservation that is applicable in remote, tropical regions and can be used for extraction of DNA, RNA and proteins.

Covalent Binding of Complement C3 to MHC II mediated Trogocytosis between Conventional Dendritic Cells and MZ B cells in the Spleen

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The role of Major Histocompatibility Complex class II (MHC II) molecules in antigen presentation to $CD4^+$ T cells and adaptive immunity has been characterised over decades of research. Complement component 3 (C3) is the central component of the complement system and its activation induces protective functions as part of the innate immune system. Here, we describe a new function for both molecules, namely the covalent binding of activated C3 to MHC II on the surface of conventional dendritic cells (cDCs) in the spleen, mediating their interaction with marginal zone (MZ) B cells.

Surface expression of MHC II in antigen presenting cells is tightly controlled by MARCH1-mediated ubiquitination. Using mice lacking this post-translational modification, and HLA-deficient human PBMCs, we observed that surface expression of MHC II in cDCs correlates with the deposition of extracellular C3. Further investigations elucidated that this is due to the covalent binding of activated C3dg/C3d to the α-chain of MHC II. Mice with altered MHC II ubiquitination, where cDC were surface-coated with C3, harboured reduced numbers of cDCs and “abnormal” MZ B cells that displayed several DC surface markers. These markers were not synthesised by the MZ B cells themselves. Rather, co-culturing experiments demonstrated transfer of membrane fractions from C3-coated cDCs to B cells, a phenomenon known as *trogocytosis*. Blocking or deleting complement receptor (CR2) in splenic B cells reduced trogocytosis.

Our results reveal a fundamental new role for MHC-II and C3 in splenic cDCs, in which surface MHC-II serves as an acceptor for covalent C3 binding. The resulting MHC-II-C3 complexes in turn act as recognition ligands for MZ B cells, mediating cell-cell interactions and directional extraction of membrane proteins. Ongoing investigations are characterizing the role of this novel mechanisms of cDC-MZ B cell interaction and membrane transfer in innate and humoral immunity.

Therapeutically targeting Myc in gastric cancer

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Background: Myc is a critical regulator of gastric tumour development and progression, and is associated with a poorer survival rate in human gastric cancer patients. Hyperactivation of inflammatory signalling cascades, including the Jak/Stat3 pathway, is crucial for gastric cancer development and can result in the overexpression of Myc. However, the pro-tumorigenic role of Myc in gastric cancer remains poorly understood.

Aim: Previous findings from our lab using the Gp130^{FF} mouse model, which spontaneously develops gastric adenomas through IL-11 dependent hyperactivation of Stat3, identified an upregulation of Myc in these tumours.

Here, we investigate the cellular requirement of Myc in gastric tumourigenesis by genetically ablating Myc within gastric epithelial cells. As well, we explore the therapeutic benefit of reducing the transcriptional activity of Myc through the use of the small-molecule inhibitor IBET-151.

Methods: Tumour-bearing Tff1^{CreERT2};Myc^{flox};Gp130^{FF} mice were treated with tamoxifen for 3 days and/or IBET-151 for 21 days. At the experimental end-point, tumour weights were recorded and tissue collected for biochemical analysis.

Results: Genetic ablation of Myc in gastric epithelial cells significantly reduced tumour growth and activation of Jak/Stat3 signalling, as observed by decreased phosphorylated Stat3.

Immunohistochemical analysis revealed a significant reduction in the percentage of Ki67+ proliferating cells. RNA-Sequencing of whole tumours subjected to KEGG pathway analysis similarly demonstrated a significant downregulation of cell-cycle related genes.

Therapeutic inhibition of Myc using the small-molecule inhibitor IBET-151 also significantly impaired tumour growth, consistent with a reduction in Myc, demonstrating its therapeutic benefit in this gastric cancer mouse model.

Conclusions: Excessive Myc activity in epithelial cells promotes gastric cancer development and progression by enhancing tumour-cell proliferation. Future work will identify the underlying mechanisms by which IBET-151 similarly impairs gastric tumour growth. Taken together, our results suggest that inhibition of Myc may be a promising therapeutic target for the treatment of gastric cancer patients.

SESSION 9

Science Bites III

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IL-23 co-stimulation drives antigen-specific MAIT cell activation and enables vaccination against bacterial infection

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MAIT cells are activated in a TCR-dependent manner by antigens derived from the riboflavin synthesis pathway, including 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil, bound to MHC- related protein-1 (MR1). However, MAIT cell activation *in vivo* has not been studied in detail. Here, we have discovered and characterised additional molecular signals required for optimal activation and expansion of MAIT cells following pulmonary *Legionella* or *Salmonella* infection in mice. We show that either bone marrow-derived APCs or non-bone marrow-derived cells can activate MAIT cells *in vivo,* depending upon the pathogen. Optimal MAIT cell activation *in vivo* requires signalling through the inducible co-stimulator of T cells (ICOS), which is highly expressed on MAIT cells. Subsequent expansion and maintenance of MAIT-17/1-type responses are dependent on IL-23. Vaccination with IL-23 plus 5-OP-RU augments MAIT cell-mediated control of *Legionella* infection. These findings reveal cellular and molecular targets for manipulating MAIT cell function under physiological conditions.

INFLAMMATORY BIOMARKERS IN PATIENTS WITH END-STAGE RENAL DISEASE CO-MORBID WITH LATENT TUBERCULOSIS

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INTRODUCTION: Patients with end-stage renal disease (ESRD) have a 52.5-fold increased risk of Mycobacterium Tuberculosis reactivation from latent to active disease (1). With a quarter of the world latently infected, chronic and acute co-morbidities are a dangerous syndemic (2). The immunological reason as to why ESRD patients reactivate tuberculosis infection is still unclear.

METHODS: This study uses Multiplex Luminex Technology and glycosylation. The cohort consists of Canadian patients with ESRD (n=10), patients with ESRD co-infected with Latent Tuberculosis (Ltb) (n=10), Ltb (n=10) and healthy controls (n=10). We assessed over 200 serological features in this cohort. These were levels of 35 different inflammatory cytokine markers, IgG and IgA binding to 17 different Mtb-specific antigens, Mtb-specific FcR binding and glycosylation patterns across each group. A systems serological approach is then completed using machine learning algorithms to determine features that differentiate our groups.

RESULTS: All patients with ESRD exhibited substantial elevations in 20 out of the 35 tested serum pro-inflammatory cytokines most notable being Osteopontin (n=0.006), IL-35 (p=0.001), Osteocalcin (p=0.003), IL-2 (p<0.0001), APRIL (n=<0.0001) and TSLP (p<0.0001). Interestingly, patients with ESRD and LTB exhibited elevations in MMP-3 (p=0.014) cf. patients with ESRD. IL-10 (p=0.001) and TWEAK (p=0.006) differentiated between Ltb and ESRD/Ltb patients. Further, agalactosylated IgG was enhanced in patients with co-morbidity compared to Ltb and fucosylation differentiated groups. Mtb-IgG1 and IgG3 differentiated ESRD/Ltb from Ltb alone.

CONCLUSION: This data reveals the specific cytokines, glycosylation patterns and IgG subclass binding differentiated ESRD/Ltb from Ltb alone. This research has implications for clinical management of latent tuberculosis in ESRD as with further investigation into the immunological state of these patients, we may elucidate markers that may correlate with tuberculosis reactivation risk and direct earlier tuberculosis treatment.

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Defining naturally acquired antibody longevity to *Plasmodium vivax* antigens in western Thailand

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Plasmodium vivax is the dominant *Plasmodium* spp. in low-transmission regions outside of Africa. Such regions often feature asymptomatic patients with sub-microscopic parasitaemia and relapses. Naturally acquired antibody responses are induced after *Plasmodium* infection, providing partial protection against high parasitaemia and clinical episodes. However, previous work has failed to address such antibody responses to *P. vivax* particularly in low-transmission regions. We followed 34 patients in western Thailand after symptomatic *P. vivax* infections to monitor antibody kinetics over 9 months with no recurrent infections. We assessed total IgG levels to 52 *P. vivax* proteins every 2-4 weeks using Luminex® assay against malaria-naïve volunteers, and identified protein-specific variation in IgG longevity. Generally, an increase in antibody level was observed within 1-week post symptomatic infection, followed by an exponential decay of different rates. For most antigens, we also observed IgG1 dominance and IgG3 sub-dominance in this population. IgM responses followed similar kinetic patterns to IgG, with some proteins unexpectedly inducing long-lived IgM response. We also monitored antibody response in 30 individuals from a similar region of western Thailand who had already acquired immunity against symptomatic disease. Our results showed most of the antigens induced robust and long-lived total IgG responses following asymptomatic infections in absence of boosting infections. With population genetics analysis, we demonstrated high antigenic sequence diversity was linked to lower antibody magnitude and greater response variation between individuals. Upon completion of this work, we will have gained insights into naturally acquired immunity development to *P. vivax* and the potential use of serology to identify populations at risk.

Dissecting the role of topical antibiotic use in the emergence of multidrug-resistant *Staphylococcus aureus*

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Topical antibiotics, such as fusidic acid or mupirocin, are used in the prevention and treatment of skin infections caused by *Staphylococcus aureus*. However, recent population-level studies demonstrate an association between extensive use of topical antibiotics and increased rates of resistance to these agents in *S. aureus* [1]. Of particular concern is the genotypic association of topical antibiotic resistance genes with mobile genetic elements encoding other unrelated antimicrobial resistance, suggesting a previously under-recognised role for topical antibiotics in co-selecting for multidrug-resistant (MDR) *S. aureus* [2].

Here, we hypothesise that topical use of these agents favours the selection of methicillin-resistant *S. aureus*. Using a targeted mutagenesis approach, we have provided one of the first experimental confirmations on the molecular basis of acquired resistance to both agents in *S. aureus*. Our ongoing contemporary investigation and risk assessment on topical antibiotic resistance among *S. aureus* in Australia will provide valuable information regarding the molecular epidemiology of major resistant clones and resistance mechanisms. Finally, our study represents the first to provide *in vitro*, *ex vivo* and *in vivo* experimental data to address the role of clinically relevant topical antibiotics in driving the emergence of MDR *S. aureus*.

These findings collectively will pave the way for the future development of antimicrobial stewardship strategies to inform the judicious use of topical antibiotics.

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Finding the Achilles Heel in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is one of the leading causes of nosocomial infections, accounting for 10% of all hospital-acquired infections, with current treatments becoming increasingly ineffective [1]. This highlights the need for novel antimicrobial agents and targets. One such target is the diaminopimelate (DAP) pathway, which is responsible for the biosynthesis of bacterial cell wall and protein building blocks, namely *meso*-DAP and lysine [2]. Importantly, these metabolites are only synthesised in bacteria and plants, indicating toxicity to humans is unlikely. The rate limiting step of the DAP pathway is catalysed by the enzyme dihydrodipicolinate synthase (DHDPS), which is typically encoded for by a single *dapA* gene in bacteria [2]. Surprisingly, sequence analyses reveal the presence of four putative *dapA* genes in *P. aeruginosa* genomes, with 3 out of 4 genes being universally present in *Pseudomonas* species. We employed a combination of complementation and enzyme kinetic assays to show that only two of these putative genes encode for functional DHDPS enzymes. Thus, *P. aeruginosa* represents the first example of a bacterium with multiple DHDPS enzymes. Although these isoforms, PaDHDPS1 and PaDHDPS2, have similar catalytic activities, only PaDHDPS2 is inhibited by the end product lysine (IC_{50} = 130 μM). Subsequently, we used mutagenesis, crystallography and homology modelling to attribute this differential inhibition to a single amino acid residue in the allosteric binding pocket. We speculate that the presence of two isoforms allow an increase in metabolic flux through the DAP pathway when required and this study provides key insights into the future development of inhibitors against this enzyme as potential new antibiotics.

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Investigating metabolic pathways in *Coxiella burnetii*, the causative agent of the zoonotic disease Q fever, and their role in pathogenesis.

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The zoonotic pathogen *Coxiella burnetii* poses a serious threat to global public health. *C. burnetii* replicates intracellularly within a unique vacuole derived from the phagolysosome, known as the *Coxiella*-containing vacuole (CCV). To cause disease, *C. burnetii* must not only survive this normally bactericidal environment but garner sufficient energy and nutrients to replicate. Exploring metabolic pathways required by *C. burnetii* to survive inside host cells may identify novel therapeutic targets or prevention strategies.

The shikimate pathway is required by bacteria, fungi, plants and some protozoa to synthesize a plethora of aromatic compounds, including aromatic amino acids. The pathway is absent in mammals and thus is an alluring potential drug target. Additionally, some bacterial strains lacking the shikimate pathway have reduced virulence and are used as vaccines. A previously generated transposon mutant library includes a mutant with a transposon inserted into the fourth enzyme within this pathway, shikimate dehydrogenase or *aroE*, (*cbu0010*), which provides an opportunity to scrutinize the importance of *aroE,* and the shikimate pathway overall, to *C. burnetii* pathogenesis.

Metabolomic analysis comparing the *aroE* mutant and wildtype suggests AroE is indeed non-functional in the *aroE* transposon mutant. There is a significant increase in abundance of protocatechuate in the mutant, and this difference has the greatest fold change of all the metabolites increased in the *aroE* mutant. If AroE were functional, 3-dehydro-shikimate would be converted to shikimate by AroE, but in the absence of AroE it is broken down to protocatechuate. Significantly reduced abundances of the aromatic amino acids phenylalanine and tyrosine in the *aroE* mutant also suggest the shikimate pathway is inactive. Significant changes in the abundance of metabolites in a number of other pathways suggest that the loss of AroE results in wider metabolic changes. Current work is focused on characterizing the intracellular behaviour of the *aroE* mutant, in order to determine if it is reduced in its ability to replicate inside host cells.

Investigating Transcriptional Correlates of Naturally Acquired Immunity to Malaria

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Malaria is one of the most important infectious diseases of humans, responsible for over 200 million clinical cases and 400,000 deaths annually. *Plasmodium falciparum*, which is endemic to Sub-Saharan Africa and South-East Asia, is responsible for the majority of malaria-associated morbidity and mortality. The clinical symptoms of malaria, which are due to the blood-stage replication of the parasite, can range from a mild febrile illness to severe presentations including renal failure, cerebral malaria and severe malarial anemia. Children under five with low levels of immunity are most susceptible to severe disease. Unlike other common infections that induce life-long protective immunity after a single exposure, naturally-acquired immunity to malaria is slow to develop and only acquired after years of repeated infections. This type of immunity prevents clinical episodes by substantially reducing parasite burden yet is not sterilising, and clinically-immune adults in endemic areas often experience asymptomatic infections.

Whereas the mechanisms underlying the slow and inefficient acquisition of immunity to malaria have been extensively investigated, correlates of naturally-acquired immunity are poorly defined. In addition, there remains a paucity of studies investigating the overall impact of persistent asymptomatic malaria infections on the host. To address this, a transcriptional analysis of peripheral blood mononuclear cells was performed on participants from a malaria-endemic region of Eastern Indonesia with either symptomatic or asymptomatic *P. falciparum* malaria infection as well as healthy, uninfected community individuals. This analysis revealed a strong proliferative and inflammatory signature in symptomatic malaria as well as evidence of malarial anemia, while immunosuppressive and immunotolerant transcriptional profiles were observed in asymptomatic malaria. These results identify the key immunological pathways that drive the development of symptomatic malaria and provide evidence of the cellular and molecular processes underlying clinical immunity to malaria.

SopF, a phosphoinositide binding effector, promotes the stability of the nascent *Salmonella*-containing vacuole.

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Salmonella enterica serovar Typhimurium utilises Type III Secretion Systems (T3SSs) to target host cell processes that enable it to invade, survive and replicate intracellularly. *S*. Typhimurium encodes two T3SSs: T3SS1 and T3SS2, on *Salmonella* Pathogenicity Islands SPI-1 and SPI-2, respectively. T3SS1 and its dedicated effector proteins are primarily involved in bacterial entry into non-phagocytic cells. We recently identified a *S*. Typhimurium gene, SL1344_1177, that is co-regulated with the SPI-1 regulon inside of mammalian cells. This gene was identified by another group to be a T3SS1 effector protein, SopF. We have confirmed that SopF is a T3SS1 substrate. Both ectopically expressed and type III translocated SopF partially associates with host cell membranes. Indirect immunofluorescence of epithelial cells ectopically expressing SopF indicates that it partially co-localises with cytoskeletal-associated markers, particularly at the leading edge of cells. We identified that recombinant SopF binds to multiple phosphoinositides in protein-lipid overlays, suggesting that it associates with host cell membranes via phospholipid interactions. This is supported by localization data in *Saccharomyces cerevisiae*, where SopF redistributes from internal membrane sites to the plasma membrane in a Mss4$^{tet\text{-}off}$ strain. Mss4 is a yeast phosphatidylinositol (PI) 4-phosphate 5-kinase (PI4P5K) that catalyzes PI4P conversion into phosphatidylinositol 4,5-bisphosphate (PI(4,5)P_2). In addition, the membrane localization in both yeast and mammalian cells is dependent on the C-terminus of SopF. SopF is not required for bacterial invasion of host cells, but is required for maintenance of the internalization vacuole membrane as infection with a *S*. Typhimurium Δ*sopF* mutant led to increased lysis of the *Salmonella*-containing vacuole (SCV) compared to wild type bacteria. We also show that SopF acts antagonistically to another T3SS1 effector, SopB. In summary, we have identified SopF as the first T3SS1 effector that contributes to stability of the nascent SCV membrane.

Poster Session 1

Poster abstracts are presented according to numerical order of presentation per the poster program table, which is mostly, but not strictly, alphabetical by surname.

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Understanding the role of NLRP3 in different cell types during severe influenza virus infection

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PUBLISH CONSENT WITHELD

Evaluation of synergistic meropenem-ciprofloxacin combination dosage regimens for critically-ill patients with altered pharmacokinetics via mechanism-based modelling and dynamic hollow fibre infection model

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Background: Infections due to *Pseudomonas aeruginosa* (Pa) are associated with high morbidity and mortality in critically-ill patients. Alterations in antibiotic concentrations at target sites are a major concern in this patient population due to augmented renal clearance (ARC) affecting renally excreted antibiotics. The impact of ARC on bacterial killing and resistance emergence has not been characterised *via* the hollow-fibre infection model (HFIM) for the combination of meropenem (MER) and ciprofloxacin (CIP).
Materials/methods: MER and CIP alone and in combinations were investigated against Pa1280 (MIC_{MER} and MIC_{CIP} 0.25mg/L) from a critically-ill patient using static-concentration time-kill (SCTK) experiments (32 profiles in total). Mechanism-based modelling (MBM) of the SCTK data and *in silico* simulations for clinical relevant regimens were conducted. Promising combination regimens were evaluated in a 10-day HFIM study (inoculum ~10^7 CFU/mL) simulating ARC (CL_{CR} 250mL/min). Total and resistant bacterial counts were determined.
Results: All CIP concentrations evaluated in SCTK achieved >3log_{10} CFU/mL killing in the first 5h followed by regrowth almost similar to control counts by 72h. MER 7.13mg/L produced >4log_{10} killing at 48h followed by regrowth. MER 25.7mg/L suppressed regrowth to <2log_{10} at 72h. All combinations yielded >5log_{10} killing and suppressed regrowth to <1log_{10} (limit of counting) over 72h. MBM well described the viable count profiles for all treatments and indicated substantial subpopulation synergy. *In silico* simulations of clinically relevant dosage regimens predicted regrowth to control values for all monotherapies and ~4log_{10} killing with regrowth suppression for combinations. HFIM results were in good agreement with *in silico* predictions. All monotherapies produced regrowth similar to control values with extensive resistance emergence (MIC_{MER} 16mg/L, MIC_{CIP} 32mg/L at 240h. Both combination regimens suppressed regrowth and resistance emergence with viable counts <2log_{10} at 240h.
Conclusions: MBM predictions based on SCTK data were successfully translated to the dynamic HFIM. For the pharmacokinetics of critically-ill patients with ARC, a combination of approved dosage regimens of MER and CIP was required to suppress regrowth and resistance over 10 days. These combination regimens are highly promising for improved clinical effectiveness and suppression of resistance emergence, even in the near-worst-case scenario of ARC.

A Role for Fibrillarin in Paramyxovirus Replication

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A genome-wide siRNA screen for host proteins impacting Hendra virus infection in viable cells identified a crucial role for the host protein Fibrillarin in the post entry stages of Hendra virus replication, namely RNA and protein production (Deffrasnes et al. 2016). Other paramyxoviruses, measles, mumps and respiratory syncytial viruses also showed dependence on Fibrillarin for infection while influenza virus did not. Fibrillarin is a nucleolar methyltransferase with established roles in ribosome biogenesis. Fibrillarin knockdown in Hela cells leads to global decreases in 2'-O-Methylation of rRNA conferring a reduced capacity to translate specific mRNAs (Erales et al. 2017). It is unclear whether Fibrillarin has a direct effect on translation of viral mRNAs or a secondary effect through changes in the host cellular milieu. This postdoctoral fellowship aims to tease these two possibilities apart using next-generation sequencing of paramyxovirus genomic and messenger RNA species in Fibrillarin-knockdown cells.

Acinetobacter baumannii employs multiple pathways for zinc and cadmium efflux

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Acinetobacter baumannii is a Gram-negative human pathogen associated with significant morbidity and mortality. The WHO has placed *A. baumannii* as the top critical pathogen in need for novel antimicrobial therapies due to the emergence of carbapenems resistant isolates. Metal ions, such as zinc, have been recognised as important antimicrobials to control bacterial infections. Thus, resistance to metal intoxication is crucial for the success of many pathogenic bacteria. *A. baumannii* is known to harbour an extensive repertoire of metal ion efflux systems, none of which have been functionally characterised. Here, we investigated the role of membrane transport systems in *A. baumannii* zinc resistance. Our analyses of transposon mutant *A. baumannii* strains revealed a role for the resistance nodulation division (RND) transporter CzcCBA in zinc resistance. This was determined by supplementing the mutant strain *czcA*::T26 with zinc, and examining the impact on growth and metal accumulation compared to the wild-type strain. The significance of this pathway was then investigated using a zinc-deficient murine infection model. This revealed that *A. baumannii* resistance to zinc stress was important in the spleen, as indicated by the reduced bacterial burden of the *czcA*::T26 strain by comparison to the wild-type strain. Our studies also identified an additional zinc resistance pathway, the cation diffusion facilitator (CDF) protein CzcD. We then investigated the contribution of these pathways in resistance of other transition metal ions. This revealed that CzcCBA contributed to cadmium resistance, and while CzcD did not provide resistance against cadmium, we identified a distinct CDF transporter, CzcE, that is crucial for *A. baumannii* survival in cadmium stress. Collectively, these analyses provide novel insights into the metal ion resistance mechanisms of *A. baumannii,* and the niches in which metal ion tolerance are important during infection.

Langerhans cells drive chronic proliferative dermatitis phenotype in SHARPIN deficient mice

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The linear ubiquitin-chain assembly complex, of which SHARPIN is a componant, regulates signalling pathways from TNF superfamily and pattern recognition receptors. A spontaneous loss of function mutation in *Sharpin* is designated cpdm due to a prominent chronic proliferative dermatitis (CPD) phenotype. Loss of TNF or TNFR1 prevents the dermatitis and crosses to *Ripk3*$^{-/-}$, *Mlkl*$^{-/-}$ and *Caspase8*$^{+/-}$ show that blocking cell death also limits the inflammatory phenotypes, implicating TNFR1 induced cell death as the main driver of the CPD.

Little is known about which cells are the source of TNF that drives the dermatitis. Immune cells can be a potent source of TNF *in vivo* and feature prominantly in the CPD pathology, though T-cells and B-cells have been shown to play no significant role in the phenotype. To investigate the possible pathogenic contribution of other immune cells to the *Sharpin*^{cpdm} cutaneous phenotype we utilised the transgenic Diphtheria Toxin Receptor (DTR) system to specifically ablate particular immune cell subsets *in vivo*.

We found that chronic systemic depletion of CD11b^{+} or CCR2^{+} cells in *Sharpin*^{cpdm} mice delays onset of disease but does not prevent progressive dermatitis, however, a similar depletion of Langerhans cells (LCs), results in a highly significant ($p<0.001$) reduction in clinical severity at the typical *Sharpin*^{cpdm} endpoint. We therefore crossed the *Sharpin*^{cpdm} mice to a strain largely absent LCs due to a defect in their ability to replicate and populate the epidermis and found that these mice had no macroscopic dermatitis at the Sharpin endpoint.

This work shows that LCs play a pivotal role in the TNF dependent, cell death mediated skin disease that arises in *Sharpin* mutant mice, placing them as a potential cellular source of pathogenic TNF in the *Sharpin*^{cpdm} skin, and highlighting a T-cell independent role for LCs in driving skin inflammation.

The effect of an indoor residual spraying intervention on the reservoir of asymptomatic *Plasmodium falciparum* infections in Bongo District, Ghana

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Plasmodium falciparum is a genetically diverse parasite that causes approximately 99% of all malaria infections in sub-Saharan Africa. Malaria control has been hampered by the high prevalence of asymptomatic infections that persist throughout seasons and remain largely undetected by traditional diagnostic methods. Putatively neutral microsatellite markers are a molecular epidemiological tool that can be used to describe parasite genetic diversity, linkage disequilibrium and population structure to monitor changes in *P. falciparum* populations over time. This research examines the temporal effects of an indoor residual spraying intervention using a longitudinal cross-sectional study of individuals of all ages with asymptomatic *P. falciparum* infections from Bongo District, Ghana. This insecticide is aimed to prevent the survival of the *Anopheles* vector, interrupting transmission of *P. falciparum.* Here we present a study to compare the *P. falciparum* populations pre-intervention and post-intervention using multilocus microsatellite genotyping and epidemiological methods.

Dendritic cells Paralysis post Systemic Inflammation: The Effect of Microenvironment

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Dendritic cells (DC) are potent antigen presenting cells which link the adaptive and innate arms of the immune response. This normal functioning of DC is attenuated after systemic inflammation when an extended period of immunosuppression causes DC apoptosis and dysfunction (called "DC paralysis"), resulting in greater risk of secondary infections and higher rates of mortality and morbidity in patients. Aiming to characterise DC paralysis, we injected mice intravenously with CpG (a mimic for bacterial DNA). It led to a transient systemic inflammation followed by three-weeks-long impairments in the priming of antigen-specific T cells *in vivo*. Use of CD11c-mOVA mice showed that DC are responsible for these impairments, but purified DC loaded with antigen *in vitro* could prime T cells normally. We therefore hypothesized that microenvironment after inflammation plays role in DC paralysis and confirmed it by showing impaired *ex vivo* presentation of different forms of antigen. Further phenotype characterisation and RNA sequencing of paralyzed DC showed up- and down-regulation of particular intrinsic factors (involved in antigen processing and presentation) as well as cytokine receptors (that regulate DC response to extrinsic signals). We tested/are testing on the involvement of these factors in DC paralysis by use of knock out mouse strains or cytokine blocking *in vivo*, making us conclude, not only paralyzed DC suffer from intrinsic functional impairments but also the spleen microenvironment post inflammation "trains" the DC for poor functionality. Understanding this memory-like behavior of paralyzed DC which is influenced by extrinsic signals, can ultimately help us to devise strategies to prevent or reduce DC paralysis in critically-ill patients by blocking such signals.

Transcriptomic analysis reveals perturbations of cellular signalling networks in human lung epithelial cells due to polymyxin treatment

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Background: Inhaled polymyxins (PMs) appear more effective and safer than intravenous administration for the treatment of pulmonary infections caused by Gram-negative 'superbugs'. Multiple mechanisms (e.g. activation of death receptor and mitochondrial apoptotic pathways) were reported for PM-induced toxicities in human lung epithelial cells. This study aimed to examine the effect of polymyxins on signaling networks in human lung epithelial cells (A549).

Methods: *In vitro* toxicity of polymyxin B (PMB, 1.0 mM) in A549 (1.0 mM, 24h, n = 3) was assessed by flow cytometry. RNA was extracted with RNeasy for microarray analysis. Significance Analysis of Microarrays (SAM) was employed to identify the differentially expressed genes (DEGs, FDR-adjusted $P < 0.01$, fold change (FC) ≥ 2) between control and PMB-treated groups, and pathway analysis was conducted with Reactome. The hub genes related to PMB-induced toxicity were examined using SIGnaling Network Open Resource (SIGNOR).

Results: PMB (1.0 mM) induced 19.0±4.2% cell death at 24 h. Overall, 2,860 out of 21,755 genes were identified as DEGs after PMB treatment. *CD86* gene, encoding a potent co-stimulator of T and B lymphocyte function, was ranked as the top significant gene (FDR < 0.001, FC = 20.4, VIP score >10). Significantly upregulated genes were enriched in pathways including cell cycle (239), DNA repair (92), DNA replication (45) and gene expression (230), while downregulated genes were enriched in pathways including immune system (206), developmental biology (57) and haemostasis (56). Captured by network analysis, the top 5 hub genes (degree) due to PMB treatment were *CDK1* (52), *CDK2* (28), *MAPK3* (27), *PLK1* (20), and *CHEK1* (17).

Conclusion: Our transcriptomic study demonstrated that significant perturbations of cell cycle and immune response play a key role in PM toxicity in human lung epithelial cells. Further studies are warranted to reveal the detailed molecular interaction networks of PM-induced respiratory toxicity, thereby facilitating the optimisation of inhaled PM in patients.

Keywords: Polymyxin, lung delivery, toxicity, transcriptomics, signaling network

Expression of immunoinhibitory molecules PD-L1 and PD-L2 by dendritic cells

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The immunoinhibitory ligands PD-L1 and PD-L2 belong to the B7 family of co-stimulatory molecules. PD-L1 and PD-L2 are important checkpoint molecules that act to limit T cell immunity. Cancer cells use increased expression of PD-L1 and PD-L2 to suppress anti-tumour immunity. While PD-L1 is expressed by both hematopoietic and non-hematopoietic cells, PD-L2 expression is more restricted to antigen presenting cells including dendritic cells.

Here we have used the murine-derived dendritic cell line MuTu DC to study cell surface expression of PD-L1 and PD-L2 in resting and activated dendritic cells under different stimulatory conditions. Using a mouse model the results obtained in vitro were also confirmed in vivo. Expression patterns on cDC were further determined in the presence of tumour pre- and post- adoptive T cell transfer using a B cell lymphoma mouse model.

PD-L1 and PD-L2 show distinct expression on the surface of dendritic cells. First, basal levels of both ligands are low on resting dendritic cells, with PD-L1 exhibiting higher surface expression than PD-L2. Second, we tested changes in the expression of PD-L1 and PD-L2 following treatment of dendritic cells with stimuli including PMA/ionomycin, bacterial agonists (LPS, CpG) and inflammatory cytokines (IFNa, IFNg, IL-4, IL-2, IL-10). Expression of PD-L1 is significantly increased by all stimuli while in contrast, PD-L2 displays a more regulated pattern of expression. In settings of cancer we found that PD-L1 expression is increased upon adoptive T cell transfer while PD-L2 expression appears on a subset of cells once the tumour has reached a certain size independent of transferred T cells.

In summary, resting and activated dendritic cells display differential and regulated expression of PD-L1 and PD-L2. The mechanisms involved will be investigated. Knowledge of how PD-L1 and PD-L2 are expressed has important consequences for understanding how these immunoinhibitory molecules act to limit T cell immunity in settings of infection and/or tumours.

Plasmodium vivax invasion: structural scaffolds and human monoclonal antibodies

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Plasmodium vivax is the most widespread relapsing human malaria and preferentially invades young red blood cells called reticulocytes. *P. vivax* invasion is mediated by the host-pathogen interaction between human Transferrin receptor 1 (TfR1) and the parasite adhesin *P. vivax* reticulocyte binding protein 2b (PvRBP2b). Longitudinal cohort studies show that individuals naturally infected with *P. vivax* develop antibodies against PvRBP2b that are correlated with protection against clinical disease. To characterize the function of PvRBP2b naturally acquired human antibodies, we sequenced PvRBP2b specific memory B cells and recombinantly expressed monoclonal antibodies from two *P. vivax* infected Cambodian individuals. Several anti-PvRBP2b human antibodies blocked PvRBP2b binding to the surface of reticulocytes and inhibited complex formation with TfR1. Crystal structures of inhibitory human antibody fragments in complex with PvRBP2b revealed that inhibitory antibodies bind PvRBP2b to cause either steric hindrance with the reticulocyte membrane or by directly binding to the sites involved in both TfR1 or Tf interaction. By combining the fine mapping of inhibitory epitopes by crystallography with field polymorphism data from hundreds of *P. vivax* field isolates, we will examine the effect of polymorphisms on the binding of inhibitory antibodies. Our insights into naturally acquired blocking antibodies will inform the rational design of a next generation *P. vivax* malaria vaccine.

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The local microenvironment drives the identity of tissue-resident memory T cells

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Tissue-resident memory T (T_{RM}) cells are non-recirculating lymphocytes that provide local immune protection across multiple tissues. Identifying ways to boost or manipulate T_{RM} cell differentiation and their functions to protect from disease may reveal more targeted approaches for immunotherapy. However, this requires a better understanding of how the local tissue microenvironment can facilitate the generation of T_{RM} cells in an organ-specific manner. Here, we assessed the factors that contribute to the heterogeneity of T_{RM} cell identity and differentiation in diverse organs such as skin, liver, salivary glands and gut. We found that tissue-specific cytokines regulate the phenotypic and transcriptional profile of T_{RM} cells amongst various sites. We also show that T_{RM} cells differ in their developmental and survival requirements, which was shown to be dictated by their local microenvironment. Importantly, the transfer of pre-existing T_{RM} cells to new tissues demonstrated that these cells possess a degree of plasticity as their developmental requirements and phenotype were dynamically regulated by local signals. Collectively, we show that the tissue microenvironment actively influences the development, survival and phenotype of T_{RM} cells residing in diverse tissues.

Targeting Host Cell Death Pathways to Promote Clearance of *Leishmania donovani*

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Leishmaniasis, a disease caused by the *Leishmania spp.* parasite, affects 700 000 to 1 million people annually worldwide. *Leishmania spp* is found throughout Asia, Central and South America, Europe and Africa, and causes chronic, severe skin and mucocutaneous ulcers but most fatally – chronic infection of internal organs; spleen, liver and bone marrow. Specific *Leishmania* species*,* such as *Leishmania donovani,* cause this visceral infection, which if left untreated is fatal in over 95% of cases, causing 25000-65000 deaths globally each year.

Current treatments for visceral leishmaniasis have severe side effects and/or are expensive, and with the rise of drug resistance and no available vaccine, those at risk are often left without safe options. Therefore, safer therapeutics are needed and drugs that specifically target the host, rather than the pathogen itself, as they are less likely to result in drug resistance, are an attractive alternative.

Intracellular pathogens, such as *Leishmania spp.* manipulate host cell signaling pathways halting cell death to survive, replicate and trigger cell death to disseminate. If infected cells can be forced, via therapeutics, to undergo apoptosis, an immunologically silent and contained form of cell death, the infection may be able to be controlled. As these therapeutics are targeting the host pathways, rather than leishmania specific proteins, these therapeutics are less likely to have drug resistance arise.

The aim of this project was to use *L. donovani* as a model organism of visceral leishmaniasis in both an *in vivo* and *in vitro* system to explore cell death pathways in infection. The hypothesis was that targeting host cell death pathways is a valid therapeutic option to reduce parasite burden and ultimately, treat visceral leishmaniasis.

We used therapeutics targeting apoptotic pathways with mouse and primary cell infection systems of *L. donovani* and determined parasitic burden and cell death, through microscopy and flow cytometry.

Preliminary data evaluating apoptotic pathways as a potential therapeutic target will be presented.

The prevalence and risk factors for obstetric complications at childbirth and in the postpartum period for women in East New Britain, Papua New Guinea

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Background. Maternal morbidity and mortality rates in Papua New Guinea (PNG) are unacceptably high and have been slow to improve. The maternal mortality ratio (MMR) is estimated to be 500 deaths per 100,000 live births, primarily due to obstetric complications including haemorrhage, prolonged labour, sepsis and hypertensive illness (1-4). The PNG National Department of Health has identified maternal health as a current priority for health service intervention, however there is currently a large knowledge gap surrounding obstetric complications in PNG (4). There are a limited number of published studies that have explored the prevalence of obstetric complications experienced by pregnant women in PNG, and no studies from the East New Britain (ENB) province. There is also limited evidence surrounding the current management of obstetric complications in health clinics in PNG. Further research is therefore needed in order to address the government's maternal health targets. This study aims to investigate the prevalence of obstetric complications, the associated risk factors, and current clinical management of complications in a cohort of women from ENB, to inform future policy, clinical practice, and research.

Methods. The Healthy Mothers Healthy Babies program (led by the Burnet Institute) conducted a prospective observational cohort study of 699 pregnant women across five health facilities in ENB. Structured bio-behavioural interviews were conducted for each woman at first antenatal visit (n=699), childbirth (n=638) and one month postpartum (n=599).

Results. This analysis describes the prevalence and types of obstetric complications experienced by women during childbirth and the postpartum period. Obstetric and sociodemographic risk factors are reported separately for each complication, and the provision of treatments for complications is described.

Discussion. We provide important new evidence on the burden of obstetric complications experienced by women in PNG. Results will help inform policy, planning and further research on maternal health in ENB and other provinces.

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The physiological role of RNAse Zc3h12c in lymphoid tissues architecture and inflammatory responses.

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High levels of the pro-inflammatory cytokine tumour necrosis factor (TNF) have been associated with many diseases including rheumatoid arthritis (RA) and inflammatory bowel disease (IBD). Our lab is studying the post-transcriptional regulation of TNF expression. Recently, we have identified the RNase Zc3h12c as a potential repressor of Tnf mRNA expression.
To study the physiological role of Zc3h12c as well as its distribution *in vivo*, we have engineered a mutant allele in which the green fluorescent protein GFP replaces Zc3h12c. Our aim is to study the consequences of the loss of Zc3h12c on the inflammatory and immune responses.
Zc3h12c-deficient mice are found at the expected Mendelian frequency and look outwardly normal. In particular, they do not present any phenotype related to an excess of TNF expression (like cachexia or arthritis), even at an advanced age.
However, loss of Zc3h12c leads to an absence of structure in secondary lymphoid tissues, and hypertrophic lymph nodes with supernumerary B cells and stromal cells in ageing mice.
Flow-cytometry analysis of our GFP-reporter mouse showed that dendritic cells (DC) are the immune cell type expressing Zc3h12c the most. We found that Zc3h12c-deficient DC have an impaired immune response when stimulated in vitro.
To evaluate the potential role of TNF in this phenotype, we generated mice lacking both TNF and Zc3h12c.
Unexpectedly, 40% of TNF/12c double knock-out mice developed lethal systemic auto-inflammation including pancreatitis, myocarditis, otitis, myositis, pyelonephritis, anaemia, extramedullary haematopoiesis and bone-marrow failure.
These observations are raising new questions on the role of Tnf and Zc3h12c in the control of immune response and inflammation.

Viperin Enhances the dsDNA mediated Type-I Interferon Response to Clear HBV and HSV-1 Infections

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Viperin is one of the most prominent interferon-induced proteins and is critical for eliciting an effective immune response against many diverse viral pathogens. It is also a member of the radical SAM enzyme family, capable of enzymatically generating the anti-Flaviviridae ribonucleotide ddhCTP through the utilization of iron-sulphur complexes. However, this capacity fails to substantiate the entirety of viperin's anti-viral activity. In an effort to determine the molecular mechanism of viperin's highly effective, pan-antiviral activity, we assessed viperin's ability to enhance the innate immune dsDNA signalling pathway to limit DNA viral infection.

Our data demonstrates viperin's ability to significantly enhance the type-I interferon response following detection of aberrant viral dsDNA. This process involved the co-localisation of viperin, and dsDNA signalling proteins STING and TBK1, via direct binding to STING; inducing enhanced K63-linked ubiquitination of TBK1. The ability of viperin to enhance dsDNA induced interferon expression was reliant on its binding to the cytosolic iron-sulphur assembly protein CIA2A; a process independent of viperin's enzymatic generation of the antiviral ribonucleotide ddhCTP. Viperin's enhancement of this pathway resulted in a significant reduction in the infectivity of two DNA viruses, Hepatitis B virus (HBV) and Herpes simplexvirus-1 (HSV-1). In the presence of overexpressed viperin, HepG2 hepatocyte cells challenged with HBV displayed reduced levels of extracellular antigens (HBsAg and HBeAg), while HeLa cells challenged with HSV-1 displayed reduced infective viral particle release. These findings provide a mechanism of viperin's previously undefined anti-HBV and anti-HSV-1 activity.

We show here that viperin's presence in the signalosome which is formed following activation of the dsDNA signalling pathway, results in an enhanced antiviral state. Moreover, viperin's interaction with the alternate cytosolic iron-sulphur assembly protein CIA2A, may represent a novel regulatory mechanism of viperin activity; switching between the innate immune regulation and the enzymatic generation of the antiviral ribonucleotide ddhCTP. This data further defines the molecular mechanism of viperin's highly effective, pan-antiviral activity, providing the foundations for the development of new antiviral therapeutics.

Exploring environmental health practitioners' perceptions of barriers and enablers to preventing cryptosporidiosis in aquatic facilities

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Cryptosporidiosis is the third most commonly reported gastrointestinal disease in Australia. Medical costs and lost productivity associated with gastrointestinal illness is estimated to cost the Australian economy over $342 million per annum including over 1 million visits to the general practitioner and over 1 million work days lost. *Cryptosporidium* oocysts are highly resistant to chlorine and outbreaks of cryptosporidiosis are commonly linked to aquatic facilities including swimming pools. Public health regulation of swimming pools has been highlighted as one important component of cryptosporidiosis prevention. In Victoria, local government environmental health practitioners (EHPs) enforce these regulations and liaise with pool operators and pool users when investigating sporadic cases and outbreaks of cryptosporidiosis linked to swimming pools. By exploring the experiences of EHPs who undertake these duties, this study aimed to identify barriers and enablers to preventing cryptosporidiosis in aquatic facilities as perceived by EHPs. A focus group discussion with a purposive sample of seven EHPs was conducted and guided by a semi-structured interview schedule using open-ended questions. Thematic analysis identified five key barriers and two key enablers to cryptosporidiosis prevention which were related to pool water testing methods, resources and training for EHPs, knowledge and behavior of aquatic facility operators and swimming pool users, regulation and aquatic facility design. One main finding from this study was that having no legislative requirement for aquatic facilities to be registered with their local council was a perceived barrier to preventing cryptosporidiosis. It was perceived that having no requirement made it difficult for EHPs to identify when and where new facilities were operating within the municipality, therefore making it difficult to monitor compliance at these facilities. Further research is needed to investigate the perceived barriers and enablers identified in this study and to ascertain the potential impact of these factors on cryptosporidiosis occurrence in aquatic facilities.

Host mobilisation of zinc following *Streptococcus pneumoniae* infection

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Zinc deficiency is estimated to affect approximately one third of the global population. Zinc is critical for optimal host defence against infection, and consequently zinc deficiency is associated with increased morbidity and mortality for infectious diseases. *Streptococcus pneumoniae* (the pneumococcus) is a host-adapted pathogen and the most common cause of bacterial pneumonia in children under the age of 5 years. Bacterial pneumoniae is highly prevalent in regions that also have endemic zinc deficiency. In this study we sought to elucidate how dietary zinc deficiency could compromise host resistance to pneumococcal disease. We investigated how dietary zinc restriction in a murine model of *S. pneumoniae* infection impacted tissue zinc concentrations and infection kinetics. Here, we generated fluorescently labelled strains of *S. pneumoniae* for use in combination with elemental bio-imaging, a novel application of laser ablation-inductively coupled plasma-mass spectrometry. By combining fluorescence microscopy with elemental bio-imaging we reveal the spatial redistribution of zinc and show that it co-localised with the invading bacteria. Further, regions that were not enriched for zinc were devoid of bacteria. Taken together, these data show that the host mobilises zinc to sites of pneumococcal infection in lungs. Collectively, this approach shows how changes in the chemistry of the host environment changes can be mapped and will aid in elucidating how dietary zinc contributes to resistance against bacterial infection.

Novel fluorescent TNF reporter mouse strains 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. Here we present the design of a fluorescent TNF translational reporter strain where knock-in of the fluorescent protein tdTomato within the TNF locus will allow for accurate representation of TNF gene expression while preserving TNF function.

Cellular accumulation of stable fluorescent protein prevents real-time observation of target gene expression and is a limitation in many fluorescent reporter models. To address this limitation, we have also designed a transgenic HaloTag-tdTomato TNF translational reporter strain. HaloTag knock-in will allow the use of proteolysis targeting chimera compounds (PROTACs) for targeted degradation of the Halo-tdTomato fusion protein, thus enabling temporal correlation between reporter protein and TNF expression. This HaloTag-tdTomato reporter strain will also allow the application of fluorescent HaloTag ligands, where synthetic dyes yield brighter fluorescence than fluorescent proteins, further aiding visualisation of TNF expression *in vivo*. These TNF translational reporter strains will permit the visualisation and observation of TNF-producing cells by a variety of approaches including intravital and live imaging. Importantly, our reporter strains will constitute novel models for the *in vivo* characterisation of TNF expression in physiological settings as well as in disease states.

Distinct immune responses elicited from cervicovaginal epithelial cells by lactic acid and short chain fatty acids associated with optimal and non-optimal vaginal microbiota

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Non-optimal vaginal microbiota (VMB), such as bacterial vaginosis (BV), is typically characterised by a depletion of beneficial lactobacilli and the presence of abundant anaerobes and is associated with subclinical cervicovaginal inflammation and increased risk of HIV infection compared to women colonised with optimal VMB dominated by *Lactobacillus* spp. Lactic acid (LA) is a major organic acid metabolite produced by vaginal *Lactobacilli* spp. that elicits anti-inflammatory effects from cervicovaginal epithelial cells and is dramatically depleted during BV. However, it is unclear if LA retains its immunomodulatory effects in the presence of the VMB metabolites comprising short chain fatty acids (SCFAs) and succinic acid at concentrations present during eubiosis. In addition, the immunomodulatory effect of SCFAs and succinic acid on cervicovaginal epithelial cells at concentrations present during BV is unknown. Here we report that VMB metabolite mixtures present during BV elicit immunomodulatory effects on cervicovaginal epithelial cells during prolonged and sustained treatments. Results were cell-type specific, and pro-inflammatory effects were evidenced by increased levels of tumour necrosis factor-α (TNFα) and the enhancement of polyinosinic:polycytidylic acid (PIC) and Pam3CSK4 (Pam) stimulated production of TNFα and IL-8, attributed mainly to acetic acid. Certain SCFAs also dampen basal RANTES and IP-10 production, as well as TLR induced IL-6, RANTES and IP-10 production in a cell-type specific manner. In contrast, the anti-inflammatory and pro-inflammatory inhibitory effects of LA were maintained in the presence of SCFAs and succinic acid at pH<4.5 at physiologically relevant concentrations present in women with lactobacillus-dominated microbiota. These findings indicate that elevated levels of SCFAs are a potential source of cervicovaginal inflammation in women experiencing BV, and support the unique anti-inflammatory properties of the VMB metabolite LA on cervicovaginal epithelial cells as well as a role for LA or LA-producing lactobacilli to reverse genital inflammation associated with increased HIV risk.

Full genome sequencing and phylogenetic analysis of Australian Canine/feline –like G3P[3], G3P[8] and G3P[9] rotaviruses from children

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Rotavirus is a pathogenic virus that causes acute gastroenteritis in humans and is a leading cause of diarrhea related mortality in children under five years of age. Rotavirus infects both animals and humans. The zoonotic transmission of rotaviruses can cause the introduction of novel strains into the human population. Hence, it is important to monitor and understand these strains as they have the potential to emerge into human populations.

Canine/feline –like G3P[3], G3P[8] and G3P[9] rotaviruses were detected as part of the Australian Rotavirus Surveillance program. These strains were isolated in Australia from 2013-2017 from pediatric patients with acute gastroenteritis.
The aim of this project was to conduct full genome sequencing and phylogenetic analysis to investigate the genetic diversity and origins of the Australian canine/feline-associated G3P[3], G3P[8] and G3P[9] rotaviruses isolated from human children.

Illumina sequencing of the full genome of the G3P[3] (n=3), G3P[8] (n=3) and G3P[9] (n=9) rotaviruses was performed. The G3P[3] and G3P[8] strains had the genome constellations: G3-P[3]-I3-R3-C3-M3-A9-N2-T3-E3-H6 and G3-P[8]-I3-R3-C3-M3-A2-N2-T3-E3-H6. The majority of the G3P[9] strains (n=7) had the genomic constellation: G3-P[9]-I3-R3-C3-M3-A3-N3-T3-E3-H6. Two G3P[9] strains had the constellations:
G3-P[9]-I3-R3-C3-M3-A3-N3-T1-E3-H6 and G3-P[9]-I2-R2-C2-M2-A3-N2-T3-E3-H3.

Maximum-Likelihood RAxML trees were generated for each Australian G3P[3], G3P[8] and G3P[9] gene using global datasets of gene sequences compiled from NCBI Virus Variation Resource.

Full genome sequencing and phylogenetic analysis allows for the detection of reassortment events which can occur during zoonotic transmission. The Australian G3P[3], G3P[8] and G3P[9] strains shared a high degree of genetic similarity to various human and animal strains suggesting these strains were derived via multiple reassortment events.

Genetic and Phenotypic Characteristics of Hyper-virulent Community-acquired *Acinetobacter baumannii*

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Acinetobacter baumannii is a highly troublesome nosocomial pathogen worldwide. In regions with hot and humid climates it is also the cause of a distinct and severe community-acquired infection, with mortality rates reported to be as high as 64%. Little is known about community-acquired *A. baumannii* (CA-Ab) infections, but given that diabetes mellitus, chronic lung conditions, smoking and alcoholism are the predominant risk factors, it has been suggested that disease severity is due to host immune deficiencies. However, we hypothesised that bacterial factors unique to CA-Ab strains were also contributing to the severe disease presentation.

In a pneumonia model using immunocompetent mice, infection with CA-Ab strains resulted in 90% mortality within 16 hours, whilst all mice infected with a geographically paired hospital-acquired strain survived up to seven days. Genomic comparisons of two CA-Ab strains with dominant hospital clones revealed only twelve genes shared by the CA-Ab strains that were absent from the hospital strains. Whether these genes play a role in virulence is under investigation. The transcriptome of a CA-Ab strain (CA-17) and hospital strain (HA-17) was determined during an *in vivo* bacteremia infection and the expression of shared genes compared. In total 217 genes (fold change ≥2, false discovery rate < 0.01) were found to be differentially expressed, including those involved in biofilm formation, cellular adherence, bacterial competition and iron acquisition. Using *in vitro* assays we confirmed that CA-17 had an increased ability to form biofilms and could grow in low iron conditions compared to HA-17.

Combined our results indicate that bacterial factors, unique to CA-Ab at least partially contribute to the severe disease presentation seen in the community.

Different *Wolbachia* strains maintain diverse pathogen blocking phenotypes in an inbred laboratory *Ae. aegypti* colony across different dengue serotypes.

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A range of human pathogens, including Zika, dengue and chikungunya are transmitted by the *Aedes aegypti* mosquito. Transinfection with *Wolbachia*, an endosymbiont present in 60% of insect species, disrupts the replication of these pathogens, significantly reducing the capacity of the mosquito to transmit disease[1]. *Ae. aegypti* infected with the *w*Mel strain of *Wolbachia* have been released as a vector control method, and many other strains have since been developed for characterisation and comparison [2, 3]. It has been shown that these different *Wolbachia* strains possess diverse fitness and vector competence phenotypes, but often on different genetic backgrounds, and testing only a single virus serotype.

We have taken a broad panel of *Wolbachia* strains - *w*Mel, *w*MelPop, *w*MelCs, *w*Ri, *w*Alb, *w*Pip, and *w*Mel*w*AlbB (superinfected line) - previously transinfected into *Ae. aegypti,* and backcrossed them onto an inbred laboratory line to remove variation in host background. To assess the vector competence of this panel, mosquitoes were challenged with DENV-1 and DENV-2 via intrathoracic injection and viral copies were determined by qRT-PCR 7-days post infection. We found that the rank order in the strength of viral blocking is consistent with earlier studies, and furthermore that this ranking is conserved across two DENV serotypes. In addition, we confirmed that despite being present in the mosquito in high density, the *w*Pip strain does not impact DENV load. This is an unexpected result given that previous data show that blocking strength appears to broadly correlate with strain density. Future work will examine this further, as well as confirm whether these phenotypes hold rank as consistently when challenged with other viral families.

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Predominantly antibody-deficient patients with non-infectious complications have reduced naive B cells, Treg, Th17 and Tfh17

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Background: Patients with predominantly antibody deficiency (PAD) suffer from severe and recurrent infections that require lifelong immunoglobulin replacement and prophylactic antibiotic treatment. Disease incidence is estimated to be 1:25,000 worldwide, and up to 68% of patients develop non-infectious complications (NIC) including autoimmunity, which are difficult to treat causing high morbidity and early mortality. Currently, the etiology of NIC is unknown and there are no diagnostic and prognostic markers to identify patients at risk.

Objectives: To identify immune cell markers that associate with NIC in PAD patients.

Methods: We developed a standardized 11-color flowcytometry panel that was utilized for in-depth analysis of B- and T-cells in 61 adult PAD patients and 59 age-matched controls.

Results: Nine males had mutations in *BTK* and were defined as having X-linked agammaglobulinemia. The remaining 52 patients were not genetically defined and were clinically diagnosed with agammaglobulinemia (n=1), CVID (31), hypogammaglobulinemia (13), IgG subclass deficiency (1) and specific antibody deficiency (6). Of the 52, 29 (56%) had one or more NIC, 35 patients had reduced B-cell numbers and 25 reduced T-cell numbers. Both PAD-NIC and PAD+NIC groups had significantly reduced Ig class-switched memory B cells, and naive CD4 and CD8 T-cell numbers. Naive and IgM^+ memory B cells, Treg, Th17, and Tfh17 cells were specifically reduced in the PAD+NIC group. $CD21^{lo}$ B-cells and Tfh cells were increased in frequencies, but not in absolute numbers in PAD+NIC.

Conclusion: The previously reported increased frequencies of $CD21^{lo}$ B cells and Tfh cells are the indirect result of reduced naive B-cell and T-cell numbers. Hence, correct interpretation of immunophenotyping of immunodeficiencies is critically dependent on absolute cell counts. Finally, the defects in naive B- and T-cell numbers suggest a mild combined immunodeficiency in PAD patients with NIC. Together with the reductions in Th17, Treg and Tfh17 numbers, these key differences could be utilized as biomarkers to support definitive diagnosis and to predict for disease progression.

Womens' knowledge of newborn danger signs during pregnancy, childbirth and the postpartum period: findings from a cohort study in rural Papua New Guinea.

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Background: Globally, over 2.7 million babies die in the first 28 days of life[1], and most of these deaths occur in low- and middle-income countries, such as Papua New Guinea (PNG)[2]. Mothers play a crucial role in early recognition of newborn danger signs and care seeking to receive timely management to prevent newborn deaths. However, there is little known about women's knowledge of newborn danger signs during pregnancy, childbirth and postpartum periods in PNG, which this project aims to examine, among one cohort in East New Britain (ENB), PNG.

Methods: Healthy Mothers, Healthy Babies (HMHB) is a collaborative research program, led by the Burnet Institute in partnership with the ENB Provincial Government, and the PNG National Institute of Medical Research. HMHB cohort study tracked 700 mothers in rural PNG, and collected data through bio-behavioural interviewed, at first antenatal care visit (n=700), delivery (n=638) and one month after delivery (n=599).

Result: The results will characterise patterns of mother's knowledge of danger signs related to their newborn. Descriptive analysis methods will examine the type and frequency of newborn danger signs reported by women and possible correlations with their socio-demographic characteristics and obstetric history.

Discussion: The findings will be of immediate usefulness to future health development programs targeting the newborn period, and more broadly to policymakers in PNG. Results will be relevant to current health policies for maternal and child health and impact on current clinical education practices, contextualised to local realities and expectations within the ENB health system.

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The *Coxiella burnetii* effector protein MceB targets the host cell mitochondrion during infection

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Mitochondria are essential organelles, fundamental to eukaryotic cell function and survival. Perhaps best known for their role in energy production, mitochondria are also central to many cellular processes, including calcium homeostasis, lipid metabolism, heme biosynthesis, immune and cell death signaling. With such diverse cellular roles, it is no surprise that virulence factors of both bacterial and viral origin target mitochondria during infection.

Coxiella burnetii is a unique intracellular bacterial pathogen and the causative agent of Q fever. The bacterium infects alveolar macrophages and replicates within a highly acidic, lysosome-like vacuole, termed the *Coxiella*-containing vacuole (CCV). During infection, *C. burnetii* translocates over 130 bacterial effector proteins into the host cytosol via a Type 4 Secretion System (T4SS). Effector proteins translocated into the cell modulate cellular functions to facilitate CCV development and bacterial replication.

We previously identified mitochondria as a *bona fide* target of *C. burnetii* effector proteins during infection (*1*). To discover additional, novel effector proteins targeted to this organelle we purified mitochondria from infected macrophages and performed mass spectrometry. This identified a subset of *C. burnetii* proteins enriched alongside the mitochondria during infection. These proteins have not previously been shown to localise to the organelle and thus represent novel, mitochondrial targeted effector proteins. Of this subset, we investigated MceB (Mitochondria *Coxiella* effector B). Using immunofluorescence we were able to demonstrate co-localisation of this effector protein with the mitochondrial network and that this targeting relies on information within the N-terminus of the protein. Furthermore, MceB is imported into an internal mitochondrial sub-compartment and forms a complex of ~500 kDa. The precise protein interacting partners of MceB and how this aids in bacterial survival and pathogenesis in the host cell is our current focus.

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Cigarette smoke-induced depletion of alveolar macrophages contributes to delayed clearance of *L. pneumophila*

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The intracellular bacterial pathogen *Legionella pneumophila* is the most common causative agent of Legionnaires' Disease (LD), a severe form of acute pneumonia. Cigarette smoke has been associated with a variety of detrimental effects on immune responses in the lung and has been strongly linked to increased susceptibility to LD caused by *L. pneumophila*. Here, we aimed to identify how cigarette smoking renders individuals more susceptible to infection.

We characterized a mouse model of concurrent acute cigarette smoke exposure and *L. pneumophila* infection. Cigarette smoke-exposed mice developed more severe disease when infected with *L. pneumophila*, and displayed increased pulmonary bacterial loads. Interestingly, quantification of lung immune cells using flow cytometry revealed that smoke exposure strongly depleted alveolar macrophages (AM). Since AM are the main replicative niche of *Legionella*, we expected their depletion would ameliorate disease. To investigate this further, we specifically depleted AM in mice by treatment with clodronate liposomes prior to infection. Similar to the cigarette smoke model, we observed delayed disease recovery and bacterial clearance in AM-depleted mice.

We hypothesize that acute cigarette smoke exposure causes more severe LD pathogenesis by depleting AM, which are clearly important contributors to *L. pneumophila* clearance. We are currently performing non-invasive whole-organ imaging using light-sheet microscopy to confirm overall AM depletion in mouse lungs as a result of smoke exposure and intend to identify mechanisms by which AM usually drive *L. pneumophila* clearance.

Organ-specific expression of fatty acid binding proteins in tissue-resident lymphocytes

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Tissue-resident memory T (T_{RM}) cells exist throughout the body where they are poised to mediate local immune responses[1]. Although studies have defined a common mechanism of residency independent of location, there is likely to be a specialization associated with a given tissue. It has been shown that skin T_{RM} cells rely on the uptake of exogenous fatty acids for their survival and consequently, upregulate fatty acid binding protein (FABP) 4 and FABP5 as part of their transcriptional program[2]. However, FABP exist as a number of isoforms that extend beyond FABP4 and 5, with varying usage in different tissues[3]. Given this, we examined whether environmental factors impact the range of FABP expression for T_{RM} cells in different locations around the body. We found this to be the case, with T_{RM} cells showing tissue-specific patterns of FABP usage. Such FABP profiles were mirrored by other tissue-resident leukocytes, arguing for common environmental selection of isoform expression. Moreover, these profiles were malleable since T_{RM} cells relocated to different organs switched their pattern of FABP expression in line with their new location. As a consequence, these results argue for a general residency program for T_{RM} cells that includes the induction of FABP expression, with a tissue-specific overlay that tailors isoform expression to suit the particular tissue of residency.

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Targeting Mind Bomb-2 and MIP-1, two New Regulators of Tumor cell Survival during TRAIL Signaling

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There are only few agents that are truly cancer cell-specific. Tumour necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) is a potent death inducing cytokine and a rare example of such molecules that kill transformed cells but spare normal ones[1,2]. Activating the "extrinsic" apoptotic pathway, selectively in cancer cells, both with the natural ligand (TRAIL) or agonistic antibodies (Mapatumumab and Conatumumab), has therefore been a goal of cancer researchers and pharmaceutical companies for many years[3]. TRAIL receptor agonists, however, have performed poorly in the clinic due to major gaps in our understanding of what drives resistance to these potent death inducers[4].

We have identified two new molecules, a RING-type ubiquitin E3 ligase Mind Bomb-2 (MIB2) and MIB2 interacting protein 1 (MIP-1) that inhibit death receptor (DR) signalling and promote tumour development. Here, for the first time we identify MIP-1 as a novel MIB2 interacting protein and demonstrate that the removal of either MIB2 or MIP-1 significantly reduces tumour development in models for colorectal cancer and is sufficient to sensitize tumour cells to TRAIL induced cell death.

We also show that MIB2 can ubiquitylate RIPK1 and MIP-1. While it has been demonstrated that MIB2 ubiquitylates RIPK1 to limit TNF-induced cell death[5], the biological requirement for the ubiquitylation of MIP-1 is still unclear, and it remains to be determined which ubiquitin linkage-type is attached. Further studies will also be conducted to confirm whether MIB2 can modulate the activities of RIPK1 or MIP-1 in TRAIL signalling.

Furthermore, we have assessed the therapeutic potential of targeting these molecules for cancer treatment in combination with TRAIL. We provide proof of principle evidence that targeting these molecules can have clinical utility. Using Proteolysis targeting chimera (PROTAC) small molecule approach we show that MIP-1 and MIB2 can be degraded using available PROTAC drugs.

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Characterisation of the *Haemophilus influenzae* PsaA ortholog, HIPsaA

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Haemophilus influenzae is a host-adapted pathogen that colonizes the human nasopharynx that can mediate diseases of the upper and lower respiratory tract . *H. influenzae* has an array of molecular mechanisms that permit growth and virulence in diverse host niches. Notably, *H. influenzae* can respond to exogenous, host-mediated and endogenous, i.e. metabolically produced, reactive oxygen and nitrogen stresses. Defence against these chemical insults employs enzyme-mediated detoxification processes, such as the molybdenum-dependent HITorZ. Transition metal ions serve crucial roles in bacterial growth, survival and stress response, but the majority of these mechanisms in *H. influenzae* remain to be determined. Bioinformatic analyses of *H. influenzae* 2019 revealed that it encoded two ATP-binding cassette (ABC) transporter solute binding proteins (SBPs) that belonged to the cluster A-I subgroup. Primary sequence analyses suggested that these were orthologs of a manganese-specific (locus tag: C645_00940) and zinc-specific (locus tag: C645_02340) SBPs. Given the central role of manganese in metabolism and resistance to oxidative stress we investigated the biochemical and biophysical properties of C645_00940. We combined recombinant protein purification with *in vitro* metal binding assays to show that C645_00940 was a manganese-binding SBP. Building on this finding, the gene was renamed as HIPsaA due to the functional and structural similarity to *Streptococcus pneumoniae* PsaA. Collectively, this work provides insight into manganese acquisition in *H. influenzae* and the contribution of this transition metal ion to bacterial virulence.

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Understanding the effect of naturally occurring human MLKL polymorphisms on necroptosis and disease

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Mixed Lineage Kinase Domain-like protein (MLKL) is the terminal effector protein in the inflammatory form of programmed cell death called 'necroptosis'. The necroptotic pathway plays an important role in the progression of a variety of disease types; in various animal models, it was shown that inhibiting key necroptotic proteins (RIPK1/RIPK3) was efficacious in reducing the severity of disease. Previous studies completed at WEHI uncovered that constitutive activity was conferred to the mouse MLKL protein as a result of a single amino acid substitution, D139V, in the first brace helix of MLKL. Homozygous mice expressing this constitutively active MLKL protein exhibit lethality and present with a range of inflammatory phenotypes. In the human population 3 in 50 people are predicted to be heterozygous for two naturally occurring first brace helix missense polymorphisms $MLKL^{S132P}$ and $MLKL^{R146Q}$. These high frequency polymorphisms fall in close proximity to the human equivalent of the lethal $Mlkl^{D139V}$ mouse variant and map to an interface between the brace and pseudokinase domain of adjacent MLKL monomers. Preliminary analysis of Chronic Recurrent Multifocal Osteomyelitis (CRMO) cohorts indicates a 10-12-fold enrichment in the occurrence of brace helix variant compound heterozygotes relative to healthy controls. We examined a CRISPR-cas9 generated mouse model containing the mouse equivalent brace helix mutation $Mlkl^{S131P}$ that when expressed in vitro exhibits a constitutively active phenotype. $Mlkl^{S131P/S131P}$ homozygotes present with deficits in circulating blood cell numbers and key serological features of inflammatory disease.

Discovering the mechanism behind Ceftazidime-avibactam killing of multidrug resistant K. pneumonia: an untargeted metabolomics study

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Introduction: The rapid emergence of multidrug resistant (MDR) pathogens stemming from the misuse of antibiotics has caused an unprecedented death toll that is expected to increase with few novel antimicrobials in the pipeline. Since being approved as a combination for treatment of MDR bacteria in 2015, ceftazidime (CAZ)-avibactam(AVI) (AVYCAZ) proved efficacious against a range of such Gram-negative pathogens including *A. baumannii*, *K. Pneumoniae* and some *P. aeruginosa* strains. The compounds individually have well characterised mechanisms of action (MOA), thus proposing a highly probable synergy for the combination. Individually, each compound has minimum inhibitory concentrations (MICs) of 128mg/L (CAZ) and >128mg/L (AVI). When used together in a ratio of 4 (CAZ): 1 (AVI), the MIC is reduced to 8mg/L.

Method: Static timekill assays using sub-MIC drug concentrations (2.5mg/L) were conducted and optimised against *K. pneumoniae* strain KPMA100-09. Samples were taken 1h, 3h, 6h and 24h after adding the compounds, purified, and the metabolites were extracted. The killing effect of the drug combination was examined at each interval (Figure 1). Untargeted metabolomics was used to study the effects of the drug combination on the complex biochemical pathway of KPMA100-09. The metabolites were quantified using LC-MS and raw data from the LC-MS was converted to metabolites using IDEOM. The significance of each metabolite in the KPMA100-09 pathways was evaluated against metabolomics databases.

Results: Analysis at 1h, 3h, 6h and 24h time points showed a time-dependent killing of the bacteria, with maximal killing at 3 hours (**Figure 1**). The MOA was shown to be from the disruption of lipopolysaccharide biosynthesis pathways, with statistically significant changes in key metabolites required for peptidoglycan synthesis and breakdown.

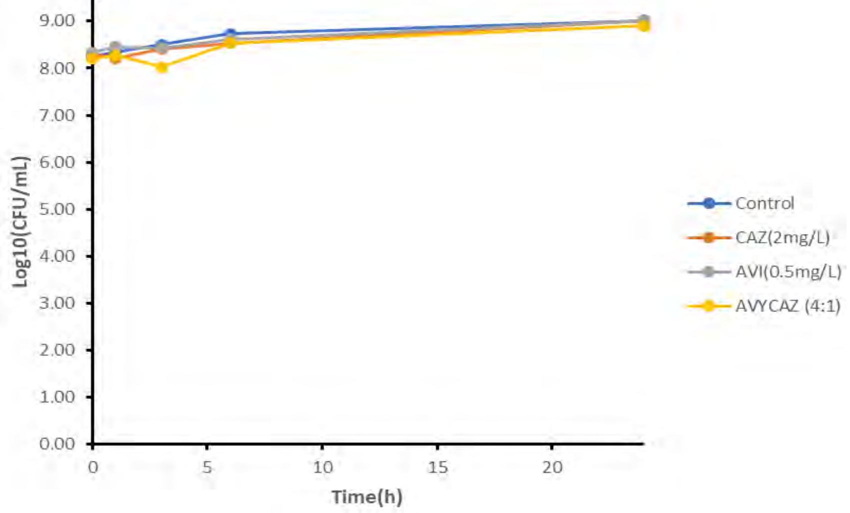

Figure 1. Time-kill curve of ceftazidime-avibactam against KPMA100-09

Conclusion: Understanding the pathways being affected by such therapeutic intervention will also be helpful in predicting the emergence of further resistance down the line.

Genomic Analysis and Exploration of Putative Drug Resistance Loci in Malaria Parasites

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Background:

Malaria is a life-threating disease caused by *Plasmodium*. The parasite has developed resistance to all known antimalarials to date, diminishing the efficacy of malaria control. Molecular markers are known to confer resistance to many antimalarials. This emphasized the need for identifying more resistant markers. Whole Genome Sequencing can be employed to underpin regions in genome which are under high drug selective pressure, notably by looking at the single nucleotide polymorphism (SNPs) and/or copy number variants (CNVs). Here, we re-analysed data from a recently published study[1] in which Cowell *el al* which identified to have plausible drug resistance inducing variants, which were either SNPs or CNVs.

Methods:

The druggable genome dataset contains 237 *P. falciparum* strains, resistant to 31 diverse compounds (24 sensitive and 213 resistant parasites) derived from three background strains: 3D7 (168), 7G8(5) and Dd2(64). As there is a considerable difference between the genomes of the three background strains, reads are aligned to the corresponding reference genome, compared to only the single reference in Cowell *el al*. We used different pipelines to call SNPs and CNVs and compared our results to the Cowell *el al* results. Variants were then compared between isogenic parent and offspring compound-resistant clones to explore mutations associated with drug resistance.

Results:

We identified 495 SNPs (305 genes) from the 3D7 samples, 220 SNPs (146 genes) from the Dd2 samples, and 9 SNPs (7 genes) from the 7G8 samples. Of these we observed 18 SNPs in known drug resistance loci (*pfcytb* (7), *pfcrt* (4), *pfmdr* (5) and *pfmrp* (2)), however, the remaining 706 SNPs have unidentified roles with respect to drug resistance, emphasizing the relevance of further experimental validation. Additionally, we were unable to identify any SNPs in 11 resistant strains (all 3D7 derived), suggesting that the drug resistance of these samples may be related to other types of mutations, such as CNVs, which we will pursue further.

1. Cowell, A.N., et al., Mapping the malaria parasite druggable genome by using *in vitro* evolution and chemogenomics. Science, 2018. 359(6372): p. 191-199.

Detection and immunophenotyping of antigen-specific memory B cells to measure influenza vaccination responses

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Background: A protective vaccination response is typically measured through serum IgG quantification. However, many immunocompromised patients receive immunoglobulin replacement therapy (IgRT) that prevents accurate assessment of subject's own immune response. As immune cells are not donated, antigen-specific cellular measurements would represent the subject's own immune response.
Objective: To examine the numbers and immunophenotype of antigen (Ag)- specific memory B cells following influenza vaccination.
Methods: Recombinant haemagglutinin (HA) antigen tetramers were generated from type A/Michigan and B/Phuket influenza strains that are included in the 2019 influenza vaccine. Following generation in the Expi293 expression system, antigens were tetramerised with fluorescently-labelled streptavidins. 16 healthy controls were recruited and blood samples were taken pre- and 4 weeks post-influenza booster vaccination to measure serum IgG to recombinant HA antigens and to immunophenotype memory B cells with antigen-tetramers with 16-colour flow cytometry.
Results: HA antigens from A/Michigan and B/Phuket were successfully purified, biotinylated and tetramerised. The IgG levels to both antigens were significantly increased post booster vaccination in our cohort of 16 controls. The 16-colour flow cytometry panel has been optimised with ~0.06% of B-cells specific for the HA of A/Michigan. We have identified a significant increase in total number of B cells specific for the HA of A/Michigan post-vaccination. Flow cytometry experiments are ongoing on the 16 healthy controls to dissect the immunophenotype, Ig isotype and IgG subclass distribution pre- and post-vaccination.
Conclusion: We have successfully identified influenza HA-specific memory B cells in healthy controls. Changes in cell numbers and immunophenotype following booster vaccination will provide a reference for immunocompetent responses. Subsequent studies in patients with predominantly antibody deficiency (PAD) will demonstrate the deficiencies in specific B-cell memory. Ultimately, analysis of antigen-specific memory B cells could provide a new means to assess immunocompetence in patients undergoing IgRT.

Bioinformatic analysis of eosinophil peroxidase and its implications for model and target species

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Gastrointestinal nematode (GIN) infections dominate sheep and goat populations worldwide, and in Australia can cost sheep producers up to $500 million per year in lost productivity. The typical immune response to GIN infections is a dominant Th2 immune response, and research using experimentally, and naturally infected sheep have indicated that eosinophils may play an important role in resistance to infection. The aim of this study was to determine whether differences in resistance and susceptibility to infection may be due to dysfunctional eosinophil proteins.

We performed a bioinformatic analysis on the most abundant cationic protein within the eosinophil, eosinophil peroxidase (EPO), which is also unique to eosinophils. Briefly, the genomic sequence for human EPO was used as a reference to BLAST the cow, sheep and goat genomes for matching sequences. Sequences were translated to protein, aligned to the reference and annotated with a tryptic peptide fragment and nitrated tyrosine.

The sheep sequence contained 1 single nucleotide polymorphism (SNP), while the goat sequence contained 2 SNPs, one of which was in the same location as in the sheep sequence. The best alignment to the reference and cow sequences was achieved when these SNPs were removed from the sheep and goat sequences. The tryptic peptide fragment for all ruminants was conserved from Met^{501} to Asn^{514}, except for one substitution (Arg^{503} to His^{503}). Additionally, the nitrated tyrosine was not conserved in the goat sequence and was substituted for a cysteine.

The nitrated tyrosine is important for EPO-mediated activities, including the post-translational nitration of eosinophil secondary granule proteins, which in turn, may influence inflammatory responses. The importance of a nitrated tyrosine in GIN infections of sheep has not yet been established, however the absence of this residue may be responsible for the relative susceptibility of goats to GIN infection. Functional studies are required to determine whether the SNPs identified in the sheep and goat sequences may be sequencing error rather than true SNPs, as well as to confirm the importance of the nitrated tyrosine in resistance and susceptibility to GIN infection.

Production and immunogenicity testing of high-stability soluble HIV-1 glycoprotein vaccine candidates based on a transmitted-founder isolate

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PUBLISH CONSENT WITHHELD

Bacterial membrane vesicles from the human microbiome contain antibiotic resistance genes.

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Antibiotic resistance is one of the biggest threats to global health, food security and development. The acquisition of antimicrobial resistance (AMR) genes contributes to the spread of antibiotic resistance and the rise in superbugs. Although there are multiple known mechanisms of horizontal gene transfer (HGT) used by bacteria to transfer AMR, an unexplored area of HGT involves bacterial membrane vesicles as secretory DNA transport vehicles. This study focuses on examining the potential of bacterial vesicles to contribute to HGT between bacteria.

All bacteria release extracellular vesicles enriched in DNA as part of their normal growth, known as outer membrane vesicles (OMVs) from Gram-negative bacteria and membrane vesicles (MVs) from Gram-positive bacteria. Our recent findings show that OMVs produced by *Pseudomonas aeruginosa* and *Helicobacter pylori* contain DNA which can encode for AMR. DNA contained within OMVs was resistant to degradation by DNases and could be transferred to recipient antibiotic-sensitive bacteria. Although other studies have also demonstrated OMV-mediated DNA transfer between single bacterial species, the transfer of AMR genes in mixed cultures such as the human gut microbiome, has not been examined.

The human gut microbiome has recently been demonstrated as a reservoir for AMR and it is thought that commensal organisms of the gut can transfer AMR genes to pathogenic or opportunistic organisms. As OMVs and MVs have already been demonstrated to contribute to dissemination of AMR genes between single bacterial species, we hypothesise that OMVs and MVs will contribute to DNA transfer in a mixed microbiome culture.

To investigate if OMVs and MVs contribute to the spread of AMR in the human microbiome, we isolated OMVs and MVs from mixed microbial cultures which are representative of the human gut. We demonstrated that after DNase treatment, these OMVs and MVs contained DNA. This DNA will be sequenced using Next Generation sequencing techniques to determine whether they contain AMR genes, which genes are most prevalent and which bacteria are releasing vesicles that contain AMR genes. Collectively, this study will advance our limited knowledge regarding the contribution of OMVs and MVs in HGT in a physiological setting such as the human microbiota.

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

Thorey Jonsdottir[1, 2*], Smitha Sudhakar[3], Ben Dickerman[1], Betty Kouskousis[1], Hayley Bullen[1], Natalie Counihan[3], Tania De Koning-Ward[3], Brendan Crabb[1, 2] and Paul Gilson[1]

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Malaria remains a major global health problem claiming the life of roughly 400 000 people every year. *Plasmodium falciparum*, responsible for the most critical form of malaria, has developed resistance to all anti-malarial drugs available indicating the urgency to identify new drugs and drug targets. The asexual blood stage of *P. falciparum* leads to the clinical symptoms of malaria and therefore attractive for therapeutic strategies. In this stage the parasite invades the red blood cell (RBC) and resides within a vacuole. The RBC is quite nutrient poor and the parasite is forced to renovate its host cell for survival. The true renovators of the host cell are proteins exported from the vacuole and into the RBC via a protein export machinery called PTEX. One of the major renovations is the opening of nutrient channels at the RBC membrane termed the new permeability pathways or NPPs. These channels allow influx of essential plasma nutrients and efflux of metabolic waste. The channel activity has been affiliated with the RhopH complex, comprised of RhopH1, RhopH2 and RhopH3. We showed previously that reducing the expression of RhopH2 results in reduced NPP function. Interestingly we also found that RhopH2 was interacting with 30 exported proteins and therefore hypothesized that these proteins might have a role in NPP activity as well. I am currently studying eleven of these proteins and found that six of them localize to the RBC surface, where the RhopH complex resides. Pulldown assays suggest that only four of eleven proteins are interacting with RhopH2. I am currently in the process of assessing NPP function when knocking down the expression of these proteins, as a direct indicator of NPP involvement. Understanding how the NPPs work and what proteins are functionally involved might help target the NPPs for future therapeutic intervention.

Manipulation of host mitochondrial protein import by *Legionella pneumophila*

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Legionella pneumophila is an opportunistic bacterial pathogen that infects human macrophages, causing Legionnaires' disease, a severe form of pneumonia. To facilitate replication and survival within a unique intravacuolar niche, *L. pneumophila* has evolved sophisticated strategies to rewire host cell functions. Once entering the host cell, this intracellular pathogen must disarm innate immune responses, activate cellular detoxification and access host nutrient supplies. *L. pneumophila* achieves this manipulation of the host through a cohort of over 300 effector proteins introduced into the host via the Dot/Icm type IV secretion system (T4SS). This T4SS is a sophisticated secretion apparatus that is essential for intracellular replication of *L. pneumophila.*

As an integrator of metabolism and energy homeostasis, the mitochondrion is a long-known intracellular target of *L. pneumophila*. Most mitochondrial proteins are translated in cytosol. Translocation of these proteins into mitochondria relies on sophisticated sets of mitochondrial protein import machineries that recognise specialised targeting information inherent in the protein sequence. A few T4SS effectors harboring similar mitochondrial targeting information have been reported to localise to the mitochondria and this is linked to modulation of mitochondrial dynamics and metabolism. We hypothesize that mitochondrial protein import machineries could be a target of *L. pneumophila* effectors to gain entry into mitochondria. One such import machine is the TIM22 complex. TIM22 facilitates the insertion of polytopic membrane proteins into the mitochondrial inner membrane including the metabolite carriers which are crucial players in metabolite exchange. Immunoprecipitation of TIM22-associated complex isolated from cells infected with *L. pneumophila* revealed marked enrichment of two novel *L. pneumophila* T4SS effectors along with known TIM22 components. This suggests that the two TIM22-localised effectors may play a role in manipulating TIM22's function for the insertion of *L. pneumophila* effectors into the host cell mitochondria. If and how these effectors exploited TIM22 for their insertion into the inner membrane of mitochondria, remains to be discovered. We anticipate that understanding of the underlying function of these novel TIM22-targeted effectors will provide better insight into molecular pathogenesis of *L. pneumophila* and further showcases mitochondria as a target of pathogenic infection.

Protein *O*-fucosyltransferase 2-mediated *O*-glycosylation of MIC2 is dispensable for *Toxoplasma gondii* tachyzoite infection.

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Toxoplasmosis is caused by the obligate intracellular parasite *Toxoplasma gondii* which is estimated to be present in one third of the world's population. Human infection results in mild flu-like symptoms while wreaking havoc in immunocompromised individuals. Protein glycosylation has been shown to play an important role in parasite invasion in many eukaryotic parasites as well as in evading host immune responses. We observed that the motility associated adhesin micronemal protein 2 (MIC2) in *Toxoplasma gondii* had highly glycosylated thrombospondin repeat (TSR) domains. Using affinity-purified MIC2 along with MS/MS analysis and enzymatic digestion assays, we observed seven *C*-linked and three *O*-linked glycosylation sites exist within MIC2, with >95% occupancy at these *O*-glycosylation sites. We determined that the addition of *O*-glycans to MIC2 is mediated by a protein *O*-fucosyltransferase 2 homolog (TgPOFUT2) encoded by the *TGGT1_273550* gene. It has previously been shown that POFUT2 homologs play an important role in stabilizing motility associated adhesins and infection in other apicomplexan parasites but the loss of POFUT2 in *Toxoplasma gondii* had only a minor effect on the levels of MIC2, and plaque formation and tachyzoite invasion were broadly similar in the presence or absence of POFUT2. These findings conclude that POFUT2 o-glycosylates MIC2 and the loss of this glycan is dispensable for *Toxoplasma gondii* infection.

Cell death modulation by *Coxiella burnetii* effectors

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Coxiella burnetii is a Gram-negative bacterium and causative agent of Q fever in humans. The bacterium is highly adapted to infect alveolar macrophages and subvert their functions, including the avoidance of TLR recognition, the inhibition of apoptosis and the modulation of diverse vesicle traffic pathways. Its virulence is dependent on the translocation of bacterial proteins (called effectors) into the host cytoplasm through the Dot/Icm T4BSS, creating a spacious intracellular vacuole that supports bacterial replication. Similar to *Coxiella*, *Legionella pneumophila* virulence depends on its T4BSS, however, *Legionella* is more suitable for genetic manipulation, a feature that supports the use of *Legionella* as surrogate host to express *Coxiella* effectors. We generated a library of 66 *L. pneumophilla flaA* mutants expressing *Coxiella* effectors. We screened this library and identified 3 *Coxiella* effectors that may be involved in the manipulation of macrophage functions. Expression of these 3 effectors by *L. pneumophila* led to decreased pyroptosis (measured by LDH release and pore formation assays) and increased cytokine production (IL-1β and IL-6). Thus, we aim to further study these effectors expressing them in eukaryotic cells, identify the possible eukaryotic partner and obtain *Coxiella* mutants for each effector. This study may help to elucidate the function of these effectors, providing information for our understanding of the evasive mechanisms used by intracellular pathogens to subvert the host cell mechanisms. In addition, this study will lead to the identification of target molecules and pathways to the development of immunological therapies.

Identification and characterization of novel hepatocyte effector proteins of the human malaria parasite *Plasmodium falciparum*

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Despite a reduction in occurrence over the past years, malaria remains a major global health concern with an estimated 216 million cases and 435.000 mortalities in 2017. Resistance to antimalarials by the most lethal human malaria parasite *Plasmodium falciparum* has raised the need for new therapeutics. During the asymptomatic liver-stage of *P. falciparum*, the parasite replicates within a parasitophorous vacuole into thousands of merozoites. These in turn invade erythrocytes initiating blood-stage infection that causes malaria-associated pathology. As the portal to blood-stage infection, the liver-stage is a primary target for prophylactic intervention. However, our current understanding of *P. falciparum* liver-stage biology is limited around the parasite's ability to evade host-defenses and exploit host nutrients. Like all intracellular pathogens, *P. falciparum* exports virulent effector proteins into the host cell. This has been studied during infection of erythrocytes. Parasitic proteins are targeted for export based on a pentameric amino-acid motif known as the PEXEL (RxLxE/D/Q), that is proteolytically cleaved in the ER by the aspartyl protease Plasmepsin V. Matured effector proteins are translocated into the host-cytoplasm by the PTEX-translocon complex. An important question is whether this pathway is activated and important during the liver-stage of the *P. falciparum* life cycle. Exported proteins would not only allow the parasite to manipulate host defense mechanisms but may also be novel vaccine candidates owing to their presence in hepatocytes.

We are addressing this question by attempting to identify and characterize novel liver-stage effector proteins using two approaches. First, a bioinformatic liver-stage expression and PEXEL motif-based search has identified over 50 effector candidates. Second, an unbiased proteomic approach involving the proximity biotin-ligase TurboID is being used to identify liver-stage specific proteins in different cellular compartments. Candidate liver-stage effector proteins are being validated using a high-content imaging screen to image the temporal dynamics during hepatocyte infection. Validated, novel effectors will be functionally characterized by CRISPR/Cas9 genome editing to disrupt expression of respective genes in *P. falciparum*. This work will delineate the function of novel *P. falciparum* hepatocyte effector proteins and may provide new drug and vaccine targets for preventive therapeutics targeting the liver-stage of *Plasmodium* infection.

Identifying Genetic Variant is Key to Treating Chronic Inflammatory Lung Disease

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While cigarette smoke is the highest risk factor for chronic obstructive pulmonary disease (COPD), only 20% of smoker develop COPD. There are strong evidence for disease susceptibility contributed by genetic variants. As these variants are now identified, the challenges lies in understanding how they contributes to the pathogenesis of COPD. The hematopoietic cell kinase (Hck) gene polymorphisms has been found to be associated with the rate of decline in lung function of COPD patients. Therefore, we have use the reverse genetic approach and created HckF/F mice that mimics this Hck gene variant. This has allow the discovery of a novel disease endotype where IL-17A-producing γδ T cells (γδT17 cells) are key mediators of not one but all the key features of COPD. We have demonstrated that Hck is expressed in γδ T cells and that cigarette smoke-exposure, a major causative factor of COPD, induces γδT17 cell expansion in the lung. Finally, loss of IL-17A ameliorates lung inflammation, emphysematous destruction and mucus metaplasia. This work when translated could mean precise treatment options by targeting γδT17 cell / IL-17A in patients harboring this lung disease endotype in the heterogeneous population of COPD patient group.

Exploring the functional interactions between *Coxiella burnetii* Dot/Icm effectors

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The causative agent of the infection Q fever, *Coxiella burnetii*, is a Gram-negative intracellular bacterial pathogen. In humans, alveolar macrophages constitute a niche for *C. burnetii* intracellular replication, which occurs in a lysosome-derived vacuole termed the *Coxiella*-containing vacuole (CCV). *C. burnetii* harbours a Dot/Icm type IV secretion system that is essential for bacterial intracellular replication. This secretion system delivers an arsenal of approximately 150 effector proteins directly from the bacteria into the human host cytosol. Studies over the past decade have offered much insights into bacterial effector functions, primarily with a focus on effectors manipulating specific host proteins. Recently it has become apparent that bacterial effectors can regulate each other once inside the host cell and this may be the primary role of a subset of effectors. A system-wide screen was performed to identify functional relationships between *C. burnetii* effectors. The nuclear *C. burnetii* effector A (NceA) was found to confer toxicity in Saccharomyces cerevisiae, and this toxicity was alleviated by co-expression of two other effectors, named Suppress toxicity of NceA A and B (StnA and StnB). This suggests that StnA and StnB can antagonise the activity of NceA. A yeast two-hybrid screen with a HeLa cell cDNA library identified two putative host binding partners of NceA; tyrosyl-DNA phosphodiesterase 2 (TDP2), a DNA repair enzyme, and stromelysin-1 PDGF-responsive element binding protein (SPBP), a transcription cofactor of various autophagy-related proteins. HeLa cells engineered to express NceA demonstrate reduced levels of the autophagy receptor SQSTM1. We hypothesise that NceA alters host transcription, through interactions with TDP2 and SPBP, impacting host cell autophagy. The antagonistic relationship of StnA and StnB with NceA suggests their potential involvement in the host biological cascades targeted by NceA. Interestingly, a stnA *C. burnetii* mutant displayed a multi-CCV phenotype which also indicates a role for this effector in modulating autophagy. This research aims to progress our understanding of these effectors and their interplay with host autophagy function.

The Application of Whole Genome Sequencing in the Prediction of Phenotypic Antimicrobial Resistance in Non-typhoidal *Salmonella* Circulating Australia

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Salmonella is the second causative agent of foodborne bacterial gastroenteritis in Australia, which also has the highest *Salmonella* notification rate in comparison to other industrialised countries. With the number of cases increasing annually, antimicrobial resistance (AMR) patterns are also emerging. Whole-genome sequencing (WGS) has paved the way for *in-silico* analysis providing improved genomic techniques and surveillance. The objective of this study was to determine whether WGS could replace phenotypic sensitivity testing at the Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL), the national *Salmonella* reference laboratory. The final dataset included 3,656 *Salmonella* isolates that had both genotypic data obtained from WGS and phenotypic data that incorporated agar dilution sensitivities on nine clinically relevant antimicrobials interpreted using Clinical and Laboratory Standards Institute guidelines. Overall, the sensitivity and specificity rates were above 98% with 0.22% (*n*=67/29,785) discrepant combinations primarily due to streptomycin. Genes encoding resistance was highest against ampicillin ($bla_{\text{TEM-1}}$) followed by sulphathiozole (*sul2*), tetracycline (*tet*A), streptomycin (*strA*-*strB*), cefotaxime ($bla_{\text{CMY-2}}$) and chloramphenicol (*floR*). The two prevalent multidrug resistant (MDR, resistance to ≥3 antimicrobial classes) antibiograms observed was resistance to ampicillin, chloramphenicol, cefotaxime, streptomycin, sulphathiozole and tetracycline (ACCfSSuT) and resistance to ASSuT, and were mainly observed in *S.* 4,[5],12:i:- ST34 isolates with the most common genotype being $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-55}}$, *catA2*, *floR*, *strA*-*strB*, *sul2*, *tet*(A) and $bla_{\text{TEM-1}}$, *strA*-*strB*, *sul2*, *tet*(B), respectively. In conclusion, this study demonstrated a high correlation between WGS and phenotypic sensitivity testing and highlighted that WGS can significantly improve AMR surveillance by simultaneously screening for AMR and emerging multidrug resistance patterns as compared to routine phenotypic methods.

Repurposing the antifungal Tavaborole as a drug candidate against visceral leishmaniasis

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Visceral leishmaniasis is a debilitating parasitic disease caused by *Leishmania donovani,* which is potentially fatal for untreated patients. Every year around 1 million new cases of leishmaniasis are reported from nearly 100 endemic countries. Currently available drugs for leishmaniasis (pentavalent antimonials, miltefosine, amphotericin B) either manifest severe side effects or lead to drug resistance in the parasite. Hence, there is an urgent need to identify novel drugs to control this disease. Drug repositioning is a strategy which helps to circumvent the costs and time involved in traditional drug discovery approaches by investigation of existing drugs for new therapeutic purposes. Aminoacyl-tRNA synthetases involved in protein synthesis are known to be good drug targets in bacterial and fungal pathogens. Tavaborole (AN2690) has been approved by the FDA in 2014 as an antifungal to treat Onychomycosis. AN2690 targets leucyl-tRNA synthetase, stalling its catalytic turnover leading to cessation of protein synthesis. The present study is focused on identification and characterization of *Leishmania donovani* leucyl-tRNA synthetase as a potential drug target and provides preliminary chemical validation of the efficacy of AN2690 against this parasite.

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Poster Session 2

Poster abstracts are presented according to numerical order of presentation per the poster program table, which is mostly, but not strictly, alphabetical by surname.

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Acinetobacter baumannii PAAR proteins: A tale of functional redundancy

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The type VI secretion system (T6SS) is a unique molecular machine found in many Gram-negative bacteria that delivers protein “effectors” directly into other cells. These effectors are delivered through direct or indirect interactions with T6SS “tip” proteins, including Hcp, PAAR, and VgrG. The nosocomial pathogen *Acinetobacter baumannii* AB307-0294 delivers three antibacterial effectors via the T6SS through direct interaction with three cognate VgrG tip proteins. *A. baumannii* encodes two PAAR proteins, located within the core T6SS structural locus (PAAR1) and outside the core locus (PAAR2). To determine the role PAAR proteins play in the delivery of *A. baumannii* T6SS effectors, both *paar* genes were deleted via homologous recombination, individually and together. Loss of either PAAR protein from *A. baumannii* alone did not result in altered T6SS activity. However, loss of both PAAR proteins resulted in complete abrogation of T6SS activity. Complementation of the double *paar* mutant strain with *paar2* completely restored T6SS activity, including delivery of all effector proteins, while complementation with *paar1* only partially restored T6SS activity. Closer examination of the architecture of the 270 amino acid PAAR2 protein revealed a predicted PAAR domain within the first 90 amino acids of the N-terminal region; no known domains were identified in the remainder of the protein. To assess the functional importance of the N-terminal region, we made a mutation approximately 303 bases into the *paar*2, leaving the PAAR domain of the encoded protein intact. This mutation alone, or combined with the loss of the *paar1* did not result in altered T6SS function. Furthermore, complementation of the double *paar* mutant with the first 101 amino acids of PAAR2 (containing the PAAR domain) resulted in full restoration of T6SS function and delivery of all effectors. Together, these results suggest that the *A. baumannii* PAAR proteins are functionally redundant; however, at least one protein is essential for activity of the T6SS. Additionally, the extended C-terminal region of PAAR2 is not required for T6SS activity. Understanding the role that PAAR proteins play in the delivery of *A. baumannii* T6SS effectors is critical to our understanding of this unique molecular weapon.

Host-pathogen interaction between *Legionella* and *Acanthamoeba*

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In the environment, *L. pneumophila* replicates in more than 20 species of amoebae and also exist as free bacteria in water or biofilms. When *L. pneumophila* containing aerosols enter human lungs, the bacteria are rapidly engulfed by alveolar macrophages which *L. pneumophila* then hijacks and replicate within, causing legionellosis in immune-compromised individuals. While the host range is diverse, the intracellular life cycle of *L. pneumophila* in them is very similar. The bacteria replicate intracellularly within a vacuole known as the *Legionella* containing vacuole (LCV) that avoids fusion with the endocytic pathway. Successful intracellular replication requires multiple virulence factors like over 330 proteins translocated Type IV secretion system. However, the majority of T4SS effector proteins remains uncharacterized, especially in the natural host *Acanthamoeba*. This is mainly attributed to functional redundancy of effector proteins and the genetic intractability of *Acanthamoeba*.

In this study, we constructed in-frame markerless mutants in *L. pneumophila* 130b strain based on the enrichment of T4SS effector proteins and use them as a tool to characterize the virulent factors during infection in *A. castellanii* and investigate how *A. castellanii* response to *L. pneumophila* infection. To date, several mutants showed defect intracellular replication in *A. castellanii* and thus defined genomic regions that are specifically essential for the ideal pathogenicity in *A. castellanii*. The further screening revealed a putative glutamate transporter to be critical for the intracellular replication in *A. castellanii*. Moreover, while encystment is applied by *A. castellanii* to endure the harsh conditions like starvation and biocides treatment, *L. pneumophila* infection inhibit the starvation-induced encystment. In addition, the inhibition was partially eliminated when infected with the mutant ΔG constructed in this study. The following study will address on characterizing the function of the putative glutamate transporter and genes responsible for encystment inhibition.

Naturally Acquired Human Antibodies to *Plasmodium falciparum* Transmission-Stage Antigen Pfs230

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Malaria is a mosquito-borne disease that affected approximately 219 million people in 2017 and led to approximately 445,000 deaths worldwide (1). *Plasmodium falciparum* is one of the deadliest parasites causing malaria in humans and is responsible for the majority of malaria-related cases, particularly in Sub-Saharan Africa (1). As recent progress towards malaria elimination have started to plateau in many countries, there is an urgent need for the development of novel treatments such as a vaccine for the long term control of malaria. Transmission-blocking vaccines have been identified as a sustainable tool in achieving long term eradication of malaria through targeting transmissible sexual-stage forms of the parasite, known as gametocytes (2). However, current knowledge on transmission-blocking immunity remains elusive which has rendered a significant hurdle in establishing a safe and effective vaccine (3).

Pfs230 is a protein expressed on the surface of *Plasmodium falciparum* gametocytes and is a leading transmission-blocking vaccine candidate (4). The role of Pfs230 as a target of naturally acquired human immunity is unknown and the functional mechanisms mediating transmission blocking immunity against Pfs230 is poorly understood. This study investigated naturally acquired immunity against Pfs230 in serum samples from children and adults from endemic populations in Kenya and Papua New Guinea. Individuals across all populations had substantial levels of naturally acquired IgG and IgM to recombinant Pfs230, with IgG1 and IgG3 being the predominant IgG subclasses. An age-dependent acquisition was observed among Kenyan children and adults (age group 0.3 to 69.2 years) suggesting an induction of immunological memory that is boosted upon successive infections. Fc-receptor cross-linking was evaluated as a measure of opsonic phagocytosis, a potential functional mechanism of gametocyte clearance within the human host. FcγRIII, was more efficient at cross-linking anti-Pfs230 antibody complexes than FcγRIIa suggesting Fc-mediated phagocytosis as a possible mechanism for parasite clearance. These preliminary results provide crucial insight into the limited field of naturally acquired sexual stage immunity to *Plasmodium falciparum* and will serve as a solid foundation to inform the development of efficacious vaccines that interrupt malaria transmission.

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Oligomerization-driven MLKL ubiquitylation during necroptosis

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Mixed-lineage kinase domain-like (MLKL) is the executioner in the caspase 8-independent form of programmed cell death called necroptosis. Once Receptor Interaction Protein Kinase 3 (RIPK3) is activated by upstream cell death signals, it phosphorylates MLKL and triggers the oligomerization and membrane translocation required for MLKL induced membrane disruption. Previous reports have shown that besides phosphorylation, MLKL also undergoes ubiquitylation during the early stages of necroptosis, yet the mode and significance of this event have not been demonstrated to date. We found that the MLKL necroptosis-specific short chain ubiquitylation happens on biological membranes, relying on its activation and oligomerisation. RIPK3 and upstream components do not directly contribute to such modification. Our data also shows that MLKL ubiquitylation is neither sufficient not necessary to induce necroptosis, instead, it may play a role in proteasome/lysosome-mediated turn over mechanism of activated MLKL.

Development and optimisation of a silkworm (*Bombyx mori*) infection model for bacteriophage therapy trials

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Due to the urgent requirement for alternatives to antibiotics, bacteriophages (phages) have received substantial attention with their recent success in treating multi-drug resistant bacterial infections. The increase in these life-threatening bacterial infections has resulted in the misuse of untested phage therapy treatments. Therefore, the development of a novel animal model that would allow the rapid testing of phages in vivo is critical to the development of safe and effective phage treatment options. To overcome limitations that arise from current animal models, such as high cost, lengthy gestation time and ethical requirements associated with mouse models or unsuitable body size associated with wax moth models, the silkworm (*Bombyx mori*) larvae provide a suitable and reliable alternative model.

Silkworm larvae possess many advantages over alternative animal models, including moderate larvae body size, low purchase and maintenance cost, low handling requirements, and have a short lifecycle for rapid collection of results, with no animal ethics required for their use. Our study has optimized and developed the silkworm model to assess the safety and efficacy of phages, potentially used to treat bacterial infections in humans and animals. To date, we have optimized the appropriate lifecycle stage, housing conditions, injection protocols and haemolymph collection for phage therapy trials in silkworms. Our work has shown the skin bacterial pathogen, *Staphylococcus pseudintermedius*, can survive and replicate within the larvae, and is quantifiable using RT-qPCR in larval haemolymph. Preliminary data suggests that the injection of four novel phages was able to increase silkworm survival in response to lethal doses of *S. pseudintermedius*.

Successful development of the silkworm infection model is essential in advancing the use of phages as safe therapeutics, used to combat an array of bacterial infections that no longer respond to current treatment options.

The neonatal Fc receptor regulates the presentation of antigens targeted to the dendritic cell receptor DEC205

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Receptor targeted vaccination is a new model of antigen delivery that relies on the use of monoclonal antibodies (mAb) to target antigen to specific dendritic cell (DC) subsets. The neonatal Fc receptor (FcRn) is a non-classical Fc receptor that binds to immunoglobulins in acidified endosomes and controls their transport and recycling. FcRn is known to participate in the antigen presentation of immune complexes, however its contribution to the presentation of mAb receptor-targeted vaccination has not previously been examined. Here we have examined the role of FcRn in antigen presentation outcomes using antigen conjugated to mAb targeting specific DC receptors including DEC205 and Clec9A. First, we show that FcRn is expressed at high levels by the conventional DC 1 (cDC1) subset, both at steady-state and following activation. Second, we observe FcRn to play a significant role in MHC I cross-presentation and MHC II presentation of antigens that are targeted to DC via mAb specific for DEC205, a receptor at the surface of cDC1. In contrast, FcRn does not play any role in the presentation of antigens conjugated to mAb specific for the cDC1 receptor Clec9A. These data highlight a new and unique role of FcRn in controlling the immunological outcome of anti-DEC205-based vaccination. To determine the mechanism behind this specificity, we are currently analysing the role of FcRn in the the persistence of antigen-conjugated mAb in vivo, their intracellular trafficking in cDC1 and the consequences for FcRn in anti-DEC205 mediated tumour immunity.

Zinc homeostasis in *Klebsiella pneumoniae*

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It is estimated that ~6% of all bacterial proteins bind the first-row transition metal ion zinc. However, despite the essentiality of zinc for diverse biological and cellular functions, it can also mediate significant toxicity when present in excess. Bacterial zinc homeostasis is achieved by highly sensitive zinc-sensing and regulatory mechanisms that maintain strict control of intracellular zinc concentrations, thereby enabling survival in zinc-restricted or -intoxicated conditions. *Klebsiella pneumoniae* is a ubiquitous opportunistic bacterial pathogen that is responsible for a diverse range of infections in humans including respiratory tract, urinary tract and bloodstream diseases. The World Health Organization has highlighted *K. pneumoniae* as a priority pathogen for urgent antimicrobial development. Although metal ion acquisition at the host-pathogen interface is critical for virulence and pathogenicity of *K. pneumoniae* the molecular mechanisms that contribute to zinc homeostasis remain poorly defined.

Here, we investigated the cellular mechanisms of *K. pneumoniae* predicted to contribute to zinc acquisition and maintenance of intracellular zinc abundance. This was achieved by combining transcriptomic profiling of the *K. pneumoniae* strain AJ218 with mutagenesis, phenotypic growth assays and analyses of cellular zinc accumulation. Building on preliminary bioinformatic analyses, mutant *K. pneumoniae* strains were generated deficient in the putative genes for *zur* (a zinc responsive transcriptional regulator), *znuA* (a periplasmic zinc import protein) and *zntA* (a zinc export pathway). These mutant strains were characterised for their respective contributions in bacterial resistance to extracellular zinc stress. Collectively, this work and the approaches therein provide a foundation for studies on *K. pneumoniae* zinc homeostasis and its contribution to the pathophysiology of this priority pathogen.

Defining the differences in functional antibody response between NAI and vaccine-induced immunity to human

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Malaria remains one of the leading global health issues and the success of current treatment strategies are waning. Malaria is caused by *Plasmodium* species primarily though *P. falciparum* in humans. With the significant health burden, vaccines represent the best approach to controlling and eliminating infection. However, despite recent advances, vaccines to malaria demonstrate poor efficacy and longevity. In comparison, naturally-acquired immunity (NAI) which develops in malaria-endemic regions displays robust and highly functional immunity. This disparity is in part due to a lack of understanding of the antibody mechanisms guiding vaccine immunity and how they compare to NAI. Recognising these differences in antibody function is crucial to develop vaccines capable of mimicking NAI order to successfully achieve an effective vaccine. Antibodies are known to be critical components in NAI against malaria, mediated through mechanisms including complement fixation and opsonic phagocytosis. Although, it is not fully understood if these mechanisms are also induced in malaria vaccine trials. To define these differences, the total level of IgG, IgG subclasses and IgM in serum samples from naturally-exposed individuals in Kenya were compared with individuals participating in a clinical trial of a phase 1 MSP2 vaccine. These samples were also tested for their ability to fix complement C1q and to interact with Fc receptors. Our data suggested that vaccination skewed IgG responses towards a IgG1 response, whereas natural-exposure preferentially induced IgG3. Additionally, complement activation was lower in vaccinated malaria naïve individuals compared to naturally-exposed individuals. Furthermore, naturally-exposed sera was able to more efficiently interact with Fc receptors than vaccinated sera. This data suggested that improvement in future vaccine design to induce functional antibodies capable of complement activation and opsonic phagocytosis may be required.

Title *Plasmodium falciparum* inhibits the formation of neutrophil extracellular traps (NETs)

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BACKGROUND
Malaria due to infection with *Plasmodium falciparum* causes severe morbidity and mortality, and young children and pregnant women are especially susceptible to disease. Neutrophils, the most abundant blood leucocytes, have a crucial role in the clearance of pathogens. However, data on neutrophil interactions with malaria parasites are limited. One important function of neutrophils is the formation of neutrophil extracellular traps (NETs). Microbial pathogens and inflammatory diseases induce NET formation but the role of *P. falciparum* parasites in NET formation is unclear. We aimed to investigate the ability of IE to influence NET formation *in vitro.*
METHODS
Human neutrophils and *P. falciparum*-infected erythrocytes (IEs) or purified merozoites were cocultured, with or without potent NETs stimulators phorbol 12-myristate 13-acetate (PMA) or lipopolysaccharide (LPS). Sytox Green was used to assess DNA release from neutrophils; confocal microscopy to assess changes in neutrophil morphology; and ELISA was used to quantify myeloperoxidase (MPO) release as a measure of neutrophil degranulation.
RESULTS
Incubation of *P. falciparum* IEs with neutrophils did not cause significant DNA release. By confocal microscopy, neutrophils stimulated with IEs displayed normal lobulated nuclei and showed only very limited NET formation. The ability of IE to inhibit NET formation was investigated. The formation of NETs increased substantially upon stimulation with PMA or LPS, but IEs were effective in inhibiting PMA- and LPS-induced NET formation in a dose dependent manner. IEs also inhibited neutrophil degranulation, decreasing PMA-induced MPO release. By contrast, *P. falciparum* merozoites did not inhibit NET formation or neutrophil degranulation.
CONCLUSIONS
We report, for the first time, that IE inhibits PMA and LPS induced NET formation and neutrophil degranulation. Inhibition of NET formation might help the malaria parasite escape host immune responses, contributing to parasite survival. Identification of the mechanism by which *P. falciparum* inhibits NET formation could contribute to the development of new treatment strategies for malaria.

Cbl-b regulates the activation threshold of Natural Killer cells

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Cancer-immunotherapy is revolutionising cancer treatment, with cells from both the adaptive and innate immune system demonstrating unprecedented efficacy as therapeutic agents. Natural Killer (NK) cells are an innate immune cell that is rapidly gaining traction in cancer immunotherapy due to their intrinsic ability to target and kill tumour cells. However, there are still many hurdles to overcome before the tumour killing ability of NK cells can be efficiently harnessed for immunotherapy. These hurdles include: 1) limited ability of NK cells to infiltrate solid tumours, 2) the expression of inhibitory receptors on NK cells or the corresponding cognate ligands on tumours, and 3) compromised cytotoxicity of adoptively transferred NK cells due to a suppressive tumour microenvironment (TME).

Cbl-b is a member of the CBL protein family of E3 ubiquitin ligases, known to modulate different immune cells by regulating the signaling events downstream of activating and inhibitory receptors. NK cells which either lack Cbl-b or express an “E3 ligase-defective” Cbl-b, display prolonged activation. This enhanced NK cell activity confers protection against metastasis in B16F10 melanoma tumour models, suggesting that inhibition of Cbl-b may be of therapeutic benefit. These studies have suggested that Cbl-b inhibits activation signalling downstream of NKG2D by ubiquitylation of inhibitory TAM receptors (1). However, the exact mechanisms by which loss of Cbl-b E3 ligase function in NK cells leads to enhanced cytotoxicity remains unclear.

In this study, we investigate the mechanism underlying the prolonged activation and persistence of NK cells observed in the absence of the inhibitory checkpoint, Cbl-b. In contrast to the previous study, our data show that direct engagement of NK cell activating receptors (without stimulating the TAM receptors) on NK cells results in enhanced production of IFNγ and degranulation. These results suggest that Cbl-b interacts directly with co-activating receptors or signalling intermediates downstream of these receptors to augment NK cell cytotoxicity. Ongoing work will examine the signalling response following NKp46, NKG2D and CD16 activation in Cbl-b E3 ligase-defective NK cells, to reveal further insights on the role of Cbl-b in NK cell function.

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Dynamics of polymorphism in a leading *Plasmodium falciparum* vaccine candidate -Reticulocyte Binding Protein Homologue 5 (RH5)

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Diversity of *Plasmodium falciparum* antigens has evolved as a means for parasites to evade host immune responses - a process known as antigenic escape. PfRh5 is necessary for erythrocyte invasion of the parasite through interaction with host erythrocyte surface protein, basigin (CD147) making PfRh5 a promising blood-stage subunit vaccine candidate. Using longitudinal cohorts of children from the endemic areas of the Papua New Guinea (PNG), we aimed to investigate the relationship between PfRh5 polymorphisms and antigenic escape using population genetic, advanced statistical and bioinformatic analyses. This provides the knowledge of critical polymorphisms that should be taken into consideration in selection of variants to include in PfRh5 vaccine. Fifteen nsSNPs were identified from a total of 674 sequences, which resulted in 44 unique haplotypes. However, the 3D7 (vaccine) haplotype was only 2.13% of infections. Cryo-EM structure of the PfRh5/CyRPA/Ripr complex showed that polymorphisms are in the Basigin binding site, and close proximity to the contact point of its interaction partner Cysteine Rich Protective Antibody (CyRPA). Both traditional and spatially derived Tajima's D analyses indicated signatures of immune or balancing selection surrounding Basigin and CyRPA binding sites. However, our newly developed statistical model indicated the lack of association between turnover of PfRh5 alleles and changes in clinical presentations at least for this longitudinal cohort. This implies the influence of other evolutionary forces rather than allele specific immunity in PfRh5 and Basigin interaction. Taken together, PfRh5 vaccine may have broad cross protective efficacy, but further analysis with older longitudinal cohorts is needed.

Keywords: *Plasmodium falciparum*, PfRH5, Vaccine, Polymorphisms, Haplotypes, Antigenic Escape

INSIGHTS INTO MECHANISMS OF ACTION OF INTERLEUKIN-1 RECEPTOR ASSOCIATED KINASE 3 (IRAK3)

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IRAK3 is known as a negative regulator of innate immunity. IRAK3 is a probable diagnostic biomarker or immunotherapy agent as it is involved in many diseases such as sepsis and asthma. IRAK3 down-regulates inflammatory responses thereby decreasing inflammatory cytokines. IRAK3 is required for endotoxin tolerance in which the production of inflammatory cytokines is diminished upon constant exposure or re-challenge of pathogens. However, mechanisms of IRAK3 actions are not fully understood. Moreover, different studies have shown a variety of different patterns of IRAK3 expression and also action on cytokine production. The aim of this study was to systematically review the literature to develop sepsis cell culture models that can be used to study the action of IRAK3. A systematic review of human and animal studies analyzed expression of IRAK3 and effects on cytokine production following endotoxin challenges. A sepsis cell culture model is under development. IRAK3 production is induced by defined endotoxin or glucocorticoid treatment. We have previously shown that IRAK3 generates cyclic guanosine monophosphate (cGMP). In the presence of nanomolar concentrations (0.1 -1.0 nM) of membrane permeable cGMP (8-bromo cGMP) and lipopolysaccharide, we observed a decrease of NF-κB expression and cytokine (IL-6 and TNF-α) production in monocytic cells. Thus we explored how cGMP modulates cytokine production during endotoxin tolerance. The sepsis model system will be used to investigate interactions between cGMP and IRAK3 in endotoxin tolerance.

Exposure to *Plasmodium* sp. blood-stage impairs liver Trm-mediated immunity

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PUBLISH CONSENT WITHHELD

Characterization of the putative cysteine protease effectors, OspD2 and OspD3, from *Shigella* species.

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Diarrheal disease caused by bacterial pathogens continues to be a major public health concern worldwide due to significant increases in mortality annually. Members of the *Shigella* genus contribute significantly to bacterial diarrheal incidences worldwide. *Shigella* is a Gram-negative facultative anaerobe that belongs to the family *Enterobacteriaceae*. They are considered highly infectious as only 10-100 organisms are required to cause disease. Like many other Gram-negative gut pathogens, *Shigella* utilizes a type III secretion system (T3SS) during infection to translocate bacterial effector proteins into host cells which interfere with host signaling pathways to benefit their survival. The exact function of many T3 effector proteins remains unknown. However recently, the T3SS effector EspL from enteropathogenic *Escherichia coli* (EPEC), was shown to contain a cysteine protease catalytic motif that targets and degrades the host RHIM domain containing proteins, RIPK1, RIPK3, TRIF and DAI, hence blocking inflammation and necroptotic cell death during infection. Homologues of EspL are also found in *Shigella*, namely: OspD2 and OspD3. Although previously labelled as *Shigella* toxins, the exact function of these effectors is yet to be elucidated. The primary aim of my study is to characterize the role of OspD2 and OspD3 and to determine their host cell targets. Co-transfection and co-immunoprecipitation experiments with OspD2 or OspD3 and the RHIM family of proteins suggest that OspD3, but not OspD2 targets and cleaves the RHIM family of proteins. Subsequent proteomap studies have led to the identification of several unique host cells targets for OspD2 and OspD3, suggesting the involvement of these proteins in the interferon signaling pathway. Currently, the exact roles of these proteins in the interferon signaling pathway are being studied. Once the functions of OspD2 and OspD3 in the interferon signalling pathway is known, the contribution of these proteins to host cell defense will be determined

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The role of ADAMTS15 versicanase in the immune response to influenza A virus infection

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Each year, influenza viruses are responsible for seasonal epidemics of acute, respiratory illness in humans, resulting in the deaths of up to half a million people globally. Although treatment and prevention strategies are available in the form of vaccination and antiviral therapeutics, these approaches are somewhat hit and miss. Mismatches between vaccine and circulating strains, and the emergence of antiviral drug resistance necessitates the development of alternative strategies. One possible alternative involves shifting the focus away from the virus and concentrating on the host. Previously, extracellular matrix (ECM) enzymes such as the a disintegrin-like and metalloprotease with thrombospondin-1 repeats (ADAMTS) family have been shown to play a role in modulating immunity. Specifically, ADAMTS5 has been shown to contribute to influenza virus-specific immunity and viral clearance by regulating T cell migration between the mediastinal lymph node and the periphery. Herein we present data from a closely related yet uncharacterised family member, ADAMTS15, and evaluate its role in influenza-specific immunity. *Adamts15* knockout and heterozygous mice were infected with X31 influenza virus and weight loss and viral titres assessed. Flow cytometric analyses of immune cell populations were also performed to fully characterise immune responses. Preliminary data suggest ADAMTS15 contributes to immunity and plays an important role in the resolution of influenza virus infection, through a yet to be determined mechanism. Targeted overexpression of ECM enzymes such as those in the ADAMTS family may therefore have the potential to reduce the burden of disease.

Investigating secreted autotransporter toxins from pathogenic *Escherichia coli* strains

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Introduction:

Gram-negative bacterium *E. coli* is one of the etiologic agents of diarrheal illnesses responsible for thousands of deaths annually. A common feature that increases the pathogenicity of enteropathogenic *E. coli* (EPEC) and enteroaggregative *E. coli* (EAEC) is the secretion of high-molecular-weight toxins known as serine protease autotransporters of *Enterobacteriacea* (SPATES). EspC and Pet are two of these SPATEs which bind and enter epithelial cells to cause cytotoxic damage during EPEC & EAEC infections respectively. However, the molecular details of how these SPATEs recognize and enter epithelial cells is unknown.

Hypothesis & Aim:

Determine the structures of both EspC and Pet toxins and use these structures to guide studies at revealing their mechanisms of action in bacterial pathogenesis.

Methodology:

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

Results & Conclusions:

In this study, I have developed reproducible protocols for the isolation and purification of both EspC and Pet from bacterial cultures. Further, I have confirmed the serine protease activity of these toxins and additionally identified the conditions for their optimal serine protease activity and inhibition. Foremost, I have crystalized and determined the first molecular structure of the EspC toxin, which will soon be followed by the Pet toxin. The structure of EspC was found to encompass a large 3-stranded β-helix with an N-terminal chymotrypsin-like serine protease domain. The large β-helix was found to contain a number of loops thought to be responsible for epithelial cell binding and internalization. Using the EspC structure I plan to design deletion mutants of these β-helix loops to uncover their potentially critical role in recognizing and promoting internalization of toxin. This information will then be used to design inhibitors to bind and block EspC from entering host tissue. The EspC structure will also be used as an alternative means to design anti-microbials, by examining the serine protease active site of EspC and its potential to be inhibited by small molecules. These two independent routes for targeting the cytotoxic activity of EspC will open new avenues to combat diarrhoeal infections.

Dissection of the Assembly and Function of the *Plasmodium* Export Machinery

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Malaria is a devastating disease that is caused by an infection from obligate intracellular *Plasmodium* parasites. These parasites thrive and replicate within the erythrocytes of their vertebrate hosts. In order to do so they are required to translocate hundreds of proteins across an encasing membrane known as the Parasitophorous Vacuole Membrane (PVM). The machinery that allows this protein transport to occur is known as the *Plasmodium* Translocon of Exported Proteins (PTEX) and without this complex the parasite cannot effectively survive, therefore implicating PTEX as a perfect target for possible future chemotherapeutic intervention.

PTEX consists of three core proteins; EXP2, HSP101, and PTEX150, as well as two auxiliary proteins; PTEX88 and TRX2. The focus of this research is on EXP2, which has been shown to be the pore of the complex, in addition to a recently identified function as a nutrient pore. The overall function, expression, and structure of EXP2 will be investigated using reverse genetic techniques, specifically through the creation of EXP2 transgenic lines, followed by analysis of the resulting phenotypes that arise.

Characterisation of bacterial effector kinases from enteropathogenic *Escherichia coli*

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PUBLISH CONSENT WITHHELD

A rapid colourimetric method to assess complement activation on Plasmodium falciparum-infected erythrocytes using 2,7-diaminofluorene

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BACKGROUND:

In *Plasmodium falciparum* malaria, infected erythrocytes (IEs) expressing the parasite-derived protein, *P. falciparum* erythrocyte membrane protein 1 (PfEMP-1), and naturally-acquired antibodies against IEs form immune complexes. The circulating immune complexes activate classical pathway of complement producing different protein fragments for effector functions like opsonisation, inflammation and target cell lysis. The involved mechanisms of protection from malaria via antibody dependent complement-mediated IE lysis are unclear. This lysis of IEs in relation to complement activation can be quantified using the amount of haemoglobin released by colourimetric methods.

OBJECTIVE:

To assess *in-vitro* the role of the classical pathway of complement on *P. falciparum*-IEs opsonised with antibodies in immune sera from individuals in malaria-endemic areas.

METHODS:

For preliminary studies, pooled immune sera (PPS) from malaria exposed individuals in a malaria endemic area and non-immune sera from gender-matched malaria-naïve individuals were used. IEs grown in type O+ blood were opsonised with heat-inactivated patient sera and type O+ fresh serum was added as a source of complement. After incubation, the supernatant was assayed using a colourimetric method. The chromogenic compound, 2,7-diaminofluorene oxidises to fluorene blue in the presence of hydrogen peroxide as a result of pseudoperoxidase activity of haemoglobin. The optical density (OD) was measured at 620 nm and compared with respect to lysis of IEs in pure water.

RESULTS:

We observed a dose-dependent lysis of IEs with the increase in immune serum concentration; this value was the highest for rabbit anti human immunoglobulins followed by PPS and almost no complement activation for malaria-naïve individuals.

CONCLUSIONS:

The pilot studies suggest that naturally acquired antibodies against PfEMP-1 may fix complement on *P. falciparum*-IEs to mediate lysis *via* classical pathway of complement activation but future experiments conducted with a cohort of malaria-infected individuals are warranted for further validation.

The health of mothers and newborns in Papua New Guinea: A narrative review

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Abstract

Papua New Guinea (PNG) is the most populous country in the Pacific with more than 9 million people (1-2). Difficult terrain, poor roads and limited infrastructure mean providing effective health care - especially in rural areas, where most people live – is challenging (1-2). Women and newborns in PNG experience high rates of preventable morbidity and mortality, however reliable data are often limited or unavailable (3). The aim of this review was to provide an overview of the maternal and neonatal (MNH) research that has been conducted in the past decade in PNG, and compare research findings to key global maternal and newborn health indicators (4).

There was considerable variation in mortality indicators (maternal mortality ratio, neonatal mortality rate and stillbirth) reported across studies in PNG, and were generally higher in rural areas. Rates of sexually transmitted infections (STIs) in pregnancy were consistently high, while anaemia in pregnancy, preterm birth and low birth weight varied widely between studies and settings. Breastfeeding seems to have been under-researched. Other indicators such as the adolescent birth rate, postnatal care provided to women and newborns, intermittent preventative treatment to prevent malaria in pregnancy and treatment to prevent mother-to-child transmission of HIV were also limited by lack of primary research.

Identified studies have demonstrated the high burden of preventable maternal and newborn morbidity and mortality across PNG. Efforts to improve MNH outcomes need to be escalated.

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Disulphide bond dependent bacterial pathogenesis

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The disulphide bond (Dsb) forming machinery and more specifically the DsbA disulphide bond catalyst, is a central mediator of bacterial pathogenesis as it promotes the assembly of multiple virulence factors required at different stages of the infection process. Extensive research in this area has focused in deciphering Dsb systems in pathogens, however to date little is known about how Dsb proteins interact with and fold virulence substrates. Uropathogenic *E. coli* (UPEC), the main causative agent of urinary tract infections, possesses two DsbA enzymes, DsbA and DsbL, for virulence factor folding. Using a combination of molecular biology, structural biology and biophysics, this study focusses on dissecting the structural and molecular determinants directing substrate specificity of these two distinct DsbA homologues. Outcomes of this research will establish how bacterial pathogens use the Dsb system to generate virulence proteins. This new knowledge is important to understand how bacteria cause disease and will provide important information to current campaigns that aim at developing inhibitors of DsbA proteins as potential antivirulence agents.

The catcher in the RIG-I: treatment of respiratory infections by RIG-I agonists

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Respiratory viruses such as influenza A virus (IAV), respiratory syncytial virus (RSV) or human metapneumovirus (HMPV) cause substantial morbidity and mortality in young children and the elderly. There is urgent need for the development of efficient therapeutic and prophylactic treatments.
Pattern recognition receptors (PRRs) such as the cytoplasmic helicase retinoic-acid-inducible gene I (RIG-I) are part of the innate immune system. RIG-I can be activated by recognition of viral nucleic acids, leading to downstream activation of interferon-stimulated genes (ISGs) and restriction of viral replication and spread. We are using synthetic RNA oligonucleotides to stimulate RIG-I in an effort to inhibit replication of respiratory viruses *in vitro* and *in vivo*.
Ferrets represent an excellent animal model to study human respiratory viral infections as they exhibit similar clinical signs to humans following infection with IAV. Moreover, viral transmission can be studied between animals. We have used *in vitro* approaches to examine how a ferret lung epithelial cell line (FRL) responds to infection with respiratory viruses in the presence or absence of pre-treatment with RIG-I agonists. We demonstrated that RIG-I treatment induces ISG expression in FRL cells and that this correlated with reduced infectivity of IAV and RSV, as well as reduced release of virus from infected cells. We have also investigated the ability of prophylactic and therapeutic treatments with RIG-I agonists to modulate clinical signs and virus load in ferrets infected with a A(H1N1)pdm2009 strain of IAV. Our results suggest that intranasal treatment with RIG-I agonists may provide some protection during IAV infection and ongoing studies aim to enhance the potency of this antiviral effect.
Overall, our results suggest that synthetic RIG-I agonists may represent an antiviral treatment strategy suitable for a wide range of respiratory viruses. Optimising the particular RIG-I agonist used, as well as the dose and route of delivery, are currently being investigated to enhance the potency of this treatment approach. These studies have the potential to facilitate the development of new antiviral treatments for a broad range of respiratory virus infections.

IFNγ controls macrophage TLR responses by activating cell death

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Pathogens molecules are sensed by the Toll-like receptors (TLRs) to activate macrophages and induce innate immune responses. In some circumstances host-derived Interferon-gamma (IFNγ) can augment the inflammatory production of tumour necrosis factor (TNF), interleukin-6 and anti-microbial nitric oxide. Intriguingly however, in murine macrophages IFNγ has been shown to induce a potent cell death when co-stimulated with TLR ligands, but the genetic requirements that determine this cell death pathway remain to be elucidated. Here, we genetically delineate the molecular mechanism underpinning IFNγ and TLR-induced cell killing, thereby defining a novel cell death pathway that might act to limit excessive innate immune responses.

Using a panel of genetic knockout mice, we demonstrate that the apoptotic caspase proteins are required for IFNγ and TLR-induced cell killing, while effector molecules of the highly inflammatory cell death pathways, pyroptosis and necroptosis, are dispensable. IFNγ and TLR stimulation synergised to increase inducible nitric oxide synthase (iNOS) levels, and the chemical inhibition of iNOS, or its genetic deletion via CRISPR/Cas9, showed that iNOS was essential for caspase activation and thus macrophage killing. Identifying the mechanism by which iNOS triggers apoptotic caspase signalling and macrophage killing may reveal new therapeutic opportunities to limit pathological innate immune cell activation and cytokine production, or promote the death and clearance of pathogen infected cells.

GLOBAL 3'-UTR LENGTH CHANGES MEDIATED BY INTERFERON BETA IN MURINE AN HUMAN MACROPHAGES

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Interferon signaling is one of the most important mechanisms shaping innate immune responses, and it needs to be tightly regulated to successfully fight infections while avoiding toxicity. Type I interferons (IFNs) induce changes in cells on many different levels, such as transcriptional, translational and metabolic. In this study, many of these changes have been characterized on a global level in murine and human macrophages using multi-omics strategies. Subsequently, this project has been focusing on 3'-UTR dynamics, a field that is only starting to be explored and its regulation as well as function remain mostly unknown.

To study type I IFN responses, we conducted time-course experiments treating murine bone marrow-derived macrophages or human blood monocyte-derived macrophages (HMDMs) with IFN-beta. We analyzed the effect of IFN-beta on these cells by RNA-sequencing and mass spectrometry to measure RNA, protein and metabolite levels.

Firstly, we observed induction of gene expression and changes in metabolite levels upon IFN-beta treatment in our datasets, consistent with findings in previous studies. RNA expression data correlated only partially with protein expression and was only partially conserved between species.

Secondly, following IFN-beta treatment, both RNA-sequencing datasets revealed an increased expression of transcript isoforms with a shortened 3'-UTR, which increased at later time points of treatment. Differential 3'-UTR usage was compared to the overall RNA and protein expression level and miRNA targeting events identified by HITS-CLIP.

In addition to this information, we incorporated data on post-translational modifications and subcellular localization from different databases and publications to characterize this novel dataset.

We selected a number of genes with differentially expressed 3'-UTR isoforms for further study. We aim to identify components involved in mediating the shortening of 3'-UTRs and the consequences for particular candidates of interest. This will involve looking for changes to IFN-beta signaling and effects on macrophage function in HMDMs after a selective knock down of 3'- UTR isoforms. This study will shed light on a new aspect of interferon signaling and how differential expression of distinct 3'-UTR transcript isoforms shapes macrophage innate immune responses.

Developing a core outcome set to measure the effects of pain management during labour and childbirth

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Background: Labour can be one of the most painful experiences that a woman encounters in her lifetime. Pain management options available to women include pharmacological (such as epidural analgesia) and non-pharmacological interventions (such as massage and heat packs). These interventions can provide pain relief; however, trials of these interventions have used a wide variety of outcomes, complicating comparison of their effects. A core outcome set is a pre-defined list of required outcomes for conducting research on an intervention. Currently there is no agreed core outcome set for the measurement of effects of pain management in labour and childbirth. This review of systematic reviews and trials is the first step towards development of a core outcome set for the management of pain during labour and childbirth.

Methods: We searched the Cochrane database (CENTRAL) for relevant systematic reviews of labour and childbirth pain management interventions. All outcomes reported within these systematic reviews (at review and trial level) were extracted and reported descriptively.

Results: We identified and extracted outcomes for 20 systematic reviews and 410 trials. Extracted outcomes were organized by importance (primary, secondary), participant (woman, infant) and type (benefit, harm, health service outcomes). Most frequent outcomes were identified for potential inclusion in a core outcome set.

Conclusion: Pain management options have varying effects on women and their newborns. The development of a core outcome set will provide future clinicians with a standardised method of recording and comparing data

Antiviral activities of interferon-inducible GTPases

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Following viral infections, type I interferons (IFNs) are rapidly induced and these, in turn, activate hundreds of interferon-stimulated genes (ISGs) in host cells. While the antiviral function of some ISGs have been well defined, there is little information regarding the mechanism of action or the spectrum of antiviral activity displayed by the majority of ISGs. Guanylate-binding proteins (GBPs) are amongst the many ISGs induced in host cells following influenza A virus (IAV) infection, but the antiviral activity of GBP-family proteins has not been well characterized. Given their (i) IFN-inducible expression in different cell types, (ii) known antimicrobial activity against intracellular bacteria and parasites, and (iii) structural and biochemical relatedness to Myxoma (Mx)-family proteins, a family of IFN-inducible GTPases known to be potent inhibitors of IAV and other viruses, we hypothesise that at least some GBP-family proteins may modulate the replication of one or more respiratory viruses. Herein, we used *in vitro* approaches to generate mouse airway epithelial cells with doxycycline(dox)-inducible expression of each of the 11 different murine (m)GBPs to determine if they can affect the growth of IAV and other respiratory viruses, including Sendai virus (SeV), parainfluenza virus (PIV)-3, herpes simplex virus (HSV)-1 and lymphocytic choriomeningitis virus (LCMV). Cells with dox-inducible expression of murine (m)Mx1 were included as a positive control known to inhibit IAV. To date, our results show that dox-inducible expression of mGBP1 does not inhibit replication of IAV, SeV, PIV-3, HSV-1 or LCMV. Of interest, in addition to inhibition of IAV, we found that mMx1 was also a potent inhibitor of HSV-1 and the mechanisms underlying this antiviral activity are currently under investigation. Furthermore, we are assessing IAV infection in knockout mice to determine if mGBP1 can modulate other aspects of viral infection (e.g. inflammatory responses) *in vivo*. Similar approaches will then be used to assess the antiviral activity of other mGBPs.

Effects of obesity on vascular and circulating immune cells in mice

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Obesity is associated with low-grade chronic inflammation and the accumulation of immune cells in the perivascular adipose tissue (PVAT) of blood vessels. In obesity, PVAT is a likely cause of vascular dysfunction but the precise mechanisms by which PVAT contributes to a pro-inflammatory state remain unclear. Therefore, we aimed to characterise the aortic immune cell profile in a mouse model of obesity. Six-week-old male C57BL/6 mice were fed a high-fat diet (HFD) for 10 weeks, after which flow cytometry was used to enumerate immune cell populations in the blood and aorta. Compared to mice on a normal diet (ND), HFD mice exhibited higher bodyweight, blood cholesterol and fasting blood glucose levels at the completion ($P<0.05$, n=9-12). The frequencies of total (CD3+) T cells and CD4+ T cells as a proportion of CD45+ cells were reduced in the aorta of HFD vs. ND mice (14±0.6% vs. 23±2% and 32±1% vs. 43±2%; both $P<0.01$). This correlated with a reduced percentage of total T cells in the blood (16±1% vs. 28±3%; $P=0.001$). Conversely, aortic, but not circulating neutrophils (Ly6G+) were more prevalent in HFD mice (35±5% vs. 15±3%; $P=0.006$). Overall, our data demonstrates a shift in the proportion of aortic immune cells during obesity which is independent of changes to circulating immune cells. Ongoing studies are aimed at better understanding the relative contributions of immune cells to vascular dysfunction during obesity.

Dissecting the origin of epitopes targeted by abacavir-specific T cells

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Drug hypersensitivity reactions (DHRs) that induce T cell-mediated immunopathology have been associated with high morbidity and mortality rates, with some being associated with specific human leukocyte antigen (HLA) molecules (1). HLA molecules present immunogenic peptides (originating from various proteins) to T cells that upon engagement elicit a response via the T cell receptor (TCR). The antiretroviral drug abacavir, which is known to cause abacavir hypersensitivity syndrome (AHS; including systemic symptoms such as fever and rash) is strongly associated with HLA-B*57:01. Abacavir has been shown to bind non-covalently within the F pocket of HLA-B*57:01, thereby altering the self-peptide repertoire and promoting the generation of drug-induced T cell epitopes (2).

This study investigates tissue-specific antigen processing and presentation of immunogenic epitopes involved in AHS. Abacavir-reactive CD8$^+$ TCRs from HLA-B*57:01$^+$ donors were transduced into a TCR-deficient T cell line. Activation of these drug reactive TCR-expressing cells was assessed by CD69 immunoassay, following stimulation with different HLA-B*57:01$^+$ antigen presenting cells (APCs; 1106 KERTr keratinocyte and HLA-B*57:01 transfected lymphoblastoid [C1R and TAP-deficient T2] cell lines) in both the presence and absence of abacavir. We have also interrogated the immunopeptidome of these HLA-B*57:01$^+$ cell lines (+/- abacavir) using mass spectrometry.

We evaluated six abacavir-reactive TCRs which showed differential immune reactivity in response to the target APCs in the presence of abacavir. This suggests that i) the TCRs have differing epitope specificities and ii) the APCs display different immunogenic HLA-drug-peptide complexes, possibly due to differences in peptide source protein expression and/or antigen processing. Future directions include identification of immunogenic peptides, via functional screening of peptides isolated

from the HLA-B*57:01^{+} APCs, to determine which peptides contribute to AHS immunopathology.

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Mutations in the guanylate cyclase centre and surrounding residues of IRAK3 can modulate NF-κB signalling

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Interleukin-1 receptor associated kinase 3 (IRAK3) acts as a negative regulator of inflammation by inhibiting inflammatory downstream signalling. Although IRAK3 is proposed as a useful diagnostic and prognostic marker in inflammation, and possibly a target for intervention, the exact mechanism of action and the selectivity of IRAK3 is still largely unclear and further evaluation is needed. Prior studies using bioinformatic search tools identified IRAK3 as a potentially novel guanylate cyclase catalyzing cyclic guanosine monophosphate (cGMP) synthesis, and IRAK3 was shown to contain a guanylate cyclase (GC) centre within its pseudokinase domain. We demonstrate that wild type IRAK3 is capable of producing cGMP, whereas point mutations in the GC centre and surrounding residues reduced cGMP production and influence distribution of the protein in mammalian cells, suggestive of changes in the interactome. cGMP alone affects downstream signalling through modulation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in the presence of lipopolysaccharide (LPS). IRAK3 mutants with reduced cGMP-generating capacity failed to supress LPS-induced NF-κB activity, suggesting that the cGMP generated by IRAK3 may be involved in regulatory function of the protein where the presence of cGMP may selectivity affect downstream signaling pathway(s) by modulating the binding and/or activity of nearby interacting proteins involved in the cascade. These findings are providing insight into the hidden functions of IRAK3 and may assist in explaining the selectivity and functionality of IRAK3 in the inflammatory signalling cascade.

Characterizing a novel anti-inflammatory activity of Genistein

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The cGAS-cGAMP-STING pathway plays a major role in innate immune responses through the production of type I interferon (IFN) in recognition to cytosolic DNA (1,2). Our laboratory has recently reported that DNA intercalating agent Acriflavine could generate mild DNA damage resulting in cGAS-STING activation (3). In this work we investigate whether other DNA intercalating agents from the flavonoid family could also engage this pathway. Surprisingly we discovered that Genistein, a known anti-inflammatory agent, suppressed propagation of the cGAS-STING signaling in cell monolayers. Our current work suggests that this inhibition is due to the modulation of gap junctional intracellular communication which is normally engaged in propagating STING mediated immune signaling in adjacent cells.

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Title: **Immunomodulatory effect of Renin-angiotensin inhibitors on T-lymphocytes in mice with Colorectal Liver Metastases**

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Abstract

Background: Colorectal cancer is the third most common cancer diagnosed in the developed world and second most frequent cause of cancer related deaths mostly caused by liver metastases. Current literature and our research show that tumor infiltrating immune cells positively or negatively contribute to tumor progression, depending on the cell type. We have shown that inhibitors of the Renin Angiotensin system (RAS) inhibit tumor growth by modulating the tumor infiltrating immune cells.
Aim: To investigate the effects of RAS inhibition on tumor T lymphocyte distribution in a mouse model of colorectal liver metastases (CRCLM).
Methods: Liver metastases were established using an orthotopic mouse model. The mouse host is immunocompetent ensuring full spectrum of immune responses. Following tumor induction, the mice were separated into two groups; control (saline) and RAS inhibitor (Captopril) treatment. Saline or Captopril was administered daily via intraperitoneal injection, from day 1 post-tumor induction to endpoint. Tumor growth was determined using stereology proliferation markers and IHC on days 15 or 21 following tumor induction. Lymphocyte subsets in the tumor and liver tissues were analysed by flow cytometry and immunohistochemistry.
Results: The results show that Captopril significantly decreased tumor viability and impaired metastatic growth. Flow cytometry analysis showed T cells ($CD3^+CD45^+$) were significantly increased in the captopril treated group compared to control for both surrounding liver and tumor at day 15. These results were also confirmed by IHC. Interestingly, flow cytometric analysis indicated that a T cell phenotype double negative for the CD4 and CD8 markers was significantly increased in the captopril treated group while the $CD3^+CD4^+$ T cells were significantly decreased compared to control group for both surrounding liver and tumor.
Conclusion: RAS inhibitors reduce tumor growth and modulate the immune response by increasing the infiltration and altering the phenotype of T lymphocytes. The exact nature of these changes needs to be further characterized, especially the identity and function of the double negative $CD3^+$ T cell population need to be elucidated.

Identifying Antibody to VAR2CSA in Pregnant Women

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Background: Malaria in pregnancy compromises the health of both mother and baby and is associated with accumulation of *Plasmodium falciparum*-infected erythrocytes (IEs) in the placenta[1]. IEs isolated from the placenta bind to chondroitin sulfate-A (CSA) expressed on placental cells[2]. Adhesion is probably mediated by *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) which is a variant parasite protein expressed on the IE surface[3]. Recent evidence suggests that the conserved PfEMP1, VAR2CSA, is a key target of antibodies associated with protection against malaria in pregnancy[4]. However, the target of VAR2CSA-specific protective antibodies remains unclear. We asses VAR2CSA-spesific antibodies in pregnant women in Papua New Guinea (PNG) and analyze their association between maternal malaria infection and maternal anthropometric status.

Methods: Plasma samples were collected from a cohort of pregnant women in PNG (≤26 gestational weeks) with mixed gravidity in which enrolment took place from November 2009 to February 2013. IgG measurement was done using ELISA and data are analyzed using logistic and linear regression STATA.

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Elucidation of novel phosphorylation sites in rabies virus P protein.

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Viruses employ diverse mechanisms to modulate the biology of infected host cells in order to facilitate replication and spread. The coding capacity of viral genomes is highly limited, so viruses exploit multiple mechanisms to increase functional diversity of their proteins, including, the expression of multiple protein isoforms, which can, take advantage of host post-translational modification mechanisms to regulate their function. Understanding of these mechanisms is critical to understanding viral replication and pathogenicity and identifying mechanisms to combat potentially lethal infections.

The rabies virus (RABV) is a member of the genus Lyssavirus and causes acute, fatal encephalitis. Although vaccines are available that provide protection from RABV, if a symptomatic infection is established, the disease is 100% fatal (currently resulting in >60,000 deaths/year). A major component of this unrivalled mortality rate is the striking capacity of lyssaviruses to evade host immunity. RABV encodes only 5 proteins, several of which hijack host cellular machinery. Among these, the phosphoprotein (P protein), has major established roles in pathogenesis, mediating innate immune evasion. It was assumed that, like related viruses, the phosphoprotein was phosphorylated at numerous sites, but the mechanisms and outcomes of this are not understood. Several potential phosphorylation sites have been proposed (S63, S64, S162, S210, S271), though confidence in the approach used is limited and direct confirmation or functional impact of these sites is lacking. The potential impact of phosphorylation has been demonstrated through phosphomimetic/phosphoinhibitory mutations that have a direct impact on subcellular trafficking of protein and potential links to immune evasion strategies, though, again, the precise residues and mechanisms remain elusive. To address this, we used mass spectrometry to determine the precise residue locations of phosphorylation, indicated by an increase in mass of 80 Da. This method identified numerous sites, distinct and exclusive to those previously proposed, with three sites of high confidence. Current research seeks to confirm these sites directly via mutagenesis and assessing the functional impact of proteins containing phosphomimetic/phosphoinhibitory mutations, at the identified sites, in cellular assays using kinase inhibitors/agonists. These data should aid in understanding how RABV hijacks host cell machinery and silences the immune response.

Salmonella Typhimurium induces cIAP degradation in macrophages in a SPI-1 dependent manner

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Salmonella enterica serovar Typhimurium (*S.* Typhimurium) is a Gram negative, motile bacterium capable of infecting human, animal or bird hosts. *S.* Typhimurium infects epithelial cells and macrophages intracellularly, enabled by use of Type III Secretion Systems (T3SSs) to translocate effector proteins directly into the host cell cytosol. These effector proteins are used to exert a range of pathogenic activities, including mediating changes to innate immune pathways, inhibiting programmed cell death responses and promoting host cell survival. Research from the Pearson Group (unpublished) has indicated that infection of immortalised mouse macrophages (RAW264.7 cells) with wild type *S.*Typhimurium SL1344 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 an inhibitor of apoptotic cell death. This cIAP1 degradation was associated with functional *Salmonella* Pathogenicity Island 1 (SPI-1) effector translocation after 10 hours of infection, and was not prevented by pan-caspase, proteasomal or lysosomal inhibitors. Consistent with our understanding of cIAP1 mediated inhibition of apoptosis, LDH assay indicated a strong association between loss of cIAP1 and increased cytotoxicity at 19 hours post infection. Later experiments detected a low molecular weight peptide specific for our cIAP1 antibody, possibly indicating that a SPI-1 effector directly cleaved cIAP1 to produce this cleavage product. Together, these data provide early support for our hypothesis that cIAP1 depletion was induced by a SPI-1 effector following infection with *S.*Typhimurium in order to promote host cell death, and potentially dissemination of the bacterium.

Large genomic deletions in *Legionella pneumophila* identify new genes influencing intracellular replication and lung infection

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Legionella pneumophila is an accidental human pathogen that causes the severe pneumonia known as Legionnaire's Disease. *L. pneumophila* evades predation and replicates within amoebae, which has equipped the bacteria with the ability to replicate in human alveolar macrophages. During infection, *L. pneumophila* establishes a replicative vacuole termed the *Legionella*-containing vacuole (LCV) that sustains intracellular replication in macrophages and amoebae. Establishment of the LCV requires the Dot/Icm type IV secretion system (T4SS), that injects more than 300 effector proteins into the infected host cell. Despite their central role in LCV biogenesis, to date most effector proteins remain uncharacterized. Therefore, to aid in the study of effector-associated phenotypes, in this study, we generated nine genomic deletions in *L. pneumophila*, which resulted in the deletion of 68 effector genes and 138 non-effector genes collectively.

Despite the loss of multiple effector genes, none of the mutants showed any replication defect in human macrophages compared to wild-type *L. pneumophila*, including one mutant (*ΔFGHI*) that was missing 42 effector genes. This supports the argument for large scale functional redundancy among Dot/Icm effector activity in mammalian cells. Interestingly, *ΔFGHI* showed a 10-fold increase in lung colonisation compared to wild-type *L. pneumophila* 24 hours after infection in A-strain mice. This finding is especially surprising as, unlike wild-type *L. pneumophila*, *ΔFGHI* was unable to replicate in *Acanthamoeba castellani*. In order to unravel this unusual observation, we will identify which *L. pneumophila* genes contribute to higher bacterial numbers in the lung using trans-complementation to restore the phenotype, and examine the immune response to infection. Together, this will shed light on to the pathogenesis of *L. pneumophila* in mammalian cells, as well as the pathogen interaction with host immune response.

The role of Flt3 in dendritic cells

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Dendritic cells (DCs) play a critical role in the activation and regulation of immune responses and are of significant interest for the design of improved vaccines against infection and cancer. The FMS-like tyrosine kinase 3 (Flt3/CD135)/Flt3L cytokine signaling cascade is a critical pathway in hematopoiesis. While it is well known that the development of DCs is regulated by Flt3, little is known about the expression and trafficking of this protein. We have characterized the expression of Flt3 in primary murine immune cells by flow cytometry. Flt3 expression is restricted to both plasmacytoid and conventional DCs, with little Flt3 expressed by T cells or B cells. Exposure to Flt3L results in a significant decrease in the cell surface expression of Flt3, which can be detected by flow cytometry and immunoblotting. To quantitate this loss of cell surface Flt3 in primary DCs, we have generated a fluorescently tagged Flt3L which can be specifically and efficiently quenched. Using this probe, we have found that DCs begin to internalize Flt3L within 5 minutes, with the majority of probe internalized within 20 minutes. Flt3 is often mutated in hematopoietic malignancies, with mutations in this gene found in ~15% of acute myeloid leukemia (AML) patients. To investigate the role of mutated Flt3 in leukemogenesis, we have characterized primary DCs from mice harboring the most common human Flt3 mutation; Flt3-ITD. Our data show that Flt3-ITD results in a significant expansion of all dendritic cell populations. The largest expansion occurs in the largely uncharacterized CD8 “look-alike” DCs (also known as CX3CR1$^+$ CD8α$^+$ DCs). To investigate how the Flt3-ITD mutation affects the function of DCs, we have carried out antigen presentation assays. Our data show that overall Flt3-ITD improves DC antigen presentation, in particular to CD4$^+$ helper T cells. Future studies will include characterizing the effect of Flt3-ITD on DC antigen uptake and processing and cytokine production. Exploring the biology of Flt3 and DCs is critical to understanding their role in normal and malignant settings. This will, in turn, provide insights for the exploitation of the Flt3/Flt3L cytokine pathway in the development of effective drugs.

Sitagliptin enhances anti-tumour immunity and delays tumour progression in a syngeneic model of ovarian cancer

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Immunity plays a substantial role in the development and progression of epithelial ovarian cancer (EOC), and the success of anti-tumour immune responses is heavily dependent on tumour-infiltrating leukocyte populations. Treatments aimed at increasing leukocyte infiltration and preventing anergy is therefore of great interest as a novel immunotherapeutic strategy for EOC. We tested the hypothesis that DPP4 inhibition with the clinically approved drug sitagliptin can restore anti-tumour immune populations in a syngeneic model of EOC. C57BL/6 mice with intrabursally-implanted near-infrared fluorescent (iRFP+) ID8 ovarian tumours were treated with sitagliptin daily (oral, 50mg/kg body weight/day), 14 days following tumour implantation until endpoint. We used multi-colour flow cytometry, immunofluorescence and cytokine assays to assess anti-tumour immune responses following sitagliptin treatment. We demonstrated that sitagliptin decreased tumour burden and delayed metastatic dissemination, as indicated by iRFP+ fluorescence and macroscopic tumour observations. Sitagliptin significantly prolonged the survival of these mice by both reinvigorating natural anti-tumour immune responses and inhibiting tumorigenic immune populations. Sitagliptin enhanced CXCR3-mediated recruitment of CD8+ T cells to the peritoneal cavity and showed increased T-effector cell infiltration within the ovarian tumour microenvironment. Additionally, sitagliptin enhanced systemic CD69+ T-effector cell activation and Ki67+ proliferation. Furthermore, sitagliptin alleviated immunosuppression by reducing myeloid-derived suppressor cells (MDSCs) within the peritoneal cavity, and by downregulating the pro-tumorigenic cytokines CCL2, CCL17, CCL22, IL-10 and IL-16 in circulation. Taken together, these results suggest that administration of sitagliptin decreases tumour burden in a syngeneic ovarian cancer mouse model by shifting the balance toward anti-tumour immunity. From a therapeutic perspective, our results provide a strategy to improve immune responses in epithelial ovarian cancer, and establishes a rationale for the use of DPP4 inhibitors as a rapidly translatable treatment for this disease.

Lytic form of cell death as a novel route for Migration inhibitory factor (MIF) release

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INTRODUCTION: Macrophage migration inhibitory factor (MIF), expressed by monocyte/macrophages, can act as a pro-inflammatory cytokine and has a pathogenic role in inflammatory diseases including rheumatoid arthritis and systemic lupus erythematosus. How MIF is secreted and released is not well understood, although one study has demonstrated that neutrophils release MIF following necrotic cell death. Here, we looked at the release of MIF in response to different forms of cell death in monocyte/macrophages.

METHODS: THP-1 monocytes were treated with inducers of necrosis (ethanol, 10%, V/V), apoptosis (staurosporine), pyroptosis (LPS + nigericin) and necroptosis (BV-6 + rhTNF-α + Z-VAD-FMK). Cell death was detected using Annexin V/PI staining and LDH release. The released concentration of MIF was measured by ELISA. In some experiments, the occurrence of cell death was abrogated using specific inhibitors.

RESULTS: MIF release was induced during all examined forms of necrotic and lytic cell detah, but not during early apoptotis. In contrast, in most conditions, LPS had additional effects on MIF release. Our findings demonstrate for the first time that MIF is released from monocytes/macrophages in response to both NLRP3-dependent pyroptosis and RIPK1-depedent necroptosis.

CONCLUSIONS: Our data suggest that MIF release is associated with necrotic but not apoptotic modes of cell death in monocytes/macrophages, indicating that it is released passively following cell lysis. Interestingly, MIF release was affected by LPS treatment which may have significant implications for sterile inflammation. However, it remains to be determined whether this released MIF is bioactive and functions as a DAMP.

The antimicrobial effect of *Pseudomonas aeruginosa* outer membrane vesicles.

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All Gram-negative bacteria produce nanoparticles known as outer membrane vesicles (OMVs) as part of their natural growth. OMVs are approximately 20 to 400nm in size and contain nucleic acids, enzymes and proteins derived from their parent bacterium. OMVs can aid bacteria in numerous bacterial functions such as cell-to-cell communication and pathogenesis through numerous mechanisms including attacking and lysing neighbouring bacterial cells. Specifically, OMVs can package autolysins, enzymes that can lyse various Gram-negative and Gram-positive bacteria. We propose that harnessing the packaging of lysins into bacterial OMVs can result in the generation of novel nanoparticle-based antimicrobials that are able to target and lyse pathogenic bacterial strains.

In this study, we have successfully isolated OMVs from *Pseudomonas aeruginosa* at the early log phase of bacterial growth. We will additionally be isolating vesicles from late log and stationary phase of *P. aeruginosa* growth and will compare whether there are changes to the antimicrobial activity of OMVs throughout bacterial growth. Additionally, we will be isolating OMVs from a mutant strain of *P. aeruginosa* that does not produce the endolysin Lys. We will then incubate cultures of the Gram-negative pathogen *Escherichia coli* and the Gram-positive pathogen *Staphylococcus aureus* with varying concentrations of the *P. aeruginosa* OMVs from different stages of bacterial growth or *P. aeruginosa* OMVs that do not contain Lys. *E. coli* and *S. aureus* have a known sensitivity to *P. aeruginosa* OMVs and therefore we hypothesis there will be a decrease in viable bacteria in the cultures of the two pathogens after co-incubation with *P. aeruginosa* OMVs that contain Lys compared to OMVs that do not contain Lys.

This work will enable us to advance our understanding regarding how bacterial growth stage can determine the packaging of lysins into OMVs and therefore the antimicrobial activity of OMVs. These findings can be used to develop new OMV-based antimicrobials that can be used against both Gram-negative and Gram-positive bacteria to combat the rise of antibiotic and multi-drug resistant bacteria.

Decipher the mechanisms of polymyxin resistance in *Acinetobacter baumannii* by genome-scale metabolic modelling

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PUBLISH CONSENT WITHHELD

Elucidating the Zn(II)-binding mechanism of *Streptococcus pneumoniae* AdcAII

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Streptococcus pneumoniae is a globally significant human pathogen responsible for more than a million deaths annually. To colonise and persist within the host, the bacterium must acquire the transition metal ion zinc [Zn(II)], which is poorly abundant in the host environment. In *S. pneumoniae*, Zn(II) import is facilitated by the ATP-binding cassette transporter, AdcCB, and two Zn(II)-specific solute binding proteins (SBPs), AdcA and AdcAII. Although both proteins deliver Zn(II) to the AdcCB transporter, AdcAII has a greater role during initial infection and survival in response to Zn(II) starvation. Despite this, the molecular details of how AdcAII selectively acquires Zn(II) remain poorly understood. To date, our understanding of the Zn(II)-binding mechanism has been based solely on the crystal structure of Zn(II)-bound AdcAII, with an open, metal-free conformation remaining refractory to crystallographic approaches. As a consequence, the conformational changes that occur within AdcAII upon Zn(II)-binding remain unknown. Here, we overcame this challenge by individually mutating each of the four Zn(II)-coordinating residues and performing structural and biochemical analyses on the variant isoforms. Structural analyses revealed specific regions within the protein that underwent conformational changes via their direct coupling to each of the metal-binding residues. Quantitative metal-binding, metal ion affinity analyses and phenotypic assays revealed that two of the four coordinating residues had essential contributions to the Zn(II)-binding mechanism of AdcAII. Intriguingly, only one of these residues had a direct role in structural rearrangements within AdcAII. These analyses also revealed that AdcAII could interact with other first-row transition metal ions, in contrast to AdcA. Collectively, our structural, biophysical and microbiological data indicate that AdcAII employs a distinct mechanism of metal binding to other Zn(II)-specific SBPs. Elucidation of this mechanism will provide the structural and biochemical data required for future antimicrobial design strategies.