Victorian Infection & Immunity Network

# Young Investigator Symposium Series

12-16 October 2020

www.viin.org.au

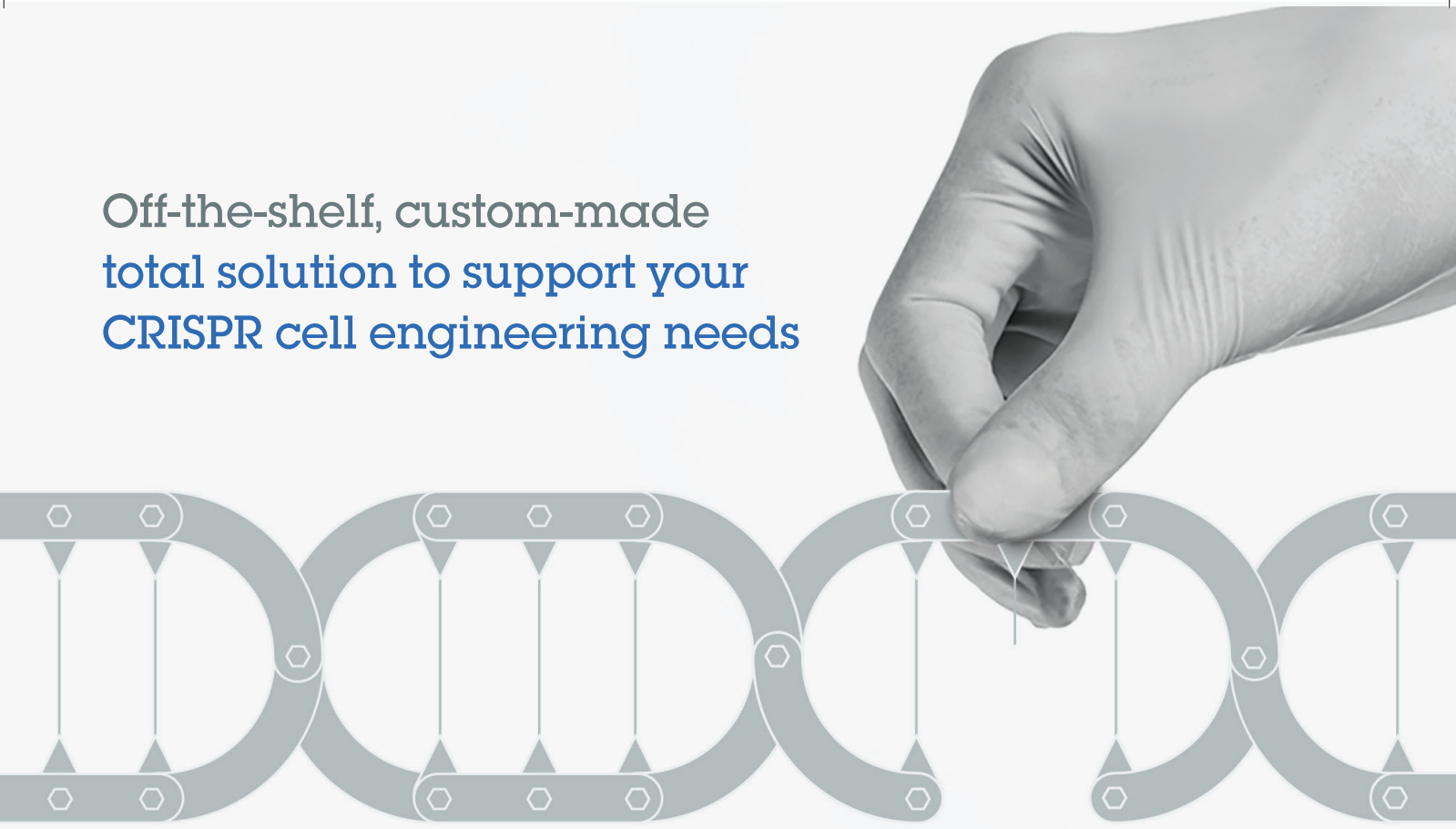
#viinyis

# VIIN Young Investigator Symposium Program-at-a-Glance 2020

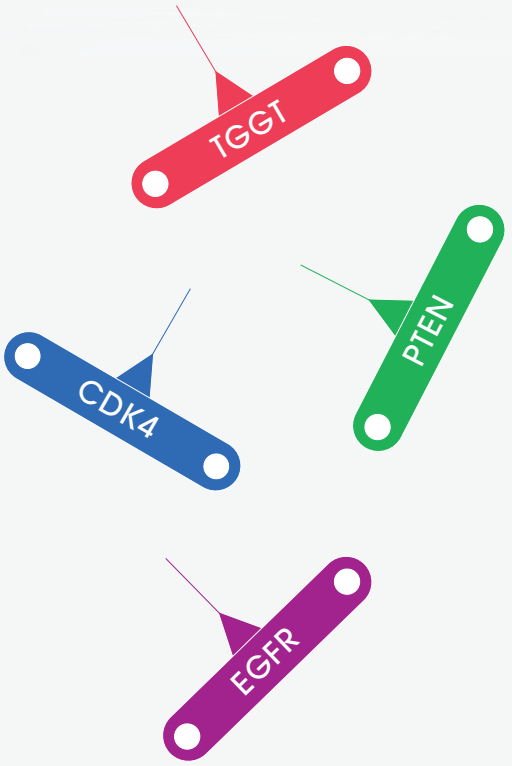
| |
|---|
| **Monday 12 October: 10am – 12.45pm** |
| **Keynote speaker: Associate Professor Kimberly Kline, Nanyang Technological University, Singapore** |
| **Talks selected from abstracts** |
| **Technical workshop** |
| **Daily prize announcement** |
| **Tuesday 13 October: 10am – 12pm** |
| **Grant writing workshop and Q&A**<br>• Associate Professor Sant-Rayn Pasricha, Walter and Eliza Hall Institute<br>• Ms Sally Roberts, veski<br>• Associate Professor Meredith O'Keeffe, Monash University and ARC College of Experts<br>• Associate Professor Michelle Tate, Hudson Institute of Medical Research and NHMRC Career Development Fellow<br>• Professor James Beeson, Burnet Institute |
| **Tuesday 13 October: 1pm – 2.30pm** |
| **Concurrent E-poster sessions (I and II)** |
| **Tuesday 13 October: 7pm – 8.15pm** |
| **Art in Science evening**<br>• Ms Marta de Menezes, Cultivamos Cultura<br>• Dr Ryan Jefferies, Science Gallery Melbourne |
| **Wednesday 14 October: 10am – 12pm** |
| **Careers panel and Q&A**<br>• Dr Krystal Evans, Medical Science Liaison, GSK<br>• Dr Jessica Borger, Lecturer, Monash University<br>• Dr Michael Christie, Principal, Shelston IP<br>• Dr Stephanie Tan, Clinical Research Associate, IQVIA<br>• Dr Ariel Achtman, Senior Project Manager, Movember |
| **Wednesday 14 October: 1pm – 2.30pm** |
| **Concurrent E-poster sessions (III and IV)** |
| **Thursday 15 October: 10am – 12.45pm** |
| **Keynote speaker: Dr Amy Chung, Peter Doherty Institute of Infection and Immunity, University of Melbourne** |
| **Talks selected from abstracts** |
| **Technical workshop** |
| **Daily prize announcement** |
| **Friday 16 October: 10am – 1pm** |
| **Keynote speaker: Dr Con Panousis, CSL Ltd** |
| **Talks selected from abstracts** |
| **Technical workshops** |
| **Daily prize announcement** |
| **Grand prize announcement** |

See more detailed program on Page 13 or click here

# Off-the-shelf, custom-made total solution to support your CRISPR cell engineering needs



With a breadth of experience working with over 1300 cell line models and over 200 distinct cell lines, we provide:




## Ready-made Knockout cell lines and lysates

- Minimal preparation format, ready to culture
- Widely used, immortalized, mammalian cell lines, including HEK-293T and HELA
- Well-characterized by Sanger sequencing and western blot to confirm KO at the genomic and proteomic level
- Parental control available

## Custom gene editing services

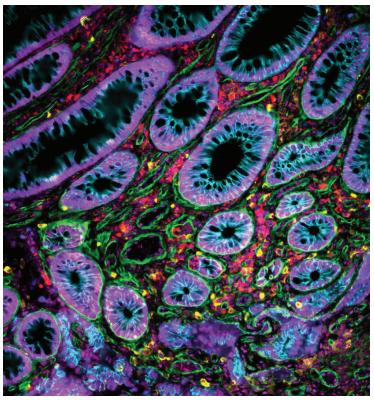
- 8-12 weeks standard project timeline from scoping to completion.
- Optional assay services, including western blot, exon sequencing, gene copy number and biomarker ID.
- FDA and IND compliant documentation to support straightforward submission

Find your KO cell lines and lysates at abcam.com/KOcells

Abcam Australia
Phone: 1800 960 553 Ordering: au.orders@abcam.com Technical: au.technical@abcam.com

abcam

Colour me in



Get Abcam colouring book

## Name of image: Lilies

**Experimental details:** Multi-epitope-ligand cartography (MELC) is a high-throughput fluorescence microscopy-based method. A tissue section is analyzed through repeated cycling of incubation with a fluorophore-labeled antibody, fluorescence imaging, and soft bleaching. This method allows staining of the same tissue section with up to 100 fluorescent markers and to analyze their toponomic expression.

**Green:** Collagen type IV, **Magenta:** Propidium iodide, **Yellow:** CD44, **Cyan:** beta-catenin, **Red:** Rac1

**Tissue sample:** Colorectal cancer

**Created by:** Miriam Jasiulionis from
Universidade Federal de São Paulo, Brazil

abcam

# Welcome to the 2020 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 2020 Young Investigator Symposium.

Special welcome to our keynote speakers: A/Prof Kimberly Kline of Nanyang Technological University, Singapore, Dr Amy Chung of Peter Doherty Institute of Infection and Immunity, University of Melbourne and Dr Con Panousis of CSL Ltd.

This is the 13th year that the VIIN has convened a symposium for young investigators but the first in virtual format. We thank VIIN program manager Dr Rebecca Smith for her tireless efforts in bringing this new format together, allowing this event to go forward.

We are indebted to many who have been vital to bringing the 2020 meeting about, namely:

- 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* Nicole Campbell, *Hudson Institute of Medical Research*
Andrew Fleetwood, *Baker Institute*
Carlo Giannangelo, *Monash Institute of Pharmaceutical Sciences*
Rhea Longley, *Walter and Eliza Hall Institute*
Emma McHugh, *University of Melbourne*
Hamish McWilliam, *University of Melbourne*
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*
Tiffany Bouchery, *Monash University*
Deb Bianco, *Victorian Infection and Immunity Network*

- Arts in Science speakers: Ms Marta de Menezes, and Dr Ryan Jefferies
- Grant Workshop Panel speakers: A/Prof Sant-Rayn Pasricha, Ms Sally Roberts, A/Prof Meredith O'Keeffe, A/Prof Michelle Tate and Prof James Beeson
- Careers Panel speakers: Dr Krystal Evans, Dr Michael Christie, Dr Jessica Borger, Dr Stephanie Tan and Dr Ariel Achtman
- The 50+ Session chairs and judges for oral presentations and posters is a reflection of the increasing popularity of this event. Thanks to each for your time an expertise.
- The sponsors and advertisers for this symposium. Your support is more and more important to the success of this event
- The 15 Academic Institutions and government agencies that support 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.

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

# Thank you to our sponsors and advertisers

abcam

BMG LABTECH
The Microplate Reader Company

**Oral Presentation prizes**

Victorian Infection & Immunity Network

abcam

**Science Bites Prizes**

millennium® science

Jomar Life Research

BMG LABTECH
The Microplate Reader Company

**Poster prizes**

Crux Biolabs

scientifix

**People's Choice Awards**

ThermoFisher SCIENTIFIC

BMG LABTECH
The Microplate Reader Company

STEMCELL™ TECHNOLOGIES

**Most Diligent Delegate Award**

**Workshop Sponsors**

abcam

BMG LABTECH
The Microplate Reader Company

GeneWorks

PerkinElmer
For the Better

**Advertiser**

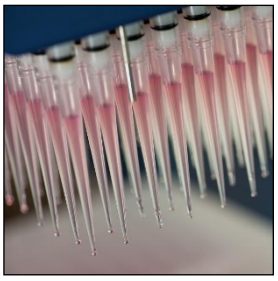
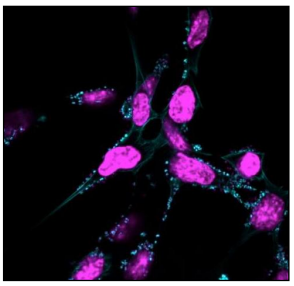
John Morris GROUP

*For links to sponsor websites:*
*https://www.viin.org.au/event/viin-young-investigator-symposium-2020*

# Victorian Infection & Immunity Network

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

The Victorian Infection and Immunity Network (VIIN) brings together researchers from diverse disciplines across various nodes within Victoria who have an interest in infection and immunity. Through events such as the Lorne Infection and Immunity Conference and annual 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:**

Walter+Eliza Hall Institute of Medical Research

MONASH University

The University of Melbourne

murdoch children's research institute

Burnet Institute
Medical Research. Practical Action.

HUDSON INSTITUTE OF MEDICAL RESEARCH

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

La Trobe Institute for Molecular Science

La Trobe University

VIDRL
Victorian Infectious Diseases Reference Laboratory

CSIRO

Swinburne University of Technology

**For enquiries contact:** info@viin.org.au

# 11th LORNE INFECTION & IMMUNITY 2021

## VIRTUAL CONFERENCE 17 - 19 FEBRUARY

**www.lorneinfectionimmunity.org**

**ORAL ABSTRACTS & CDA AWARDS DUE:** FRIDAY 23 OCTOBER 2020
**EARLY-BIRD REGISTRATION CLOSES:** FRIDAY 27 NOVEMBER 2020
**POSTER ABSTRACTS CLOSE:** TUESDAY 12 JANUARY 2021

## INTERNATIONAL SPEAKERS

**Leah Cowen,** University of Toronto, Canada
**Kate Fitzgerald,** University of Massachusetts, USA
**Akiko Iwasaki,** Yale University School of Medicine, USA
**Elizabeth Mann,** University of Manchester, UK
**Dana Philpott,** University of Toronto, Canada
**Nassos Typas,** EMBL, Germany
**Marit Vangils,** Amsterdam UMC, Netherlands
More speakers to come

## ECR Career Development Awards:

**3** on offer! Apply as you submit your abstract



**ADAPTIVE IMMUNITY • COVID-19 • HOST-PATHOGEN INTERACTIONS • INFECTIOUS DISEASES AND INFLAMMATION • INNATE IMMUNITY • MICROBES AND THE MUCOSA • MOLECULAR BASIS OF INFECTION • SYSTEMS BIOLOGY AND 'OMICS • CLINICAL • VACCINES / THERAPEUTICS / DIAGNOSTICS / PUBLIC HEALTH**

## www.lorneinfectionimmunity.org

The Annual Meeting of the

Victorian Infection & Immunity Network

AID | Australian Infectious Diseases Research Centre


# The Hartland Oration at Lorne

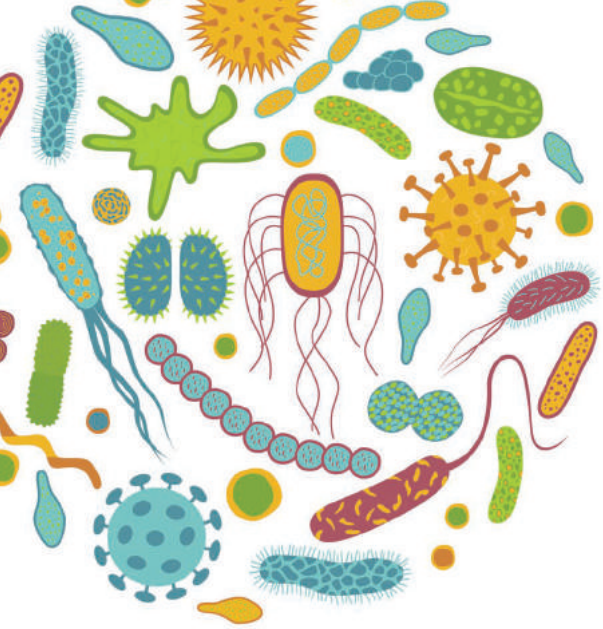
Professor Elizabeth Hartland was VIIN Co-Convenor between 2009 and 2017. Professor Hartland had key roles in co-convening the Lorne Infection and Immunity Conferences (2012-2017), the VIIN Industry Alliance (2012-2014) and the VIIN Young Investigator Symposia (2009 – 2016). Together with Co-Convenor 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 2021 Lorne Infection and Immunity Conference.

## Additional prizes at the VIIN Young Investigator Symposium

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

- ePoster 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 – stay tuned!
- Most Diligent Delegate – attend each session and answer our week-long quiz to be recognised as our Most Diligent Delegate. More details to follow.



BMG LABTECH

*The Microplate Reader Company*

# Streamline your microbial growth assays to increase your research throughput

From ELISA assays, to studying the effect of antibiotics, nutrient requirements of microbes, biofilm growth & gene expression ...

... we've got your growth assay covered!


- Measure multiple samples in a single batch, automated for walk-away solutions & tracked in real time
- Replicate specific growth conditions: Temp. & gas control
- Withstands extensive shaking & long-term kinetic assays
- Multiple wavelengths, performed in combination with other read modes - including LUM & FI
- A range of other dedicated features for a variety of assays
- Robot compatible
- Free customer support for the lifetime of your instrument
- Deliver accurate results, publication ready in free software

## Detection Modes*

UV/vis | FI | Lum | TRF | TR-FRET | AS | FP

* Availability depends on reader specifications

## BMG LABTECH microplate readers have the most sensitive & reliable performance

*"After extensive testing of five different brands of microplate readers, only BMG LABTECH instruments were found to have the sensitivity, durability and reliability required for our applications."*

*Associate Professor Christopher McDevitt, Group Leader*
*The Peter Doherty Institute for Infection and Immunity,*
*The University of Melbourne, Australia*

Download BMG LABTECH's Microbiology Brochure

For further information, an instrument demonstration or quotation request, click here.

W: www.bmglabtech.com
E: australia@bmglabtech.com.au
T: (03) 5973 4744

# Notes

**Capture and transmission of data:**

Please do not record or take photographs of any data slide in an oral presentation or Science Bite, or any data on a poster.

**Social media:**

**Slack for VIIN 2020**

Please join our Slack workspace for VIIN 2020!

Click on this link to join

Introduce yourself to other attendees in the #bio channel

The Slack workspace is a place to chat to other attendees during the online symposium

**Twitter**

We encourage you to join the conversation on Twitter with #viinyis and #VIIN2020.

Follow us @The_VIIN

Use #VIINYIS and #VIIN2020 when tweeting the symposium

*Please remember: no data! Thank you.*

# Monday 12 October: 10am – 12:45pm

| | |
|---|---|
| **10:00 – 10:10** | **Welcome and Acknowledgement of Country** |
| **10:10 – 10:40** | **Keynote Speaker I**<br>**Chair: Jason Paxman, La Trobe University, on behalf of the Organising Committee** |
| 10:10 | *Pathogenesis of Enterococcus faecalis biofilm-associated infections*<br>**Keynote speaker: A/Prof Kimberly Kline, Nanyang Technological University** |
| **10:40 – 11:10** | **Sciences Bites I**<br>**Chairs: Rebecca Ambrose, Hudson Institute of Medical Research, Carlo Giannangelo, Monash Institute of Pharmaceutical Sciences (MIPS)** |
| 10:40 | Expression and induction of the glucocorticoid-induced protein GILZ are inhibited by type I IFN<br>**Wendy Dankers, Centre for Inflammatory Diseases, Monash University School of Clinical Sciences at Monash Health, Monash University (PD)** |
| 10:43 | Exposure to *Plasmodium* sp. blood-stage impairs liver Trm cell-mediated immunity<br>**Maria de Menezes, Department of Microbiology and Immunology, Peter Doherty Institute, University of Melbourne (PD)** |
| 10:46 | Bacterial outer membrane vesicles induce mitochondrial dysfunction and intrinsic apoptosis-mediated inflammation<br>**Pankaj Deo, Biomedicine Discovery Institute, Department of Biochemistry and Molecular Biology, Monash University (PD)** |
| 10:49 | Molecular Assessment of Thoracic Empyema (MATE)<br>**Jonathan Jacobson, Infection and Immunity, Murdoch Children's Research Institute (PD)** |
| 10:52 | Using genomics to inform vaccine design against *Streptococcus pyogenes* in remote Indigenous communities<br>**Taylah James, Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunology, University of Melbourne (PG)** |
| 10:55 | Dissection of the Assembly and Function of the *Plasmodium* Export Machinery<br>**Ethan Pitman, School of Medicine, Deakin University (PG)** |
| 10:58 | Antigen-Sampler Microfold Cells Orchestrate Microbe-Immune Interactions<br>**Wang Cao, Walter and Eliza Hall institute of Medical Research (PG)** |
| 11:01 | The Role of the Short Chain Fatty Acid Butyrate in CD4+ T cell Immunity<br>**Ariane Lee, Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, University of Melbourne (PG)** |
| **11:10 – 11:25** | **Technical workshop – GeneWorks, Brenton Short**<br>Ultra sensitive immunoassays in infection and immunity |
| **11:25 – 12:25** | **Oral Presentations I**<br>**Chairs: Rebecca Ambrose, Hudson Institute & Carlo Giannangelo, MIPS** |
| 11:25 | 4D Lattice-light sheet microscopy reveals dynamic membrane reorganisation during *P.falciparum* invasion of erythrocytes<br>**Niall Geoghegan, Walter and Eliza Hall institute of Medical Research (PD)** |
| 11:35 | Molecular interplay between SARS-CoV-2 and Human proteins for viral activation and entry, potential drugs and scope for new therapeutics<br>**Naveen Vankdari, Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University (PD)** |

<-- Click on shaded box to go to abstracts

| | |
|---|---|
| 11:45 | Interferon ε as a novel regulator of intestinal homeostasis<br>**Eveline de Geus, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research (PD)** |
| 11:55 | The chromatin remodeler RSC of *C. albicans* is compositionally distinct with important roles in the pathobiology of the fungus<br>**Vinutha Balachandra, IITB-Monash Research Academy, Indian Institute of Technology Bombay, Mumbai, India & Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University (PG)** |
| 12:05 | Characterising the role of B cells in gastric cancer development<br>**Lokman Pang, Olivia Newton John Cancer Research Institute (PG)** |
| 12:15 | Evaluation of serosurveillance to understand the micro-heterogeneity of sub-microscopic malaria and other malaria risk factors in Western Cambodia.<br>**Merryn Roe, Burnet Institute & School of Public Health and Preventive Medicine, Monash University (PG)** |
| **12:25 – 12.40** | **Concluding Remarks and Daily Prize Announcement** |

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

Copyright © 2020 PerkinElmer, Inc. All rights reserved. PerkinElmer® is a registered trademark of PerkinElmer, Inc. All other trademarks are the property of their respective owners.


# The perfect combination of convenience and results

Drug development and disease prevention are a race against time – so researchers need the confidence in their biology to enable quick solutions.
AlphaLISA® Technology delivers a fast, cost-effective, no-wash ELISA alternative that reduces user error, prevents washing away weak interactions, and makes walkaway, overnight immunoassays possible. With **wider dynamic range and better sensitivity,** you'll get more confidence in your findings especially when coupled with our VICTOR® Nivo™ Alpha enabled-Multimode microplate reader for a complete assay solution.

## Main Advantages of AlphaLISA vs ELISA:

| | ELISA | AlphaLISA® |
|---|---|---|
| **Wash Steps** | Yes | No |
| **Time of Assay** | 6-8 Hours | 2-3 Hours |
| **Sample Volume** | 50-100µL | **1-5µL** |
| **Scalable** | No | Yes |
| **Dynamic Range** | ~2-3 Log | **~4-5 Log** |
| **Sensitivity** | 10pg/mL | 1pg/mL |
| **Signal Stability** | <15min | Up to 24 hours |

Contact your local sales representative for more information.

PerkinElmer®
*For the Better*

LASSalesAU@perkinelmer.com ☎ 1800 033 391 www.perkinelmer.com/au/category/alpha-reagents

# Tuesday 13 October: 10am – 12pm

| | |
|---|---|
| **10:00 – 10:10** | **Welcome and Acknowledgement of Country** |
| **10:10 – 11:50** | **Grant-writing workshop**<br>**Co-Chairs: Linda Reiling, Burnet Institute and Tiffany Bouchery, Monash University** |
| 10:10 | *Building a funding strategy*<br>**A/Prof Sant-Rayn Pasricha, Division Head, Walter and Eliza Hall Institute** |
| 10:25 | *veski opportunities & best practice when applying*<br>**Ms Sally Roberts, Fellowships Coordinator, veski** |
| 10:40 | *Preparation is key for writing ARC grant applications*<br>**Associate Professor Meredith O'Keeffe, Laboratory Head, Monash University and ARC College of Experts** |
| 10:55 | *Standing out as an ECR in the fellowship schemes*<br>**Associate Professor Michelle Tate, Hudson Institute of Medical Research and NHMRC Career Development Fellow** |
| 11:10 | *NHMRC grant schemes: tips and advice for getting funded*<br>**Professor James Beeson, Deputy Director, Burnet Institute** |
| 11:25 | **Q&A with audience** |
| **11:50 – 12:00** | **Concluding Remarks** |

# Tuesday 13 October: 1pm – 2:30pm (POSTER I)

| | |
|---|---|
| **13:00 – 13:10** | **Welcome and Acknowledgement of Country** |
| **13:10 – 14:30** | **E - POSTER SESSION I (concurrent with POSTER SESSION II)**<br>**Chairs: TBC** |
| 13:10 | Fosfomycin fails to eradicate *Klebsiella pneumoniae* in a dynamic bladder infection in vitro model.<br>**Iain Abbott, Department of Infectious Diseases, The Alfred Hospital and Central Clinical School, Monash University (PG)** |
| 13:13 | Rational design of antisense oligonucleotides modulating the activity of TLR7/8 agonists.<br>**Arwaf Alharbi, Centre for Innate Immunity and Infectious Diseases, Monash University and Hudson institute of Medical Research (PG)** |
| 13:16 | Langerhans cells drive Chronic Proliferative Dermatitis phenotype in SHARPIN mutant mice.<br>**Holly Anderton, Inflammation, Walter and Eliza Hall Institute of Medical Research (PD)** |
| 13:19 | The Development of a Novel Antimalarial Class with a Delayed Mechanism of Action.<br>**Brodie Bailey, Chemical Biology, Walter and Eliza Hall Institute of Medical Research (PG)** |
| 13:22 | Neonatal BCG vaccination induces long-term changes in circulating immune cells<br>**Samantha Bannister, Infectious Diseases Research Group (MCRI), Department of Paediatrics (UoM), Murdoch Children's Research Institute and University of Melbourne (PG)** |
| 13:25 | Tick Evasins: Inhibitors of Chemokine-mediated Inflammation<br>**Ram Bhusal, Department of Biochemistry and Molecular Biology, Monash University (PD)** |

| | |
|---|---|
| 13:28 | Synergistic ceftazidime and tobramycin combinations for clinical hypermutable *Pseudomonas aeruginosa* isolates; an innovative dosing approach to enhance bacterial killing and mitigate resistance in a dynamic biofilm model<br>**Hajira Bilal, Centre for Medicine Use and Safety, Monash Institute of Pharmaceutical Sciences, Monash University (PG)** |
| 13:31 | The effects of endogenous activin activity on the number and functional properties of testicular macrophages in adult mice<br>**Sneha Biniwale, Hudson Institute of Medical Research, Monash University (PG)** |
| 13:34 | Investigating the link between Gut Microbiota and Cerebral Malaria Pathogenesis<br>**Lars Capule, School of Life Science, Department of Physiology, Anatomy, and Microbiology, La Trobe University (Hons)** |
| 13:37 | A 'multi-omics' analysis of the novel aminobenzimidazoles reveals a depletion in haemoglobin derived peptides<br>**Matthew Challis, Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences (PG)** |
| 13:40 | Glycolipid-peptide vaccination induces liver-resident memory CD8+ T cells that protect against rodent malaria<br>**Yu Cheng Chua, Department of Microbiolgy & Immunology, Peter Doherty Institute, University of Melbourne (PG)** |
| 13:43 | Unleishing' Host Cell Death Pathways to Promote Clearance of Leishmania donovani<br>**Michelle Clark, Infectious Disease and Immune Defence, The Walter and Eliza Hall Institute of Medical Research (PG)** |
| 13:45 | Novel fluorescent TNF reporter system for characterisation of TNF expression<br>**Destiny Dalseno, Inflammation, Walter and Eliza Hall Institute (PG)** |
| 13:48 | Identifying a specific inhibitor of the invasion of red blood cells by *Plasmodium falciparum*<br>**Madeline Dans, Life Sciences, Burnet Institute (PG)** |
| 13:51 | Serum IgA inhibits HIV-specific broadly neutralising antibody Fc functions<br>**Samantha Davis, The Peter Doherty Institute for Infection and Immunity, The University of Melbourne (PG)** |
| 13:54 | The role of RIP kinases in bacterial gut infection<br>**Vik Ven Eng, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research (PG)** |
| 13:57 | The Flexible Usage of Diverse Cell Death Pathways Ensures Host Protection against *Salmonella* Typhimurium Infection<br>**Sven Engel, Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, University of Melbourne (PG)** |
| 14:00 | Investigating the role of the unfolded protein response during *Salmonella* Typhimurium infection<br>**Qingqing Fan, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research (Hons)** |
| **14:03 – 14:15** | **Concluding Remarks and Daily Prize Announcement** |

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

GeneWorks

**www.geneworks.com.au**

# Tuesday 13 October: 1pm – 2:30pm (POSTER II)

| | |
|---|---|
| **13:00 – 13:10** | **Welcome and Acknowledgement of Country**<br>**Andrew Fleetwood, Baker Institute** |
| **13:10 – 14:30** | **E - POSTER SESSION II (concurrent with POSTER SESSION I)**<br>**Chairs: Ursula Norman, Monash University and**<br>**Andrew Fleetwood, Baker Institute** |
| 13:10 | Association between antibody profile, infecting serotypes and viral load in dengue-infected patients.<br>**Jocelyn Foo, Department of Immunology and Infectious Disease, Monash University Malaysia (Hons)** |
| 13:13 | The efficacy of oral-fed geraniin as an antiviral agent against DENV-2<br>**Wen Han Foo, Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia (Hons)** |
| 13:16 | Group A streptococcal Enn proteins bind numerous human plasma proteins<br>**Hannah Frost, Infection and Immunity, Murdoch Children's Research Institute (PD)** |
| 13:19 | Investigating the cargo selection mechanism of the Plasmodium translocon of exported proteins<br>**Mikha Gabriela, Biomedical Research, Burnet Institute (PG)** |
| 13:22 | The Salmonella Efffector SseK3 Targets Small Rab GTPases<br>**Jiyao Gan, Department of Microbiology and Immunology, University of Melbourne and Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research (PG)** |
| 13:25 | Using combination immune checkpoint blockade to reinvigorate exhausted T cells in people living with HIV (PLWH) on antiretroviral therapy (ART)<br>**Celine Gubser, Peter Doherty Institute of Infection and Immunity, University of Melbourne (PD)** |
| 13:28 | Pulmonary inflammation alters the lung disposition of a PEGylated liposome based inhalable nanomedicine<br>**Shadab Haque, D4, Monash Institute of Pharmaceutical Sciences (PD)** |
| 13:31 | Evaluation of immune longevity through quantification and immunophenotyping of SARS-CoV2-specific memory B cells<br>**Gemma Hartley, Immunology and Pathology, Monash University (PG)** |
| 13:34 | Characterising a novel Type VI DNase effector and immunity protein pair from *Acinetobacter baumannii*<br>**Brooke Hayes, Department of Microbiology, Monash University (PG)** |
| 13:37 | Defining novel components of the innate immune response to unedited endogenous dsRNA<br>**Jacki Heraud-Farlow, Cancer and RNA biology lab, St Vincent's Institute (PG)** |
| 13:40 | Modulation of host immune responses by ST34 *Salmonella* typhimurium<br>**Sahampath Hettiarachchi, Department of Microbiology, Monash University and Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research (Hons)** |
| 13:43 | Lymphoma and metastatic melanoma induce phenotypic changes in fibroblastic reticular cells in secondary lymphoid organs<br>**Thomas Hueneburg, Department of Microbiology and Immunology, Peter Doherty Institute of Infection and Immunity, University of Melbourne (Masters)** |

| | |
|---|---|
| 13:46 | Influences on surgical antimicrobial prophylaxis decision making by surgical craft groups, anaesthetists, pharmacists and nurses in public and private hospitals.<br>**Courtney Ierano, National Centre of Antimicrobial Stewardship, University of Melbourne and Peter Doherty Institute (PD)** |
| 13:49 | Epigenetic profiling of B cells in food allergy<br>**Samira Imran, Murdoch Children's Research Institute (PG)** |
| 13:52 | Lactic acid produced by an optimal vaginal microbiota promotes cervicovaginal epithelial barrier integrity: implications for HIV transmission<br>**Brianna Jesaveluk, Life Sciences, Burnet Institute and Department of Microbiology, Monash University (PG)** |
| 13:55 | Antimicrobial genes are packaged, protected and transferred by bacterial membrane vesicles produced by pathogenic bacteria.<br>**Ella Johnston, Department of Physiology, Anatomy and Microbiology, La Trobe University (PG)** |
| 13:58 | Using serology to explore heterogeneity in malaria transmission in Southeast Myanmar<br>**Ellen Kearney, Life Sciences, Burnet Institute (PG)** |
| 14:01 | A New Class of Antimalarials with an Unknown Mechanism of Action<br>**Jomo Kigotho, Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences (PG)** |
| 14:04 | Integrated immune dynamics define correlates of COVID-19 severity and antibody responses<br>**Marios Koutsakos, Microbiology & Immunology, University of Melbourne, Peter Doherty Institute (PD)** |
| **14:07 – 14:15** | **Concluding Remarks and Daily Prize Announcement** |

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

# Tuesday 13 October: 7pm – 8:15pm

| | |
|---|---|
| **19:00-19:10** | **Welcome and Acknowledgement of Country**<br>**Co-Chairs: Catarina de Almeida, Peter Doherty Institute of Infection and Immunity, University of Melbourne and Nicole Campbell, Hudson Institute of Medical Research** |
| 19:10 – 20:10 | **Art in Science Evening** |
| 19:10 | *Self and Non-Self: A lifetime commitment to art and biology*<br>**Ms Marta de Menezes, Cultivamos Cultura** |
| 19:40 | *Creative Collisions: Art + Science at Science Gallery Melbourne*<br>**Dr Ryan Jefferies, Science Gallery Melbourne** |
| 20:10 – 20:15 | **Concluding Remarks** |

# Wednesday 14 October: 10am – 12pm

| | |
|---|---|
| **10:00 – 10:10** | **Welcome and Acknowledgement of Country**<br>**Linda Reiling, Burnet Institute & Rhea Longley, Walter & Eliza Hall Institute** |
| **10:10 – 11.50** | **Careers panel and Q&A** |
| 10:10 | Dr Krystal Evans, Medical Science Liaison, GSK |
| 10:25 | Dr Jessica Borger, Lecturer, Monash University |
| 10:40 | Dr Michael Christie, Principal, Shelston IP |
| 10:55 | Dr Stephanie Tan, Clinical Research Associate, IQVIA |
| 11:10 | Dr Ariel Achtman, Senior Project Manager, Movember |
| 11:25 | Q&A with audience |
| **11:50 – 12:00** | **Concluding Remarks** |

# Wednesday 14 October: 1pm – 2:30pm (POSTER III)

| | |
|---|---|
| **13:00 – 13:10** | **Welcome and Acknowledgement of Country**<br>**Ursula Norman, Monash University** |
| **13:10 – 14:30** | **E - POSTER SESSION III (concurrent with POSTER SESSION IV)**<br>**Chairs: Ursula Norman, Monash University & Andrew Fleetwood, Baker Institute** |
| 13:10 | Characterization of a new *Coxiella burnetii* effector that blocks cell death at host mitochondria<br>**Robson Kriiger Loterio, Department of Microbiology and Immunology, The University of Melbourne / University of São Paulo (PG)** |
| 13:13 | Superinfection exclusion can prevent Chikungunya infection in Aedes mosquitoes<br>**Mathilde Laureti, Vector Borne diseases, CSIRO (PG)** |
| 13:16 | Sleep disturbance and its impact on children with primary ciliary dyskinesia<br>**Rachel Leong, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne (Masters)** |
| 13:19 | Neuroimmune interaction of the sympathetic nervous system and antiviral immune responses<br>**Keit Loi, Peter Doherty Institute, University of Melbourne (PG)** |
| 13:22 | Outcomes in patients who are overweight or obese hospitalised with COVID-19: an international, multi-centre analysis<br>**Danielle Longmore, Infection and Immunity, Murdoch Children's Research Institute (PG)** |
| 13:25 | Transcriptional regulation of CCL17 production by Dexamethasone<br>**Tanya Lupancu, Department of Medicine, University of Melbourne (PG)** |
| 13:28 | Prevention of Asplenic Pneumococcal Infection (PAPI): Protecting Asplenic Children and Adults Against Pneumococcal Disease and Identifying Optimal Immunisation Regimens<br>**Sarah Luu, Faculty of Medicine, Nursing and Health Sciences, Monash University (Hons)** |

| | |
|---|---|
| 13:31 | Antibiotic exposure across pregnancy and early life, and maternal and infant metabolomic and lipidomic profile<br>**Toby Mansell, Inflammatory Origins, Murdoch Children's Research Institute (PD)** |
| 13:34 | The Immunomodulatory and Anti-Inflammatory Effects of the Dietary Compound, L-Sulforaphane<br>**Nadia Mazarakis, Faculty of Veterinary and Agricultural Sciences, University of Melbourne (PG)** |
| 13:37 | *Withdrawn* |
| 13:40 | Insights into mechanism of action of Interleukin-1 receptor associated kinase-3 (IRAK3)<br>**Trang Nguyen, La Trobe University (PG)** |
| 13:43 | CD1a-restricted T cells: a subset of "unconventional" T cells like no other<br>**Catriona Nguyen-Robertson, Department of Microbiology and Immunology, University of Melbourne, Peter Doherty Institute for Infection and Immunity (PG)** |
| 13:46 | High Seroprevalence Of Viral Hepatitis Among Animal Handlers In Abeokuta, Nigeria<br>**Adeolu Oluremi, Medical Laboratory Science, Ladoke Akintola University of Technology, Nigeria (PG)** |
| 13:49 | Fatal attractants: antibiotics that enhance neutrophil clearance of S*taphylococcus aureus* infections<br>**Jennifer Payne, Biomedicine Discovery Institute and Department of Biocheistry and Molecular Biology, Monash University (PD)** |
| 13:52 | Comparison of Two Pre-clinical Animal Models of Non-Alcoholic Steatohepatitis<br>**Cheng Peng, Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences (RA)** |
| 13:55 | Transcriptomic analysis of naïve CD4+ T cells in peanut allergic adolescents<br>**Stephen Peng, Department of Pediatrics, University of Melbourne (Hons)** |
| 13:58 | The role of citrullination on defensin function in innate defence and cancer<br>**Minuri Ratnayake, Department of Biochemistry and Genetics, La Trobe University (Masters)** |
| 14:01 | Identifying novel host resistance factors in *Toxoplasma gondii* infection<br>**Ushma Ruparel, Department of Infectious Disease and Immune Defence, The Walter and Eliza Hall Institute of Medical Research (PG)** |
| 14:04 | Investigating the role of macrophages in dying cell fragmentation<br>**Jascinta Santavanond, Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science (PG)** |
| **14:07 – 14:15** | **Concluding Remarks and Daily Prize Announcement** |

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

# Wednesday 14 October: 1pm – 2:30pm (POSTER IV)

| | |
|---|---|
| **13:00 – 13:10** | **Welcome and Acknowledgement of Country**<br>**Nicole Messina, Murdoch Children's Research Institute** |
| **13:10 – 14:30** | **E - POSTER SESSION IV (concurrent with POSTER SESSION III)**<br>**Chairs: Nicole Messina, Murdoch Children's Research Institute and Carlo Giannangelo, Monash Institute of Pharmaceutical Sciences** |
| 13:10 | High burden of reproductive tract infections and other poor sexual and reproductive health indicators in pregnant women in East New Britain Province of Papua New Guinea<br>**Michelle Scoullar, Maternal, child and adolescent health, Burnet Institute (PG)** |
| 13:13 | A synergistic exacerbation between COVID-19 and tuberculosis predicted by RNA-seq comparative profiling<br>**Dylan Sheerin, Infectious Diseases and Immune Defence , The Walter and Eliza Hall Institute of Medical Research (PD)** |
| 13:16 | Maurer's cleft tethers – is tethering important for malarial adhesion?<br>**Mohini Shibu, Biochemistry and Molecular Biology, The University of Melbourne (PG)** |
| 13:19 | Genomic surveillance for antimicrobial resistance in non-typhoidal *Salmonella enterica*<br>**Cheryll Sia, Department of Microbiology and Immunology, University of Melbourne (PG)** |
| 13:22 | Novel 3D-compounds to fight malaria in a time of drug resistance<br>**Liana Theodoridis, Department of Physiology, Anatomy and Microbiology, La Trobe University (Hons)** |
| 13:25 | Interleukin-18 is crucial to the development of 1 kidney/DOCA/salt-induced renal inflammation and elevated blood pressure<br>**Jordyn Thomas, Department of Physiology, Anatomy and Microbiology, La Trobe University (PG)** |
| 13:28 | Resolving IncL/IncM plasmid incompatibility using CRISPR-Cas9 system<br>**Aurelie Tsee, Department of Physiology, Anatomy and Microbiology, La Trobe University (PG)** |
| 13:31 | The Impacts of Influenza Virus NS1 Proteins on Viral Compatibility and Regulation of Innate Immune Responses<br>**Yeu-Yang Tseng, The WHO Collaborating Centre for Reference and Research on Influenza, The Peter Doherty Institute for Infection and Immunity, University of Melbourne (PD)** |
| 13:34 | Compatibility and Regulation of Innate Immune Responses<br>**Muhammad Ikhtear Uddin, Department of Microbiology, Biomedicine Discovery Institute, Monash University (PG)** |
| 13:37 | Novel antigen within the RPL6 protein of Plasmodium berghei confers sterile immunity against malaria in mice<br>**Ana Maria, Valencia-Hernandez, Department of Microbiology and Immunology, Peter Doherty Institute, University of Melbourne (PG)** |
| 13:40 | Establishing a High Throughput Sequencing Approach for Mapping Thymocyte-stromal Interactions During T Cell Development |

| | |
|---|---|
| | **Yi (Angela) Wang, Department of Microbiology & Immunology, University of Melbourne (Hons)** |
| 13:43 | *Salmonella* Typhimurium induces cIAP1 degradation to induce death in macrophages<br>**Madeleine Wemyss, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research (PG)** |
| 13:46 | Large genomic deletions in *Legionella pneumophila* identify new genes influencing intracellular replication and lung infection<br>**Rachelia Raissa Wibawa, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research (PG)** |
| 13:49 | Autoimmune epididymo-orchitis in the mouse: Indolamine deoxygenase-1 (Ido-1) expression and the effects of exogenous follistatin treatment<br>**Rukmali Wijayarathna, Centre for Reproductive Health, Hudson Institute of Medical Research (PD)** |
| 13:52 | Development and Homeostasis of Unconventional T cells<br>**Calvin Xu, The Peter Doherty Institute of Infection and Immunity, Department of Microbiology and Immunology, The University of Melbourne (PG)** |
| 13:55 | Untangling the roles of T follicular helper (Tfh)-germinal center (GC) response to helminth infection<br>**Aidil Zaini, Monash Biomedicine Discovery Institute, Monash University (PG)** |
| 13:58 | Unravelling immunity towards seasonal influenza vaccine in haematopoietic stem cell transplant recipients<br>**Wuji Zhang Peter Doherty Institute University of Melbourne (PG)** |
| 14:01 | The development and functional fitness of type 1 conventional dendritic cells require the transcription factor DC-SCRIPT<br>**Shengbo Zhang, Walter and Eliza hall institute and University of Melbourne (PG)** |
| 14:04 | Antimicrobial peptides and their antibiofilm effects<br>**Maryam Ghahri, Department of Biochemistry and Biotechnology, Faculty of Science, Engineering and Technology, Swinburne University of Technology (PG)** |
| **14:07 – 14:15** | **Concluding Remarks and Daily Prize Announcement** |

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

# Thursday 15 October: 10am – 12:45pm

| | |
|---|---|
| **10:00-10:10** | **Welcome and Acknowledgement of Country**<br>**Rebecca Ambrose, Hudson Institute on behalf of the Organising Committee** |
| **10:10 – 10:40** | **Keynote Speaker II**<br>**Chair: Rebecca Ambrose, Hudson Institute** |
| 10:10 | *Profiling protective humoral immunity against infectious diseases using Systems Serology*<br>**Keynote speaker: Dr Amy Chung, Doherty Institute, University of Melbourne** |
| **10:40 – 11:10** | **Sciences Bites II Chairs: Nicole Messina, Murdoch Children's Research Institute and Rebecca Ambrose, Hudson Institute** |
| 10:40 | Allergen-specific memory B cell numbers and their transcription profile are affected within 16 weeks of sublingual immunotherapy for ryegrass pollen allergy<br>**Craig McKenzie, Department of Immunology and Pathology, Central Clinical School, Monash University (PD)** |
| 10:43 | Mode of birth and risk of infection-related hospitalisation in childhood: A population cohort study of 7.17 million births from four countries<br>**Jessica Miller, Murdoch Children's Research Institute (PD)** |
| 10:46 | Functional overlap of different cell death pathways ensures host protection against intracellular bacterial pathogens<br>**Marcel Doerflinger, Walter and Eliza Hall institute of Medical Research (PD)** |
| 10:49 | Characterizing the molecular basis of *Klebsiella pneumoniae* zinc tolerance<br>**Eve Maunders, Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne (PD)** |
| 10:52 | TCR repertoire and transcriptome differs between optimal HLA-A*02:01- and high-risk HLA-A*24:02-restricted CD8+ T cell immunity against influenza A virus<br>**So Young Chang, Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, University of Melbourne (PG)** |
| 10:55 | Investigating the role of *Plasmodium falciparum* exported proteins that bind the new permeability pathway complex protein RhopH2<br>**Thorey Jonsdottir, Burnet Institute (PG)** |
| 10:58 | Is ovarian infection the missing piece in the puzzle of *Chlamydia*-associated infertility?<br>**Urooza Sarma, Department of Anatomy and Developmental Biology, Monash Biomedicine Discovery Institute, Monash University (PG)** |
| 11:01 | Anti-pneumococcal defences are altered in house-dust mite aeroallergen challenged mice<br>**Angelica Papanicolaou, School of Health and Biomedical Sciences, RMIT University (PG)** |
| **11:10 – 11:25** | **Technical workshop – BMG Labtech, Justin Robin**<br>Walk-away solutions to streamline your bacterial growth assays |
| **11:25 – 12:25** | **Oral Presentations II**<br>**Chairs: Faye Morris, Monash University and Catarina Almeida, University of Melbourne** |
| 11:25 | Quantification of membrane vesicles for investigation of their immunological properties<br>**Natalie Bitto, Department of Physiology, Anatomy and Microbiology, La Trobe University (PD)** |

| | |
|---|---|
| 11:35 | Identification of neoepitopes from tumour biopsies: how low can you go?<br>**Sri Ramarathinam, Department of Biochemistry and Molecular Biology, Infection and Immunity Program, Biomedicine Discovery Institute, Monash University (PD)** |
| 11:45 | Uncovering mammary gland-resident macrophages by 3D and intravital imaging<br>**Caleb Dawson, Walter and Eliza Hall institute of Medical Research (PD)** |
| 11:55 | A V Delta 3+ subset of MR1 reactive gamma delta T cells recognise the side of the MR1 molecule<br>**Michael Rice, Infection and Immunity Program and Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University (PG)** *(Abstract Withheld)* |
| 12:05 | The molecular basis for zinc uptake via *Streptococcus pneumoniae* AdcAII<br>**Marina Župan, Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity Peter Doherty Institute for Infection and Immunity, University of Melbourne (PG)** |
| 12:15 | Liver resident CD4 T cell in malaria infection<br>**Matthias Enders, Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, University of Melbourne (PG)** |
| **12:25 – 12.40** | **Concluding Remarks and Daily Prize Announcement** |

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

# Friday 16 October: 10am – 1pm

| | |
|---|---|
| **10:00 – 10:10** | **Welcome and Acknowledgement of Country**<br>**Emma McHugh, on behalf of the Organising Committee** |
| **10:10 – 10:40** | **Keynote Speaker III**<br>**Chair: Emma McHugh, Department of Biochemistry and Molecular Biology, University of Melbourne** |
| 10:10-10:40 | *Antibody-mediated targeting of factor XII: from bench to clinic*<br>**Keynote speaker: Dr Con Panousis, Senior Director - Head of Molecular Biology, CSL Ltd** |
| **10:40 – 11:10** | **Sciences Bites III**<br>**Chairs: Hamish McWilliam, University of Melbourne and Nicole Campbell, Hudson Institute of Medical Research** |
| 10:40 | Neural regulation of leukocyte trafficking and immunity<br>**Sapna Devi, Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, University of Melbourne (PD)** |
| 10:43 | Polymyxins bind to the cell surface of unculturable *Acinetobacter baumannii* and cause unique dependent resistance<br>**Yan Zhu, Biomedicine Discovery Institute, Monash University (PD)** |
| 10:46 | Selectively targeting the nlrp3 inflammasome to attenuate diabetes-associated atherosclerosis<br>**Arpeeta Sharma, Baker Heart and Diabetes Institute (PD)** |
| 10:49 | β-adrenergic regulation of macrophage immunometabolism<br>**Amanda Peterson, Monash Institute of Pharmaceutical Science, Monash University (PG)** |
| 10:52 | A novel role of apoptotic dendritic cells as antigen presenting entities within immune settings<br>**Amy Hodge, La Trobe Institute for Molecular Science, La Trobe University (PG)** |
| 10:55 | The epidemiology of invasive *Staphylococcus aureus* and Group A *Streptococcus* in Fiji, a prospective study from 2018-2019<br>**Li Jun Thean, Murdoch Children's Research Institute (PG)** |
| 10:58 | Using Polyhydroxyalkanoate Beads as Vaccine Delivery Carriers<br>**Devi Jenika, Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne (PG)** |
| **11:05 – 11:20** | **Technical workshop – Perkin Elmer, Dr Shima Hamidi**<br>AlphaScreen: No Wash alternatives to ELISA and Western blots |
| **11:20 – 12:20** | **Oral Presentations III**<br>**Chairs: Hamish McWilliam, University of Melbourne and Nicole Campbell, Hudson Institute of Medical Research** |
| 11:25:11:35 | The importance of MAIT cells revealed through the discovery of a rare human allele that renders MR1 unable to present microbially-derived stimulatory ligand<br>**Lauren Howson, Infection and Immunity Program and Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University (PD)** |
| 11:35-11:45 | SUR2, a novel therapeutic target for *H. pylori* associated diseases<br>**Sohinee Sarkar, Murdoch Children's Research Institute (PD)** |
| 11:45-11:55 | Discovering the anticipatory functions of innate cells<br>**Cyril Seillet, Walter and Eliza Hall Institute (PD)** |

| | |
|---|---|
| 11:55-12:05 | Investigating transcriptional correlates of naturally-acquired immunity to malaria<br>**Stephanie Studniberg, Walter and Eliza Hall Institute (PG)** |
| 12:05-12:15 | TBK1 and IKKepsilon act redundantly to mediate NF-kappaB activation downstream of Stimulator of Interferon Genes (STING)<br>**Katherine Balka, Department of Anatomy and Developmental Biology, Monash Biomedicine Discovery Institute, Monash University (PG)** |
| 12:15-12:25 | Suboptimal SARS-CoV2-specific CD8+ T-cell response associated with the prominent HLA-A*02:01 phenotype<br>**Jennifer Habel, Department of Microbiology and Immunology, University of Melbourne, at the Peter Doherty Institute for Infection and Immunity, University of Melbourne (PG)** |
| **12:25 – 12:40** | **Technical workshop – Abcam, Dr Brent Thomson**<br>Walk-away solutions to streamline your bacterial growth assays |
| **12:40 – 13:00** | **Daily and Grand prize announcement and closing remarks**<br>**Chairs: Heidi Drummer, Burnet Institute and Paul Hertzog, Hudson Institute of Medical Research** |

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

# Monday 12 October

# Monday
# 12 October

## 10:10-10:40am

# Keynote Speaker I

Click here to go back to program table

**Pathogenesis of Enterococcus faecalis biofilm-associated infections**

Associate Professor Kimberly Kline

*Nanyang Technological University*

The Gram-positive Enterococci are commensal inhabitants of the gastrointestinal tract, as well as opportunistic pathogens associated with endocarditis, urinary tract infections, and wound infection. Many Enterococcal infections are difficult to treat due to their multi-drug resistance, association with bacterial biofilms, and polymicrobial nature. The goal of our research is to understand the molecular mechanisms by which Enterococcus faecalis interacts with other bacterial species and the host in the context of these polymicrobial, biofilm-associated infections. In this talk, I will present our latest research exploring how E. faecalis modulate the host immune response to create a hospitable environment for itself and co-infecting microbes and to promote chronic wound infections.

# Monday
# 12 October

## 10:40-11:10am

# Science Bites I

Click here to go back to program table

# Expression and induction of the glucocorticoid-induced protein GILZ are inhibited by type I IFN

Wendy Dankers[1,a*], Melissa Northcott[1,a], Taylah Bennet[2], Brendan Russ[2], Wendy Zhu[2], Rochelle Sherlock[1], Akshay D'Cruz[1], Sebastian Scheer[3], Keigo Ikeda[4], Jacqueline Flynn[1], Jamie Gearing[5,6], Paul Hertzog[5,6], Eric Morand[1,b] and Sarah Jones[1,b]

*[1]Rheumatology Research Group, Centre for Inflammatory Diseases, Monash University School of Clinical Sciences at Monash Health, Clayton, Victoria, Australia, [2]Department of Microbiology, Monash University, Clayton, Victoria, Australia, [3]Infection and Immunity Program, Monash Biomedicine Discovery Institute, Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia, [4]Department of Internal Medicine and Rheumatology, Juntendo University Urayasu Hospital, Japan, [5]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Victoria, Australia, [6]Department of Molecular and Translational Sciences, Monash University, Clayton, Victoria, Australia, [a,b]contributed equally*

* = presenting author

Glucocorticoids (GC) are broadly used in the treatment of inflammatory diseases, including systemic lupus erythematosus (SLE). Despite their widespread use, most SLE patients do not reach a state of low disease activity on GC treatment. Currently it is not completely understood what factors play a role in the response to this treatment. It is thought that many anti-inflammatory effects of GC are mediated through upregulation of glucocorticoid-induced leucine zipper (GILZ). GILZ expression is decreased in the blood of SLE patients compared to healthy controls. Interestingly, we have previously shown that this is inversely correlated with the interferon (IFN) signature induced by type I IFN, including IFNα and IFNβ. Given the important role of type I IFN in SLE pathogenesis, we studied whether IFNα could suppress GILZ and thereby reduce the effectiveness of GC.

First peripheral blood mononuclear cells (PBMC) were isolated from healthy individuals and treated with 100 nM of the glucocorticoid dexamethasone (DEX), 1000 IU IFNα2a, or both. IFNα treatment reduced the expression of both GILZ mRNA and protein in human PBMC in a dose- and time-dependent manner. Interestingly, it also reduces the DEX-induced upregulation of GILZ. This corresponds to data in SLE patients, where GC treatment is less effective at inducing GILZ in patients with a high IFN score than in patients with a low IFN score. Mechanistically, we found that IFNα2a reduces GILZ expression via the Jak1/Tyk2 signaling pathway, as treatment with the specific inhibitor tosylate salt reversed the effects of IFNα2a. In public datasets, we subsequently found that the transcription factor STAT1, downstream of Jak1/Tyk2, has multiple DNA binding sites surrounding the GILZ locus. These STAT1 binding sites coincide with binding sites of the glucocorticoid receptor (GR), which may explain the mechanism by which IFNα2a reduces the DEX-induced GILZ upregulation.

In conclusion, we found that in human PBMC, IFNα2a reduces GILZ expression and the DEX-induced upregulation of GILZ via the Jak1/Tyk2 signaling pathway and potential competition between STAT1 and GR. These data reveal a potential mechanism by which type I IFN suppress the effectivity of GC, which could be targeted to improve therapeutic efficacy in SLE.

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

Maria N. de Menezes[1*], William R. Heath[1], Daniel Fernandez-Ruiz[1]

[1]*Department of Microbiology and Immunology, The Peter Doherty Institute, University of Melbourne, Parkville, VIC 3010, Australia*

Malaria parasites develop shortly in the liver before progressing to the blood and causing potentially fatal clinical disease. In endemic regions, sterilising immunity against either liver or blood stage parasites is never achieved through natural exposure to infection. We have recently demonstrated that liver-resident memory CD8 T (Trm) cells can protect efficiently against liver stage infection. These cells can form and accumulate in the liver upon successive rounds of exposure to antigen. It is therefore puzzling that liver Trm cells, accumulating over the life of people living in malaria endemic areas, do not reach a threshold where they can afford sterile protection. Trm cells are formed in the liver in response to liver stage infection and, due to the liver architecture, they are constantly exposed to the blood and to the profound alterations caused by the systemic blood-stage infection. We hypothesized that such exposure could compromise the establishment and maintenance of the liver Trm cell compartment and the protection conferred by it. Using TCR transgenic CD8 T cell lines, we have found that Trm numbers in the liver are reduced by long-exposure to blood stage infection, regardless their antigen specificity. However, the remaining Trm cells preserve their functionality, as they are still able to produce cytokines and protect against the sporozoite challenge. We found that type-I IFN, a cytokine produced during blood stage infection that has been associated with impaired liver Trm cell maintenance after malaria vaccination, was not responsible for the decay in numbers of liver Trm cells. In summary, blood-stage infection can interfere with liver Trm response by mechanisms independent of type-I IFN, potentially impairing liver Trm mediated immunity.

**Bacterial outer membrane vesicles induce mitochondrial dysfunction and intrinsic apoptosis-mediated inflammation**

**Pankaj Deo,** Seong Hoong Chow, Mei Ling Han, Subhash Dhital, Kate E. Lawlor Thomas Naderer
Biomedicine Discovery Institute, Department of Biochemistry and Molecular Biology, Monash University

*Pankaj Deo

Outer membrane vesicles (OMVs) released by Gram-negative bacteria contribute to infectious diseases. The OMV-associated endotoxin activates cytoplasmic receptors triggering macrophage death, inflammation and sepsis. We have recently shown that OMVs from pathogenic bacteria, such as *N. gonorrhoeae*, deliver protein toxins to mitochondria to kill macrophages. How OMVs that target mitochondria trigger macrophage death and whether inflammation is induced or not remain unclear. We now have deciphered that OMVs derived from pathogenic bacteria cause mitochondria dysfunction, which activates intrinsic apoptosis and the NLRP3 inflammasome. Mechanistically, mitochondrial dysfunction reduces host cell protein translation and triggers the loss of the short-lived pro-survival BCL-2 family member, MCL-1, thereby unleashing the mitochondrial death factor, BAK. BAK-induced apoptosis in OMV treated macrophages and potassium ion efflux trigger NLRP3-dependent secretion of the inflammatory cytokine, IL-1ß. Importantly, mice deficient in intrinsic apoptosis contain reduced IL-1 ß serum levels in response to *N. gonorrhoeae* OMVs. These findings identify OMVs as activators of the host cell mitochondrial apoptosis machinery to trigger both macrophage death and inflammasome activation. Collectively, our findings suggest a host immune surveillance mechanism that monitors mitochondrial health to detect pathogenic bacteria

# Molecular Assessment of Thoracic Empyema (MATE)

Jonathan Jacobson* [1,2], Loraine Fabri[1,7], Eileen Dunne[1], Joshua Osowicki[1,3,4,6], Shivanthan Shanthikumar[1, 3, 4], Anna-Maria Costa.[4], Warwick Teague[1,4], Jim Buttery[5,6], Andrew Steer [1,4], Andrew Daley[3,4], Sarath Ranganathan[1,3,4], Catherine Satzke[1,2,3]

[1] *Infection and Immunity, Murdoch Children's Research Institute, Victoria, Australia.* [2] *Department of Microbiology & Immunology, The University of Melbourne, Victoria, Australia.* [3] *Department of Paediatrics, The University of Melbourne, Victoria, Australia.* [4] *Royal Children's Hospital Melbourne, Victoria, Australia* [5] *Department of Paediatrics, Monash University, Victoria, Australia.* [6] *Monash Children's Hospital Victoria, Australia.* [7] *Department of Paediatrics, Université Libre de Bruxelles, Belgium*

**Introduction:**
Pleural empyema is a serious complication of bacterial pneumonia. Affected children often require empiric antibiotic therapy and surgical intervention to drain the purulent pleural fluid (PF) that collects around the lungs. Identifying the bacterial cause of empyema by culturing the PF is unreliable, especially with prior antibiotic use. In most cases, the bacterial cause remains unidentified, necessitating prolonged broad-spectrum antibiotics, increasing risk of side-effects and antimicrobial resistance. We have developed a multiplex-qPCR to improve identification of the bacterial causes of empyema, to direct rational antibiotic therapy.

**Methods:**
We are recruiting 150 empyema patients, under 18 years old, across two tertiary hospitals in Melbourne, and testing PF using our multiplex-qPCR. Our assay targets the most common causes of empyema including: *Streptococcus pneumoniae, Streptococcus pyogenes*, *Staphylococcus aureus* and *Haemophilus influenzae*. We will compare the performance of our multiplex-qPCR assay against culture and assess its potential clinical impact.

**Results:**
We have recruited 50 empyema patients, median age (IQR) 3.2 (2.0-4.2) years. Using our multiplex-qPCR we detected a bacterial species in 45/50 (90%) of PF samples, compared with 9/50 (18%) by culture ($p$<0,0001, Fisher's exact test). Results from the m-qPCR were 100% concordant with culture for the four target species. By multiplex-qPCR, the most common pathogen was *S. pneumoniae* (n=36, 72%), followed by *S. pyogenes* (n=6,12%), *H. influenzae* (n=2, 4%) and *S. aureus* (n=1, 2%).

**Conclusion:**
Preliminary results show our multiplex-qPCR is 5-fold more sensitive than culture in identifying the bacterial cause of pleural empyema. We will further assess the performance of our multiplex-qPCR, and its potential clinical impact as a rapid, reliable diagnostic, enabling efficient antibiotic stewardship and improved clinical outcomes for patients.

# Using genomics to inform vaccine design against *Streptococcus pyogenes* in remote Indigenous communities

Taylah James[1*], Jake Lacey[2], Mark Davies[1] and Steven Tong[2]

*[1]Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunology, The University of Melbourne [2]Doherty Department at the Peter Doherty Institute for Infection and Immunology, The University of Melbourne*

Streptococcus pyogenes (GAS) causes Rheumatic heart disease (RHD) and hyperendemic levels of impetigo in remote Indigenous communities of Northern Australia. Control programs to reduce the disease have not been effective. An effective vaccine against GAS will be essential to controlling disease prevalence and reducing morbidity and mortality.

Several vaccine candidates are in various stages of development. However, most of the epidemiology informing vaccine design is centered around high-income counties (HIC) and the theoretical coverage of these vaccines in remote regions where the disease burden is the highest is poorly understood.

In this work, we aim to better inform vaccine design by utilizing the whole genome sequences of 1,051 GAS isolates from remote Indigenous communities, spanning 15 years within the Northern Territory (NT). We screened for 3 groups of vaccines candidates; a 30mer M protein vaccine, 74 peptides and 23 whole antigens.

We found that the coverage of the 30mer vaccine in the NT would be poor, with only 50.9% of NT isolates covered compared to 80.2% of HIC isolates. Screening for vaccine peptides showed that the distribution of peptides varied significantly between NT and HIC regions. The SpyCEP peptides S2 and S2.1 were found to be the best peptide candidates with the most conserved coverage. The distribution of prevalences of the theoretical vaccine antigens was similar; with 14/23 vaccine antigens screened having prevalence levels of >90% in both endemic NT and non-endemic HIC isolates.

We concluded that the best potential vaccine candidates for coverage in the NT included; ADI, TF, Spy0762 and Spy0942 which were prevalent in >99% of all GAS isolates screened. A multiantigen vaccine formula including a combination of these antigens could theoretically increase the protective coverage of GAS isolates in hyperendemic regions like the NT and reduce the burden of RHD.

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

Ethan Pitman[1*], Callie Webb[2], Sheena McGowan[2] Kat Matthews[1] and Tania deKoning-Ward[1]

*[1]Deakin University, School of Medicine [2]Monash University, Monash Biomedicine Discovery Institute*

For *Plasmodium falciparum* parasites to effectively survive within their erythrocyte host cell and cause the disease malaria, they must export hundreds of effector proteins across an encasing membrane known as the Parasitophorous Vacuole Membrane (PVM) into the erythrocyte. 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 survive, therefore implicating PTEX as a target for possible future chemotherapeutic intervention.

PTEX is made up of three core constituents, with exported protein 2 (EXP2) being the component that forms the pore through the PVM. Recent studies have revealed that EXP2 is not only a protein export pore but is also capable of acting as a nutrient transfer pore, independent of the rest of the PTEX complex. This can be particularly seen through the capability of EXP2 to compliment the nutrient pore function of GRA17, a homolog found in the closely related parasite, *Toxoplasma gondii*.

In this study, we have used reverse genetic techniques to knockdown EXP2 in *P. falciparum* and complement with derivatives of EXP2, GRA17 and GRA17/EXP2 hybrids. We reveal that conversely to the studies in *T. gondii,* GRA17 cannot complement the function of EXP2 in *P. falciparum*. EXP2 contains a unique C-terminal acidic tail (AT) sequence, which structural studies have shown interacts with the molecular motor of PTEX. We show the AT is required for PTEX function and parasite survival and assess whether appendage of the AT to GRA17 is sufficient to allow assembly of GRA17 into the PTEX complex to facilitate protein export. We also reveal how modulating the timing of EXP2 expression, which is different to other PTEX components, impacts on parasite survival and protein export.

# Antigen-Sampler Microfold Cells Orchestrate Microbe-Immune Interactions

Wang Cao[1*], Nancy Wang[2], Richard Strugnell[2], Stephen Nutt[1] and Gabrielle Belz[1,3]

*[1] Walter and Eliza Hall institute of Medical Research, Melbourne, Australia; and Department of Medical Biology, University of Melbourne, Melbourne, Australia, [2]Peter Doherty Institute, University of Melbourne, Melbourne, Australia, [3]University of Queensland Diamantina Institute, The University of Queensland, Brisbane , Australia*

* = presenting author

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

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

# The Role of the Short Chain Fatty Acid Butyrate in CD4$^+$ T cell Immunity

Ariane Lee[1*], Annabell Bachem[1] and Sammy Bedoui[1]

[1]*Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Parkville, VIC 3010, Australia*

* = presenting author

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

We demonstrated that butyrate promotes CD4$^+$ T cell mitochondrial respiration upon activation. These metabolic changes were accompanied by an elevated T-bet expression, indicating greater polarisation into Th1 cells even under Th0 polarising conditions. Moreover, Eomes co-expression suggested differentiation into CD4$^+$ cytotoxic lymphocytes – an underappreciated but important subset that can directly eliminate target cells. This cytotoxicity was evaluated using *in vitro* killing assays, which showed that butyrate induced a 50% increase in CD4$^+$ T cell-mediated direct killing of B16 melanoma cells. This correlated with significantly upregulated expressions of IFN-$\gamma$, granzyme B and perforin. Greater polarisation of butyrate-treated CD4$^+$ T cells into Th1 cells was maintained long-term after adoptive transfer and HSV-1 infection *in vivo*. These findings highlight the importance of metabolite availability, such as butyrate, in the microenvironment for CD4$^+$ subset specification and hence immune responses to infection and cancer.

# Technical Workshop I
# GeneWorks

**Click here to go back to Program table**

## Technical Workshop I

### Ultra sensitive immunoassays in infection and immunity

Brenton Short, Scientist (Immunoassay)

*GeneWorks Pty. Ltd.*

Patients infected with SARS-CoV-2 have demonstrated a range of immune responses that correspond with COVID-19 disease severity. Examination of immune cells in COVID-19 patients with a range of symptom severities revealed that those patients with severe and critical illness demonstrated impaired type I IFN response that correlated with a continual viral load and a major inflammatory immune response. Moreover, neutralizing auto-antibodies against type I IFNs have been identified in at least 10% of patients with severe COVID-19 pneumonia but not in asymptomatic or mild cases of the disease. These findings suggest that type I IFN deficiency may be indicative of severe COVID-19 and that treatments could be targeted to address the insufficient IFN response.

# Monday
# 12 October

## 11:25am-12:25pm

# Oral Presentations I

Click here to go back to program table

BMG LABTECH

*The Microplate Reader Company*

# RT-LAMP Assays as an alternative to RT-PCR & qPCR for COVID-19 testing

**Research Paper**

## A role for Biofoundries in rapid development and validation of automated SARS-CoV-2 clinical diagnostics

Two SARS-CoV-2 detection assays based on CRISPR-Cas13a & colorimetric RT-Loop-mediated isothermal Amplification (RT-LAMP) approaches using the CLARIOstar Plus

Download Nature Communications research paper

Michael A. Crone, Miles Priestman, Marta Ciechonska, Kirsten Jensen, David J. Sharp, Arthi Anand, Paul Randell, Marko Storch & Paul S. Freemont

*Nature Communications* **11**, Article number: 4464 (2020)

For further information, an instrument demo or quotation request, click here.

### Miles Priestman,
Imperial College, UK


*"... we can track the activity of enzymes using the microplate reader... and automate work-flows with setting up experiments using our liquid handlers and moving to the plate readers automatically using our robot arms. It creates a very nice, seamless work-flow"*

### Kirsten Jensen
Imperial College, UK


*"My relationship with BMG LABTECH started in 2009..." "We tested lots of different plate readers and BMG LABTECH was our first choice". "It's not only the equipment - but also the service and support that we get from BMG LABTECH that is outstanding."*

W: www.bmglabtech.com E: australia@bmglabtech.com.au T: (03) 5973 4744

# 4D Lattice-light sheet microscopy reveals dynamic membrane reorganisation during *P.falciparum* invasion of erythrocytes

**Niall D Geoghegan[1,2], Cindy Evelyn[1], Lachlan Whitehead[1,2], Michal Pasternak[1,2], Jennifer K. Thompson[1], Julie Healer[1,2], Alan Cowman[1,2] and Kelly Rogers[1,2].**
**The Walter and Eliza Hall Institute of Medical Research[1]**
**The University of Melbourne[2]**
**1G Royal Parade, Parkville, Melbourne, VIC, Australia 3052**
**E-mail: Geoghegan.n@wehi.edu.au**

**KEY WORDS:** 4D Imaging, Lattice-light Sheet Microscopy, Live Cell Imaging, Membrane Biophysics

Invasion of erythrocytes during the asexual blood stage of malaria is an area of intense research owing to its promise a target point for anti-malarial treatments. To date significant attention has been paid to the biomolecular mechanisms that govern this process, but little research has focussed on the biophysical contributions of the host cell. Recent studies elude to the importance of the host membrane's composition and mechanical properties to successful invasion using computational modelling [1]. The invasion process has been studied by various microscopy techniques at fixed points in time [2, 3, 4], however, these studies lack the necessary temporal information to fully understand the molecular and biophysical basis for invasion in real time.

An important part of the invasion process is the formation of the parasitophorous vacuole membrane (PVM) at the point of entry to the host red blood cell. This membrane provides a physical barrier and an exchange surface between the parasite and the host cell. The formation of the PVM and subsequent remodelling of the host membrane during invasion are incredibly dynamic events and are very challenging to study in real time [5].

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

## References

[1] S. Dasgupta, "Membrane-wrapping contributions of the malaria parasite invasion of the human erythrocyte," *Biophysical Journal,* vol. 107, pp. 43-54, 2014.

[2] J. C. Volz, "Essential role of the PfRh5/PfRipr/CyRPA complex during Plasmodium Falciparum invasion of Erythrocytes," *Cell Host and Microbiome,* vol. 20, pp. 60-71, 2016.

[3] G. E. Weiss, "Revealing the Sequence and Resulting Cellular Morphology of Receptor-Ligand Interactions during Plasmodium falciparum Invasion of Erythrocytes," *Plos Pathogens,* vol. 11, no. 2, 201.

[4] D. T. Riglar, "Super-resolution dissection of coordinated events during malaria parasite invasion of the human erythrocyte," *Cell host and microbiome,* vol. 9, no. 1, pp. 9-20, 2011.

[5] C. Gruring, "Development and host cell modifications of Plasmodium Falciparum blood stages in four dimensions," *Nature Communications,* vol. 2, no. 165, 2011.

# Molecular interplay between SARS-CoV-2 and Human proteins for viral activation and entry, potential drugs and scope for new therapeutics

Naveen Vankadari[1*]

[1]*Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Victoria 3800, Australia.*

* = presenting author

The pandemic Coronavirus Disease 2019 (COVID19) caused by SARS-CoV-2 is a serious public health concern with global mortality reaching 1 million. Whilst the search for a vaccine is underway, there a several antiviral and antibody treatments being clinically evaluated to fill the "therapeutic gap". The development of potential drugs requires an understanding of SARS-CoV-2 pathogenicity and mechanism of action. Thus, it is essential to understand the full repertoire of viral proteins and their interplay with host factors. Here, we show how the SARS-CoV-2 spike protein undergoes 3 stages of processing to allow virion activation and host cell infection. Our comprehensive structural studies reveal why COVID19 is hypervirulent and the reason for the failure of several antibody treatments to date. We demonstrate via molecular dynamics and functional studies how the host proteins CD26, Furin and TMPRSS2 process the viral spike glycoprotein and assist in the viral entry in addition to ACE2. These results cognize the detailed mechanism of spike glycoprotein and reveal new avenues for potential therapeutics to block different stages of viral entry and new pathways for vaccine development.

*Key References:*

1. **Vankadari, N**. & Jacqline, A., Wilce. Emerging COVID-19: glycan shield and structure prediction of spike glycoprotein and its interaction with human CD26. *Emeging Microbes & Infections* **9**(1), 601-604 (2020).
2. **Vankadari, N**. Arbidol: A potential antiviral drug for the treatment of SARS-CoV-2 by blocking the trimerization of viral spike glycoprotein. *Int. J. Antimirobial Agents*, **56**(2),105998 (2020).
3. Qibin Liu,.... **Vankadari, N** *et al*., Arbidol treatment with reduced mortality in COVID-19 patients: a retrospective cohort study in 3 hospitals of Wuhan. *Plos Med*. (2020) (under review)
4. **Vankadari, N**. Structure of Furin protease binding to SARS-CoV-2 spike glycoprotein & implications for potential targets & virulence. *J Phy Chem Let* **11**(16), 6655-663 (2020).

# Interferon ε as a novel regulator of intestinal homeostasis

Eveline D. de Geus[1*], Jennifer S. Volaric[1,2], Gemma L. D'Adamo[1], Vanessa R. Marcelino[1], Linden J. Gearing[1], Jodee A. Gould[1,] Nicole A. de Weerd[1], Niamh E. Mangan[1], Samuel C. Forster[1], Edward M. Giles[1,2], Paul J. Hertzog[1]

*[1]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research and Department of Molecular and Translational Sciences, Monash University, Clayton, Victoria*

*[2]Department of Paediatrics, Monash University, Clayton, Victoria*

* = presenting author

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

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

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

# The chromatin remodeler RSC of *C. albicans* is compositionally distinct with important roles in the pathobiology of the fungus

Vinutha K Balachandra[1,2,3,4*], Jiyoti Verma[2], Ana Traven[2], Ralf Schittenhelm[2,3] and Santanu Ghosh[4]

*[1]IITB-Monash Research Academy, Indian Institute of Technology Bombay, Mumbai, India, [2]Infection and Immunity Program and the Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia, [3]Monash Proteomics & Metabolomics Facility, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia, [4]Department of Biosciences and Bioengineering, Indian Institute of Technology, Bombay, Mumbai, India*

* = presenting author

Diverse microorganisms thrive on and inside the human body, many of which are acquired during birth. Some of these microbes majorly exist as harmless commensals but can cause opportunistic infections with serious clinical implications when the host immune response is weakened. *Candida albicans* is one such fungal pathogen accounting for majority of the invasive infections, and a leading cause of nosocomial infections. As result of years of coevolution with its host, this pathogen has acquired a range of traits that determine its success both as a commensal and a pathogen. These include morphological plasticity and adaptive responses to various stress factors. While it is widely appreciated that precise regulation of gene expression programs is crucial for these responses, the role of factors that alter the chromatin structure are poorly explored in *C. albicans*.

Here, we identified the only essential chromatin remodelling complex in the model yeasts, RSC (Remodel the Structure of Chromatin) in *C. albicans* (CaRSC) and characterized its functions in the pathobiology of *C. albicans* by combining multi-omics with genetic and phenotypic characterization. Our biochemical (AP-MS) and genetic studies indicate substantial divergence in the composition and functions of this complex in *C. albicans*. Intriguingly, we discovered two novel subunits in the CaRSC. Transcriptomic profiling (RNA-Seq) of a conditional *rsc* mutant revealed alterations in the expression of genes involved in phenotypic switching, host pathogen interaction and stress responses in addition to growth-modulating processes. Consistent to this, deletion of a novel subunit identified here led to defective hyphae formation and, growth retardation when challenged with stress inducers *in vitro*. We also showed that deletion of a non-essential RSC subunit attenuates the systemic virulence of *C. albicans* in a mammalian host. Together, our findings attract the possibility of the CaRSC serving as a clinically relevant drug target to treat *C. albicans* infections in the future.

# Characterising the role of B cells in gastric cancer development

**Lokman Pang**[1, 2*], Frederik Masson[3], Matthias Ernst[1, 2], Jennifer Huynh[1, 2]

1. *Olivia Newton-John Cancer Research Institute, Heidelberg, VIC, Australia*
2. *La Trobe University, School of Cancer Medicine, Bundoora, VIC, Australia*
3. *Centre of Physiopathology Toulouse-Purpan, INSERM UMR 1043, Toulouse, France*

## Aims

Gastric cancer (GC) is the third leading cause of cancer-related death worldwide and affects more than 1 million individuals per year, with limited treatment options. Our laboratory has generated mounting evidence that chronic inflammation, as a result of dysregulated immune cell function, is a key driver for GC. Thus, we utilised different animal mouse models to delineate the contribution of different B cell subsets in the development of GC.

## Methods

Three mouse models with different B cell compartments were utilised in this project. The **GP130$^{F/F}$** preclinical mouse model of GC, in which the B cell compartment is intact, was used as controls. Mature B cells were ablated from GP130$^{F/F}$ mice by crossing GP130$^{F/F}$ mouse with B cell deficient uMT$^{-/-}$ mice (**GP130$^{F/F}$;uMT$^{-/-}$**). Furthermore, the contribution of plasma B cells in GC development was investigated in a mouse model of plasma cell depletion (**GP130$^{F/F}$;Blimp1$^{fl/fl}$;CD23Cre$^{+/-}$**). Tumour burden was determined at 14 weeks of age for each respective mouse model. Spleens and stomachs were harvested for downstream histology and flow cytometric analysis.

## Results

Characterisation of the **GP130$^{F/F}$;uMT$^{-/-}$** mice demonstrated that depletion of mature CD19$^{+}$ B cells protected mice from the development of gastric tumours. Furthermore, immunophenotyping of the gastric epithelium from **GP130$^{F/F}$;uMT$^{-/-}$** mice suggested that absence of B cells reduced the proportion of CD4$^{+}$ helper T cells while increasing the CD8$^{+}$ cytotoxic tumour-killing T cell population. Despite the prominent role of B cells in secreting antibodies, plasma cell depletion did not have an effect on GC growth in **GP130$^{F/F}$;Blimp1$^{fl/fl}$;CD23Cre$^{+/-}$** mice.

## Conclusion

Our results provide evidence that B cells may contribute to GC tumourigenesis by altering the development of T cells in the tumour microenvironment. Modulation of the B cell compartment alone, or in combination with standard of care chemotherapy, may represent an effective therapeutic strategy for patients with GC.

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

Merryn Roe[1,2*], Katherine O'Flaherty[1], Rupam Tripura[3], Thomas J Peto[3,5], David Price[4], Aung Pyae Phyo[1,10], Ellen Kearney[1], Herbert Opi[1,3,5], Jeremy Chalk[3], Sue J Lee[3,5], Chea Nguon[7], Mehul Dhorda[7], Richard J Maude[3,5,8], Nicholas Day[3,5], Mallika Imwong[3,9], James Beeson[1,11], Arjen M Dondorp[3,5,11], Von Lorenz Seidlein[3,5,8], Julie Simpson[4], Freya JI Fowkes[1,2,4].

*1 Burnet Institute, Australia. 2 School of Public Health and Preventive Medicine, Monash University, Australia. 3 Mahidol Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. 4 Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Australia. 5 Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK. 7 National Centre for Parasitology, Entomology, and Malaria Control (CNM), Trapeng Svay Village, Sangkat Phnom Penh Thmei, Khan Sen Sok, Phnom Penh, Cambodia. 7 WorldWide Antimalarial Resistance Network, Asia Regional Centre, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. 8 Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, MA, 02115, USA. 9 Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. 10Myanmar Oxford Clinical Research Unit, Yangon, Myanmar.11 Department of Microbiology and Central Clinical School, Monash University, Melbourne Australia.*

* = presenting author

In low transmission settings approaching malaria elimination, such as the Greater Mekong Subregion, a large proportion of *Plasmodium* spp. infections are sub-microscopic. Sub-microscopic infections are molecular-detectable low parasite density infections which go undetected, and therefore untreated, using routine field diagnostics. Antibody serosurveillance, has the potential to detect sub-microscopic infections, as both current and recent exposure events. In order understand the potential use of serosurveillance to identify sub-microscopic infections and high-risk populations in low transmission settings, we determined seroprevalence and levels of antibodies specific for *Plasmodium* spp. antigens by ELISA in 990 participants living in 20 Western Cambodian villages. Within this population sub-microscopic *Plasmodium* spp. prevalence was at 9.2% (91/990), with few (n=7) detectable by microscopy. Seroprevalence was high, with 77.7% (769/990) of the total cohort being seropositive to *Plasmodium falciparum* apical membrane antigen 1 (Pf-AMA1). Both Pf-AMA1 levels and seroprevalence varied across villages ($p<0.001$), with seroprevalence ranging from 65% (28/50) to 100% (50/50). Variation of seroprevalence was quantified using multivariate mixed effects regression which found marked heterogeneity between villages (adjusted ICC Rho [95%CI]) (0.280 [0.147, 0.467]). Risk factors for seropositivity identified from the multivariate models included the presence of sub-microscopic infections and frequency of self-reported malaria history whereby the odds of sero-positivity approximately doubled in those with current or more than 2 historical infections. The odds of sero-positivity also increased with age, but no association was found with sex, overnight forest stays, international travel, or bed net use. This study supports the utility of malaria antibodies as a serosurveillance tool to determine the micro-heterogeneity of malaria transmission in low transmission areas as well as current sub-microscopic infections and historical (clinical) infections. Its application will allow the micro-stratification of malaria risk in a population to enable spatially targeted interventions to advance progress towards the target of malaria elimination in the Greater Mekong Subregion by 2030.

# Tuesday 13 October

# Tuesday
# 13 October

## 1:10 - 2:30pm

# E-Poster Session I

Click here to go back to program table

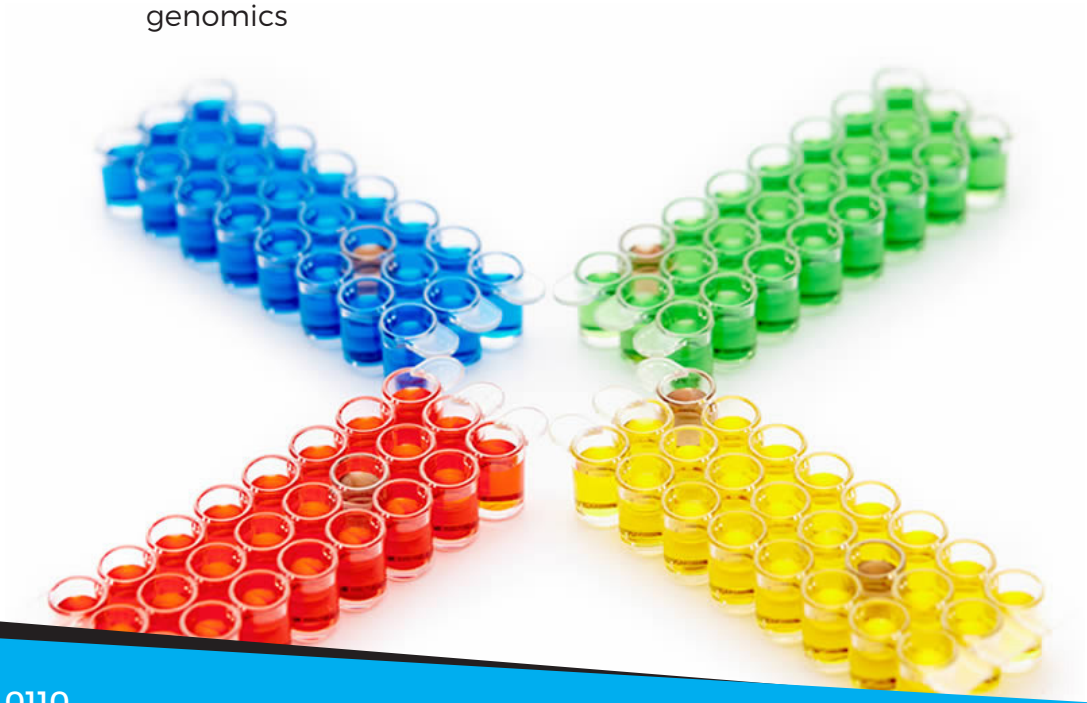
# Crux Biolabs

## Our PC2 (BSL-2) laboratory in Melbourne, Australia, is NATA accredited to ISO 17025 for clinical and pre-clinical studies.

NATA
WORLD RECOGNISED ACCREDITATION

Crux Biolabs' range of expert immunology services include:

- PBMC Processing
- Flow Cytometry
- In vitro Cell Culture
- ELISA Development
- Biomarker & Cytokine Panels
- Clinical project management, including kit production and laboratory manuals
- Co-ordination with accredited partner labs for virology and microbiology, oncology and immunotherapies and clinical genomics



+61 3 9763 0110
www.cruxbiolabs.com
enquiries@cruxbiolabs.com
Building 21, 885 Mountain Highway,
Bayswater VIC 3153 Australia

# Fosfomycin fails to eradicate *Klebsiella pneumoniae* in a dynamic bladder infection *in vitro* model

Iain J. Abbott[1*], Elke van Gorp[1] and Anton Y. Peleg[1,2]

*[1] Department of Infectious Diseases, The Alfred Hospital and Central Clinical School, Monash University, Melbourne, Victoria, Australia. [2] Infection and Immunity Program, Monash Biomedicine Discovery Institute, Department of Microbiology, Monash University, Clayton, VIC, Australia*

**Objectives**: Urinary tract infections (UTIs) are extremely common, with *Klebsiella* spp. being a frequent uropathogen. Single-dose oral fosfomycin is first-line therapy for UTIs (USA/EU) or reserved for resistant-uropathogens (Australia). Limited data, however, supports efficacy against *K. pneumoniae*.

**Methods**: Clinical *K. pneumoniae* urinary isolates underwent fosfomycin susceptibility testing by agar-dilution. Selected isolates were exposed to single-dose fosfomycin in a dynamic bladder-infection model, simulating changing urinary fosfomycin concentrations concurrently with urodynamics (continuous dilution/intermittent voiding). Standard media (Mueller-Hinton-broth with 25mg/L glucose-6-phosphate [MHB+G6P]) and synthetic human urine (SHU) were used. Low and high starting-inoculums were tested. Initial bacterial kill (>3 $\log_{10}$ reduction CFU/mL), re-growth (≥7 $\log_{10}$CFU/mL) and emergence of resistance were assessed.

**Results**: Fifty-isolates underwent fosfomycin susceptibility testing ($MIC_{50/90}$ 16/512mg/L). Fourteen representative isolates, with a range of fosfomycin MICs (MIC: 2 - >1024mg/L), and ATCC-13883 strain (MIC 64mg/L), were selected for detailed evaluation. Compared to MHB+G6P, drug-free dynamic incubation in SHU demonstrated slower growth (generation time 66.6 ±23.7min versus 29.5 ±4.3min) and reduced growth capacity (7.9 ±0.1 $\log_{10}$CFU/mL versus 8.8 ±0.2 $\log_{10}$CFU/mL). A resistant-subpopulation (RSP; MIC ≥1024mg/L) was detected in all isolates in MHB+G6P and 8-isolates in SHU (proportion $10^{-5}$-$10^{-7}$). With low starting-inoculum (5.5 ±0.3 $\log_{10}$ CFU/mL), fosfomycin exposure promoted an initial kill in 13-isolates in MHB+G6P, but only 2-isolates in SHU. Re-growth occurred in 9-isolates in MHB+G6P by 72h, compared with all isolates in SHU by 48h. Post-exposure rise in MIC occurred in 2-isolates in MHB+G6P and 1-isolate in SHU. In contrast, with high starting-inoculum (7.2 ±0.1 $\log_{10}$CFU/mL), only 3-isolates had an initial kill in MHB+G6P and 13 had re-growth after 24h (14-isolates by 48h). All re-growth isolates had a post-exposure MIC ≥1024mg/L. In SHU, initial kill occurred in 2-isolates, re-growth in 11-isolates by 24h, and in all isolates by 48h. Only 2-isolates had a rise in MIC.

**Conclusions**: Regardless of baseline MIC, fosfomycin failed to eradicate *K. pneumoniae*. *In vitro* media and inoculum are critical for fosfomycin efficacy in a simulated UTI. Reduced time to re-growth and emergence of resistance was promoted by a high starting-inoculum. Fosfomycin had reduced activity in SHU, despite slower growth-rate and reduced growth capacity in this media.

# RATIONAL DESIGN OF ANTISENSE OLIGONUCLEOTIDES MODULATING THE ACTIVITY OF TLR7/8 AGONISTS

Arwaf Alharbi[1,2*] and Michael Gantier[1,2]

[1]*Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Australia,* [2]*Department of Molecular and Translational Science, Monash University, Clayton, Victoria 3800, Australia*

Oligonucleotide-based therapeutics have become a reality, and are set to transform management of many diseases. Nevertheless, the modulatory activities of these molecules on immune responses remain incompletely defined. Here, we show that gene targeting 2′-O-methyl (2′OMe) gapmer antisense oligonucleotides (ASOs) can have opposing activities on Toll-Like Receptors 7 and 8 (TLR7/8), leading to divergent suppression of TLR7 and activation of TLR8, in a sequence-dependent manner. Through a screen of 192 2′OMe ASOs and sequence mutants, we characterized the structural and sequence determinants of these activities. Importantly, we identified core motifs preventing the immunosuppressive activities of 2′OMe ASOs on TLR7. Based on these observations, we designed oligonucleotides strongly potentiating TLR8 sensing of Resiquimod, which preserve TLR7 function, and promote strong activation of phagocytes and immune cells. We also provide proof-of-principle data that gene-targeting ASOs can be selected to synergize with TLR8 agonists currently under investigation as immunotherapies, and show that rational ASO selection can be used to prevent unintended immune suppression of TLR7. Accordingly, we propose that rational selection of TLR8-potentiating ASOs could present new opportunities in the therapeutic development of bifunctional ASOs with gene-targeting and immunostimulatory activities. Taken together, our work characterizes the immumodulatory effects of ASOs to advance their therapeutic development.
doi: 10.1093/nar/gkaa523

# Langerhans cells drive Chronic Proliferative Dermatitis phenotype in SHARPIN mutant mice

Holly Anderton*[1,2], Michael Chopin[1,2], Caleb Dawson[1,2], Stephen Nutt[1,2], Najoua Lalaloui[1,2], John Silke[1,2]

[1] *The Walter and Eliza Hall Institute for Medical Research, Parkville, Melbourne, VIC 3050, Australia*
[2] *Department of Medical Biology, University of Melbourne, Parkville, VIC 3050, Australia*

The linear ubiquitin-chain assembly complex, of which SHARPIN is a component, regulates signalling pathways from TNF superfamily and pattern recognition receptors. Loss of function in *Sharpin* impairs normal survival signaling making Sharpin mutant cells more prone to TNF induced cell death. A spontaneous mutation in the mouse *Sharpin* gene is designated cpdm due to a prominent chronic proliferative dermatitis (CPD) phenotype, though mice also have systemic inflammation. 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 prominently in the CPD pathology, though T-cells and B-cells have previously 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 used the transgenic Diphtheria Toxin Receptor system to specifically ablate CD11b$^{+}$, CCR2$^{+}$, CD11c$^{+}$, or Langerin$^{+}$ cells *in vivo.* We found that chronic systemic depletion of CCR2$^{+}$ cells delays onset of the disease but does not prevent dermatitis. However, depletion of Langerhans cells (LCs), results in a highly significant ($p<0.001$) reduction in clinical severity.

We therefore crossed the *Sharpin*$^{cpdm}$ mice to a NOVEL mouse strain that is largely absent LCs. These mice had no macroscopic dermatitis at the *Sharpin*$^{cpdm}$ endpoint. Somewhat remarkably we also observed a reduction in severity of the systemic inflammatory phenotype when LCs were absent.

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 Development of a Novel Antimalarial Class with a Delayed Mechanism of Action

Brodie Bailey[1,2*], Dr Brad Sleebs[1], Prof. Alan Cowman[1], Dr William Nguyen[1], and Dr Paul Jackson[3]

*[1] Department of Chemical Biology, Walter and Eliza Hall Institute of Medical Sciences, VIC, Australia, [2] Department of Medical Biology, University of Melbourne, VIC, Australia, [3]Janssen Pharmaceuticals, California, USA.*

* = presenting author

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

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

**References**

(1) White, N. J.; Pukrittayakamee, S.; Hien, T. T.; Faiz, M. A.; Mokuolu, O. A.; Dondorp, A. M. *Lancet (London, England)* **2014**, *383* (9918), 723–735.
(2) World Health Organization: Geneva, **2018**.
(3) Cui, L.; Mharakurwa, S.; Ndiaye, D.; Rathod, P. K.; Rosenthal, P. J. *Am. J. Trop. Med. Hyg.* **2015**, *93*, 57–68.

# Neonatal BCG vaccination induces long-term changes in circulating immune cells

Samantha Bannister[1,2,3*], Melanie Neeland[1,4], Nicole Messina[12], Susie Germano[2], Boris Novakovic[1,4], Richard Saffery[1,4] and Nigel Curtis[1,2,3]

*[1] Department of Paediatrics, The University of Melbourne; [2]Infectious Diseases Research Group, Murdoch Children's Research Institute; [3]Infectious Diseases Unit, Royal Children's Hospital; [4]Epigenetics Research Group, Murdoch Children's Research Institute*

* = presenting author

**Background**: Bacillus Calmette-Guérin (BCG), the vaccine given to protect against tuberculosis, also has beneficial non-specific effects. BCG vaccine reduces all-cause infant mortality in high-mortality settings, is used to treat some malignancies and may prevent the progression of certain autoimmune diseases. The mechanisms underlying these 'off-target' effects of BCG vaccine are hypothesised to result from immunomodulatory effects on both the innate and adaptive immune systems, including metabolic and epigenetic reprogramming of monocytes. This study aimed to characterise the long-term changes in innate immune cell profiles in infants following BCG vaccination.

**Methods**: At 13 months of age, blood samples were collected from 64 BCG-vaccinated and 68-BCG naïve infants enrolled in our randomised controlled trial of neonatal BCG vaccination ('MIS BAIR'). PBMC were isolated from these 132 infants and profiled using a 13-parameter flow cytometry panel. Major PBMC populations were characterised, including CD4+ T cells, CD8+ T cells, monocytes, natural killer (NK) cells, dendritic cells and their functional subsets. Data analysis was done using manual gating and unsupervised analysis in parallel.

**Results**: Infants who received BCG vaccine at birth had increased frequencies of both classical (median 5.03% vs. 4.36% of live PBMC, p=0.096) and non-classical (median 0.51% vs. 0.41% of live PBMC, p=0.059) monocytes compared with BCG-naïve infants at 13 months of age. While there was a similar frequency of circulating Tregs in BCG-vaccinated and BCG-naïve infants (median 2.45% vs 2.35% of live PBMC, p=0.84), BCG-vaccinated infants had a higher proportion of activated Tregs (median 34.7% vs 29.9% of Tregs, p=0.054). No differences in other key immune cell subsets were observed.

**Conclusions**: Neonatal BCG vaccination increases the proportion of circulating monocytes and induces activation of Tregs in infants. These changes persist over the first year of life. This is the first study to show that BCG vaccine induces long-term changes in circulating immune cells in children, the population in whom BCG vaccine reduces all-cause mortality.

# Tick Evasins: Inhibitors of Chemokine-mediated Inflammation

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

*1. Infection and Immunity Program, Monash Biomedicine Discovery Institute, and Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia*

* = presenting author

Inflammation is a complex immune response to tissue injury or infection. Leukocyte migration, a ubiquitous feature of inflammation, is regulated by small secreted proteins called chemokines. Ticks are hematophagous organisms that parasitize humans, livestock and both domestic and wild animals and often transmit viral or bacterial pathogens to their hosts. Ticks have been found to secrete numerous glycoproteins called Evasins that bind and block chemokine activity, enabling ticks to avoid detection and prolong blood feeding. Using bioinformatics methods, we have identified more than 250 putative Evasins and we have validated representative Evasins as chemokine-binding and inhibitory proteins. Recently, we have used structural and mutational analysis to define the requirements for selective recognition of particular chemokines. Overall, our study has provided insights into the role of tick Evasins to avoid host immune response and how Evasins can be repurposed or engineered as therapeutic anti-inflammatory agents.

**Synergistic ceftazidime and tobramycin combinations for clinical hypermutable *Pseudomonas aeruginosa isolates*; an innovative dosing approach to enhance bacterial killing and mitigate resistance in a dynamic biofilm model**

Hajira Bilal[1*], Phillip J. Bergen[1], Wee L. Lee[1], Anton Y. Peleg[2], Antonio Oliver[3], Roger L. Nation[4], Cornelia B. Landersdorfer[1,4]

*Centre for Med Use and Safety[1], MIPS, Monash Univ.; Dept. Infect. Dis.[2], Central Clinical School, Monash Univ.; Hosp. Univ. Son Espases[3], Palma de Mallorca, Spain; Drug Deliv Dispos Dynam[4], MIPS, Monash Univ., Melbourne, Australia.*

*Pseudomonas aeruginosa* chronically infects patients with cystic fibrosis and is associated with increased morbidity and mortality. Ceftazidime and tobramycin are considered first-line treatments. However, hypermutability and biofilm formation results in treatment failure due to selection of resistant mutants. We systematically investigated the pharmacodynamic effects of intravenous *versus* inhalation dosage regimens of tobramycin with and without intravenous ceftazidime in the dynamic *in vitro* CDC biofilm reactor (CBR). Two clinical hypermutable *P. aeruginosa* isolates CW30 ($MIC_{CAZ}$ 0.5 mg/L, $MIC_{TOB}$ 2 mg/L) and CW8 ($MIC_{CAZ}$ 2 mg/L, $MIC_{TOB}$ 8 mg/L) were investigated for 120 h. Clinically relevant treatments were: continuous infusion ceftazidime 9 g/day (33% lung penetration); intravenous tobramycin 10mg/kg Q24h (50% lung penetration); and tobramycin 300 mg Q12h as inhalation, and their combinations. Total and less-susceptible planktonic and biofilm bacterial counts were carried out over 120 h. All treatments in monotherapy were ineffective for both isolates, with a regrowth of planktonic (≥4.7$log_{10}$ CFU/mL) and biofilm (>6.6$log_{10}$ CFU/$cm^2$) bacteria, and amplification of less-susceptible planktonic and biofilm bacteria by 120 h. Both combination treatments demonstrated synergistic bacterial killing, not only for planktonic but also biofilm bacteria; however, greatest bacterial killing against both modes of bacterial growth was observed with the combination simulating tobramycin inhalation. In addition, the combination regimens resulted in a very substantial suppression of resistance of planktonic and biofilm bacteria to each of the antibiotics for both isolates. Thus, ceftazidime combinations with intravenous or, especially, inhaled tobramycin hold promise to treat challenging infections caused by hypermutable *P. aeruginosa* strains and warrant clinical investigation.

# The effects of endogenous activin activity on the number and functional properties of testicular macrophages in adult mice

Sneha Biniwale[1,2*], Rukmali Wijayarathna[1,2], Sudhanshu Bhushan[3], Andreas Meinhardt[1,2,3], Kate L Loveland[1,2] and Mark Hedger[1,2]

*[1]Centre for Reproductive Health, Hudson Institute of Medical Research, [2]Department of Molecular and Translational Sciences, School of Clinical Sciences, Monash University, Clayton, Victoria, Australia; [3]Institute of Anatomy and Cell Biology, Justus-Liebig-University, Giessen, Germany*

Macrophages are potent regulators of infection, inflammation and immunity, and play key roles in development, vascular remodeling and organogenesis. They are important regulators of immunological privilege in the testis, but the factors responsible for the development and maintenance of the testicular macrophage population remains to be fully elucidated. There are two major populations of testicular macrophages: tissue-resident macrophages within the interstitium and stellate-like macrophages within the peritubular layers of the seminiferous tubules. Macrophages in the sub-capsular region and rete testis, which are prime sites of disease onset in autoimmune orchitis, have received less attention. Activin A is a widely-expressed immunoregulatory cytokine of the TGF-β superfamily. It is principally produced by Sertoli cells (SC) and regulates SC and spermatogenic cell development. Activin is inhibited by an endogenous activin-binding protein, follistatin. This study investigated the effect of altered activins and follistatin on testicular macrophages using activin- and follistatin-deficient adult mice: *Inhba*$^{+/-}$ (reduced activin), *Inhba*$^{SCKO}$ (reduced activin of SC origin), and Tgh*Fst315* (reduced follistatin). Macrophages were identified and enumerated by morphometry in Bouin's-fixed, paraffin-embedded sections using an F4/80 antibody. Macrophage-related gene expression was measured by qRT-PCR in whole testis. Interstitial macrophages were more than twice as numerous as peritubular macrophages, but significant populations of macrophages were also present in the subcapsular and rete testis regions. In the *Inhba*$^{+/-}$ testis, interstitial and peritubular macrophage numbers were normal, but significant reductions were observed in subcapsular and rete testis macrophages. Some macrophage-specific chemokines and their receptors (*Csfr1*, *Ccl2*) were elevated, while others (*Cx3cr1*, *Ccr2*) were unchanged. The key macrophage-specific immunoregulatory gene, *Cd86,* also increased in expression, but *Cd80* was not altered. Macrophage numbers were not affected in the *Inhba*$^{SCKO}$ and Tgh*Fst315* testis. Interestingly, expression level of genes related to Sertoli cells gap junction, steroid synthesis and peritubular myoid cells were altered in Tgh*Fst315* testis whereas a few Sertoli cell functional genes in *Inhba*$^{SCKO}$ testis. This study indicates that activin A regulates the number and functions of macrophage subsets in the adult testis. This action is region-specific and may be dependent upon proximity to the cell of origin.

# Investigating the link between Gut Microbiota and Cerebral Malaria Pathogenesis

Lars Capule[1*], Anya Shindler[1], Sarah A Knowler[1], Rachele Gore[1, 2], Elisa L Hill-Yardin[2], Tania De Koning-Ward[3], Ashley E Franks[1] and Teresa G Carvalho[1]

*[1]Department of Physiology, Anatomy and Microbiology, La Trobe University, Melbourne, [2] RMIT University, Melbourne, [3] Deakin University, Geelong*

Malaria is the most prevalent parasitic disease worldwide, claiming half a million lives annually. Clinical presentations of human *Plasmodium* infections can range from asymptomatic to severe malaria. A subset of malaria patients progresses into severe malarial symptoms including neurological disorders such as cerebral malaria. Pathological manifestations of cerebral malaria are characterised by impaired consciousness, primarily entering an unrousable state of coma. Cerebral malaria disproportionately affects children under the age of five, yet its pathological mechanisms require further investigation. Patients with severe *Plasmodium* infections commonly report gastrointestinal symptoms including abdominal pain, vomiting and diarrhoea. The human gut microbiota plays an important role in health and disease and has been implicated in the progression of numerous enteric diseases; however, the relationship between the gut microbiota and the pathology of malaria is yet to be fully elucidated. In the present study, C57BL/6 mice were infected with *Plasmodium berghei* ANKA strain (*PbA*), an established mouse model of cerebral malaria. Faecal samples were collected daily from infected (n=5) and non-infected (n=5) mice for six days. On day six, as mice displayed onset of cerebral malaria symptoms, gut tissue was collected. Although no significant differences were observed between infected and non-infected groups in relation to body weight, small intestine length or colon lengths, the caecal weight of *PbA*-infected mice was significantly lower (*p*-value = 0.000865) compared to uninfected mice. In addition, 16S rRNA amplicon sequencing was performed on the faecal samples across all timepoints. Differences in the composition of the gut microbiota were detected between the two groups of mice. Samples of small intestine, colon, and caecum have been prepared for histopathology analysis to investigate gut morphology. Our data provide preliminary results that may connect the changes in gut structure and microbial composition with the progression of severe malaria.

# A ‘multi-omics’ analysis of the novel aminobenzimidazoles reveals a depletion in haemoglobin derived peptides

Matthew Challis[1*], Ghizal Siddiqui[1], Raymond S. Norton[2], Peter J. Scammells[2], Shane M. Devine[2] and Darren J. Creek[1]

[1]Monash Institute of Pharmaceutical Sciences, D4, Monash University, Parkville, VIC 3052,
[2]Monash Institute of Pharmaceutical Sciences, Med Chem, Monash University, Parkville, VIC 3052

* = presenting author

Current anti-malarial treatments are failing due to the emergence of resistance to the only frontline antimalarials available, the artemisinins, and therefore the identification of novel antimalarials is urgently needed. The aminobenzimidazoles (ABIs) are a novel class of antimalarial that have excellent potency against the blood stage of the malaria parasite, *Plasmodium falciparum*, and retain activity against a number of strains that are resistant to current antimalarials. However, the mechanism of action of these compounds remains unknown, limiting the scope for further development.

A multi omics study was undertaken in order to explore the mechanism of action of ABIs. An untargeted metabolomics analysis was performed on ABI-treated *P. falciparum*-infected red blood cells and following a 1 h incubation, over 600 metabolite features were detected. Multivariate analysis revealed a clustering of ABIs with mefloquine and dihydroartemisinin, primarily due to the depletion of haemoglobin-related short chain peptides. Untargeted peptidomics was performed to analyse the abundance of longer chain peptides associated with haemoglobin degradation; revealing a significant depletion of all haemoglobin-related peptides following treatment. Finally, an untargeted proteomics analysis of ABI-treated *P. falciparum* identified proteins associated with translation or transcription processes as the most significantly impacted proteins following ABI treatment.

The ABIs are a novel class of anti-malarial compounds with attractive *in vitro* potency and activity against known drug-resistant strains of *P. falciparum*. Multi-omics analysis suggests that they act to inhibit the breakdown of haemoglobin, a key pathway for the growth of the malaria parasite inside the host red blood cell as well as disrupting important transcription and translational mechanisms. Further work will investigate the molecular mechanism(s) through which this inhibition occurs.

# Glycolipid-peptide vaccination induces liver-resident memory CD8$^+$ T cells that protect against rodent malaria

Yu Cheng Chua[1*], Lauren E Holz[1,2], Geoffrey I McFadden[3],

Ian F Hermans[4,5,6], Gavin F Painter[5,7], William R Heath[1,2]

*[1]Department of Microbiology and Immunology, The Doherty Institute for Infection and Immunity, The University of Melbourne, Australia, [2]Australian Research Council Centre of Excellence in Advanced Molecular Imaging, University of Melbourne, Australia, [3]School of BioSciences, University of Melbourne, Parkville, Australia, [4]Malaghan Institute of Medical Research, Wellington, New Zealand, [5]Avalia Immunotherapies Limited, Lower Hutt, New Zealand, [6]Maurice Wilkins Centre for Molecular Biodiscovery, Wellington, New Zealand, [7]Ferrier Research Institute, Victoria University of Wellington, Lower Hutt, New Zealand.*

* = presenting author

Liver resident-memory CD8$^+$ T cells ($T_{RM}$ cells) can kill liver-stage *Plasmodium*-infected cells and prevent malaria, but simple vaccines for generating this important immune population are lacking. Here, we report the development of a fully synthetic self-adjuvanting glycolipid-peptide conjugate vaccine designed to efficiently induce liver $T_{RM}$ cells. Upon cleavage *in vivo*, the glycolipid-peptide conjugate vaccine releases an MHC I-restricted peptide epitope (to stimulate *Plasmodium*-specific CD8$^+$ T cells) and an adjuvant component, the NKT cell agonist $\alpha$-galactosylceramide ($\alpha$-GalCer). A single dose of this vaccine in mice induced large numbers of endogenous malaria-specific liver $T_{RM}$ cells that were long-lived (half-life ~425 days) and were able to maintain >90% sterile protection to day 200. These cells could be further increased in number upon vaccine boosting, and an interval of 60 days between homologous prime and boost is optimal for liver $T_{RM}$ cell generation. Our findings describe an ideal synthetic vaccine platform for generating large numbers of liver $T_{RM}$ cells for effective control of liver-stage malaria and, potentially, a variety of other hepatotropic infections.

# ‘Unleishing’ Host Cell Death Pathways to Promote Clearance of *Leishmania donovani*

Michelle P. Clark[1,2*], Marcel Doerflinger[1] and Marc Pellegrini[1]

*[1]The Walter and Eliza Hall Institute of Medical Research, [2]The University of Melbourne*

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

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

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

Our results suggest there is no role for pyroptosis as mice deficient in either Caspase-1/11/12$^{-/-}$ or Gasdermin-D$^{-/-}$ showed no difference in parasite burden compared to C57Bl/6 controls. Additionally, targeting intrinsic apoptosis using BCL-2, MCL-1, BCL-$X_L$ inhibitors also did not affect parasite burdens *in vitro*.

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

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

# Novel fluorescent TNF reporter system for characterisation of TNF expression

Destiny Dalseno[1,2*], Andreas Strasser[1,2], Philippe Bouillet[1,2], and John Silke[1,2]

*[1]The Walter and Eliza Hall Institute of Medical Research, [2]Department of Medical Biology, University of Melbourne*

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.

To generate a faithful transgenic TNF translational reporter mouse strain, we first developed and characterised fluorescent reporter constructs for validation *in vitro*. The tdTomato sequence (for optimal *in vivo* characteristics) was inserted at several locations within the ORF of the TNF genomic sequence. Using a number of tests, we selected the construct that best reports TNF gene expression while preserving TNF function. We have confirmed successful expression of the tdTomato-TNF fusion protein in cell lines, and have demonstrated membrane localization of the fusion protein. We have also shown that tdTomato-TNF is cleaved in a TACE-dependent manner, producing functional soluble TNF.

Our data support physiological behaviour of the tdTomato-TNF fusion protein, which indicates this fusion strategy represents a viable approach to produce a fluorescent TNF reporter mouse strain where functional TNF protein is preserved. The knock-in reporter mouse is currently being generated, and will permit the visualisation and observation of TNF-producing cells by a variety of approaches including intravital and live imaging. Importantly, our TNF reporter strain will constitute a novel model for *in vivo* characterization of TNF expression in physiological settings, as well as in disease states.

# Identifying a specific inhibitor of the invasion of red blood cells by Plasmodium falciparum

Madeline G. Dans[1,2*], Greta E. Weiss[1], William Nguyen[3], Zahra Razook[1,2,] Somya Mehra[1,2], Danny W. Wilson[4], Brad E. Sleebs[3], Brendan S. Crabb[1,5], Alyssa Barry[1,2], Tania de Koning Ward[2] and Paul R. Gilson[1]

*[1]Burnet Institute, Melbourne, Victoria 3004, Australia, [2]Deakin University, Geelong, VIC, Australia, [3]Walter and Eliza Hall Institute, Parkville, Victoria 3052, Australia, [4]The University of Adelaide, Adelaide, South Australia 5005, Australia, [5]The University of Melbourne, Parkville, Victoria 3010, Australia.*

* = presenting author

With emerging resistance to frontline treatments, it is vital that new antimalarial drugs are identified to target *Plasmodium falciparum*. A critical process during the parasites' lifecycle is the invasion of red blood cells (RBCs); an action that requires many unique parasite proteins that could be exploited as druggable targets. We set out to identify inhibitors of invasion by screening the MMV Pathogen Box compound library using our high-throughput luminescence-based assay. At a concentration of 2 µM, we identified 24/400 compounds that reduced invasion to <10%. Using cell-based assays and live cell microscopy, we arrived at one compound, a sulfonylpiperazine termed MMV020291, that specifically inhibited parasite invasion of RBCs. To identify the potential target(s) of MMV020291, we performed resistance selection by exposing parasites to high concentrations of the compound. Whole genome sequencing and bioinformatic analysis was conducted on three MMV020291 resistant populations revealing three non-synonymous SNPs in two genes involved in generating the force required for parasite invasion of RBCs. Currently, we are aiming to confirm these targets by genetically engineering the SNPs into wildtype parasites using CRISPR-Cas9. This study should provide insight into the mechanisms underlying parasite invasion of RBCs, in addition to advancing the development of a novel antimalarial.

# Serum IgA inhibits HIV-specific broadly neutralising antibody Fc functions

Davis SK[1*], Kent SJ[1,2,3], Chung AW[1].

*[1] Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Victoria, [2] Melbourne Sexual Health Centre, Department of Infectious Diseases, Central Clinical School, Monash University, Melbourne, Victoria, [3] ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, University of Melbourne, Melbourne, Victoria.*

* = presenting author

Macaque passive transfer studies of HIV-broadly neutralizing antibodies (BnAbs) suggest a vital role of Fc functions in protection. The importance of antibody Fc functions were highlighted in the human HIV RV144 vaccine trial, however, serum IgA reduced vaccine efficacy and protective Fc functions. Serum IgA can influence Fc effector cell functions, such as phagocytosis, via FcαR. When in complex with antigen, serum IgA can induce Fc functions, however, free IgA can inhibit Fc functions. Elucidating how serum IgA modulates Fc responses is essential. Here we endeavour to determine if serum IgA influences the Fc capacity of IgG from people living with HIV or BnAbs.

Pooled purified IgG from HIV individuals (HIVIG) along with a panel of HIV BnAbs including PGT121 and VRC01, currently in human clinical trials, were assessed for their Fc functional capacity. The influence of IgA upon IgG was assessed by adding pooled HIV-specific IgA (n=10), pooled HIV-negative IgA (n=10), IgA1 and IgA2.

HIV-specific IgA showed minor inhibition of phagocytosis (median=10.38%, IQR=8.09%, $p>0.05$). Intriguingly, significant inhibition was observed when HIV-negative IgA was added (median=21.24%, IQR=14.28%, $p<0.001$). Similarly, significant inhibition was observed with IgA1 (median=23.11%, IQR=18.18%, $p<0.001$) and IgA2 (median=19.88%, IQR=4.60%, $p<0.001$) when added to HIVIG and BnAbs. Addition of FcαR block to these assays was capable of reconstituting Fc functions, suggesting that IgA inhibition is mediated through IgA-FcαR binding.

HIV-negative serum IgA, and to a lesser extent HIV-positive IgA, reduced the functional capacity of HIVIG and BnAbs, suggesting IgA may inhibit through IgA-FcαR mediated inhibitory mechanisms. Understanding the mechanisms behind why IgA inhibits Fc responses could lead to improved future HIV vaccine design and educate passive transfer monoclonal antibody therapies. Elucidating the extent of IgA inhibition of Fc functions of different BnAbs will help inform tailor-made passive transfer treatments for HIV prevention, control and cure.

# The role of RIP kinases in bacterial gut infection

Vik Ven Eng[1,2*], Annabell Bachem[3], Nikola Baschuk[1,4], Sammy Bedoui[3], Elizabeth L. Hartland[1,4] and Jaclyn S. Pearson[1,2,4]

*[1]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, [2]Department of Microbiology, Monash University, [3]Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, [4]Department of Molecular and Translational Sciences, Monash University*

* = presenting author

Enteropathogenic *Escherichia coli* (EPEC) is a diarrhoeagenic gut bacteria that utilises a type III secretion system to translocate effector proteins into host cells and modify cell signaling. One such effector is EspL, which has been characterised to inhibit host cell necroptosis and associated inflammatory pathways by directly cleaving RIPK1 and RIPK3 at their conserved RHIM domains. This suggests a novel role for RIP kinases in the clearance of enteric bacteria.

Preliminary experiments on *Ripk1*$^{-/-}$*Ripk3*$^{-/-}$*Casp8*$^{-/-}$ mice infected with the EPEC-like mouse pathogen, *Citrobacter rodentium,* demonstrated heightened susceptibility to infection. As the physiological importance of RIP kinases remain unclear, this study sought to clarify the role of these proteins and the underlying pathway mediating exacerbated pathology in *Ripk1*$^{-/-}$*Ripk3*$^{-/-}$*Casp8*$^{-/-}$ mice during gut infection.

Examination of disease in various single and compound knockout mice revealed that both RIPK1 and RIPK3 provides protection against *C. rodentium* infection. Notably, RIPK3 plays a significant role in moderating local gut pathology. More interestingly, flow cytometry analysis of the colonic lamina propria from infected *Ripk1*$^{-/-}$*Ripk3*$^{-/-}$*Casp8*$^{-/-}$ mice showed a marked reduction in the T-helper 17 and T-regulatory cell populations, which are important for mediating bacteria-induced colitis. This is consistent with the improved disease phenotype exhibited by wildtype mice infected with an *espL* deleted *C. rodentium* mutant. Here, qPCR evaluation of inflammatory cytokine levels found an increased expression of *Il22*, but not *Il17a* in the colons of mutant infected mice compared to wildtype controls. Thus far, these results show for the first time, a link between RIPK1/3 (innate immunity) and T cell responses (adaptive immunity), which will be further investigated to better inform the significance of RIP kinases in bacterial pathogenesis and maintenance of gut homeostasis.

# The Flexible Usage of Diverse Cell Death Pathways Ensures Host Protection against Salmonella Typhimurium Infection

Sven Engel[1*], Marcel Doerflinger[2], Annabell Bachem[1], Andreas Strasser[2], Marco Herold[2] and Sammy Bedoui[1]

*[1]Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Parkville, VIC, Australia, [2]The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia*

* = presenting author

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

To investigate this, we used two different strategies. We infected host cells with *Salmonella* mutants with different potential to induce distinct forms of host cell death. This approach demonstrated how several bacterial factors such as flagellin or invasive protein A impact host cell death. Additionally, we infected mice and cells lacking individual or multiple cell death initiators and effectors to measure bacterial burdens and cell death kinetics. Surprisingly, the loss of pyroptosis, necroptosis or apoptosis alone had only minor impacts on *Salmonella* control, demonstrating that host defense can employ several cell death pathways to limit intracellular infection. However, combined deficiency of these cell death pathways caused loss of bacterial control in mice and macrophages, indicating that killing of infected cells is required for the clearance of intracellular pathogens. All together, these findings uncover a highly coordinated and flexible backup system between several programmed cell death processes that protects the host from intracellular infections.

# Investigating the role of the unfolded protein response during *Salmonella* Typhimurium infection

Qingqing Fan[1,2*], Rebecca Ambrose[1,3], Jaclyn S. Pearson[1,2,3]

*[1]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, [2]Department of Microbiology, Monash University, [3]Department of Molecular and Translational Sciences, Monash University*

* = presenting author

An important innate immune mechanism of host cells is the unfolded protein response (UPR), which aims to restore homeostasis in a cell after it encounters misfolded proteins due to increased stress in the endoplasmic reticulum. The present study explored the mechanisms through which non-typhoidal *Salmonella* Typhimurium interacts or activates UPR pathways. The study evaluated the expression of various mRNAs and proteins that are related to the three UPR activation pathways, IRE-1, PERK, and ATF6 upon infection of HT-29 colonic epithelial cells with *S.* Typhimurium. Three variants of the pathogen were used; wild-type *S.* Typhimurium, and two type III secretion system mutants (*ΔSPI-1* and *ΔSPI-*2). The study endorsed previous findings that showed SPI-1 mediators are required for the initial virulence and infection of host cells while the SPI-2 mediators ensured replication levels of the bacteria within host cells, by postponing apoptotic mechanisms that could threaten the replication and survival of the pathogen in the experimental cell line. The study further showed that *S.* Typhimurium infection activates all three UPR pathways, amongst which the IRE-1 pathway is activated at later stages of the infection. These findings suggest that *S.* Typhimurium may regulate the activation of UPR pathways to influence survival of *S.* Typhimurium inside host cells.

# Tuesday
# 13 October

## 1:10 - 2:30pm

# E-Poster Session II

Click here to go back to program table

# Millennium Science and Jomar Life Research Unite to Form Australia and New Zealand's Premier Life Science Supply Business

millennium® science

JOMAR LIFE RESEARCH

**The two companies will work closely together to offer researchers throughout Australia and New Zealand access to an extensive range of the world's most innovative technologies for life science research. Jointly the two companies represent more than one hundred leading life science product manufacturers including 10X Genomics, PacBio, BioTek, Roche, InvivoGen, Biotium, Sino Biological, and ThermoFisher, among others.**

10x GENOMICS

Combines hardware, chemistry, & software to give single cell & spatial views of biological systems.

BioTek® A part of Agilent

Life science instrumentation, including imaging & microscopy, multi-mode detection, liquid handling & automation systems.

SB Sino Biological

A world leader in recombinant technology with +6000 proteins and antibodies in stock. We also provide CRO service upon client's needs.

InvivoGen Innovation within reach

Providing life scientists globally with the largest range of high quality & innovative products for innate immunity research

PACBIO®

Highly accurate long-read sequencing provides the most comprehensive view of genomes, transcriptomes & epigenomes

Roche

Instrumentation, reagents & consumables for all researchers including life science, agriculture & applied research.

biotium

Innovative dyes for science. Expert solutions for scientists.

ThermoFisher SCIENTIFIC

eBioscience reagents for immunology, multicolor flow cytometry, and biological system analysis

**To celebrate our strategic alliance, we are giving all VIIN Young Investigator Symposium e-delegates a chance to win a stand up desk for your home office!**

Simply email us in 150 words or less how your research has adapted, changed or pivoted due to the COVID-19 pandemic. We can't wait to hear your stories!

Email entries to sarah.fardy@jlresearch.com.au by COB 16th October. Every entry gets a prize and overall winner will be contacted and announced on Twitter @mscienceaustnz and @jomar_LR!

www.mscience.com.au www.jlresearch.com.au

# Association between Antibody Profile, Infecting Serotypes and Viral Load in Dengue-Infected Patients

Huimin Foo[1*], Wen Han Foo[1], Chin Fang Ngim[2], Amreeta Dhanoa[1] and Sharifah Syed Hassan[1]

*[1]Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia,*
*[2]Clinical School Johor Bahru, Monash University Malaysia*

* = presenting author

**Background**: Dengue continues to become a global public health concern in many countries, including Malaysia. Despite extensive research, a safe and effective vaccine against dengue virus (DENV) has not been introduced. There is still a significant knowledge gap regarding the association of antibody profile and viral load which is hindering the development of dengue vaccine. Antibody dependent enhancement (ADE) is a major problem in which subneutralizing antibody titers can lead to enhanced clinical severity in patients secondarily infected with DENV. In the current study, we intend to investigate the dynamic interplay between the DENV-infecting serotype, antibody titer and viral load among patients in an endemic setting.

**Objective**: To investigate the association between antibody, infecting serotype and viral load of dengue infected patient.

**Methodology**: Twenty four serum samples collected from patients diagnosed with dengue fever in Mahmoodiah Community Clinic, Johor Bahru, Malaysia were analysed. Nested RT-PCR was performed to determine the infecting serotype. Viral load of the infecting serotype was determined by quantitative RT-PCR. $FRNT_{90,}$ a WHO-recognised gold standard technique for antibody titer quantification was conducted to determine the antibody titer of each DENV serotype.

**Results**: Although there was no statistically significant correlation between antibody titer and viral load, we observed that while high antibody titers of more than 320 conferred protection, patients with medium antibody titers between 21 to 320 were susceptible to high viral loads. A multitypic antibody profile was significantly associated with low viral load whereas a monotypic antibody profile was significantly associated with high viral load. In addition, patients with more than two antibody titers higher than 320 were significantly associated with low viral load.

**Conclusions**: The relationship between antibody titer and viral load is not linear. An intermediate antibody titer can potentially result in high viral load through a suspected mechanism of ADE. Development of vaccine should aim to produce multitypic antibody patterns with more than two high antibody titers to confer adequate protection.

Word Count: 317

# The efficacy of oral-fed geraniin as an antiviral agent against DENV-2

Wen Han Foo[1*], Sharifah Syed Hassan[1], Uma Devi Palanisamy[1], Kyi Kyi Tha[1], Vinod RMT. Balasubramaniam[1]

*[1]Jeffrey Cheah School and Health Sciences, Monash University Malaysia*

**Background**: Globally, dengue is a prevalent and debilitating mosquito-borne disease. Nonetheless, there is no effective antiviral agent to inhibit dengue virus (DENV). Geraniin, a polyphenolic compound with a myriad of health benefits seem to show some promise. Previous studies have demonstrated the efficacy of geraniin *in-vitro* and intravenous geraniin *in-vivo*. As oral route is the most convenient and economical way of drug administration, in current study, we sought to use geraniin extracted from *Nephelium lappaceum* to investigate its *in-vivo* efficacy against DENV-2 via oral administration.

**Methodology**: Male BALB/c mice aged 4-5-week-old were intraperitoneally inoculated with $10^5$ $TCID_{50}$ of non-neuroadapted DENV-2 on day 0 to establish infection. Geraniin was administered via oral gavage to the mice at 25mg/kg/day for 4 days consecutively. At day 4 post infection, mice were euthanised to obtain serum, liver and spleen. Spleen-to-body weight ratio (SBR) was calculated. DENV-2 viral load in serum and liver was quantified using qRT-PCR. Histopathology of the liver was assessed using subjective scale of semi-quantitative analysis. The efficacy of oral-fed geraniin was determined by comparing the viral load and organ damage of geraniin-treated and untreated DENV-2 infected mice.

**Results**: No significant difference in serum DENV-2 viral load and SBR was observed between treatment and non-treatment group. Liver DENV-2 viral load was below detectable level in both treatment and non-treatment group. Histologically, there was slight exacerbation of liver injury among geraniin-treated DENV-2 infected mice.

**Conclusions**: Oral-fed geraniin at 25mg/kg/day for 4 days is ineffective in reducing viral load and organ damage induced by DENV-2 in BALB/c mice. More studies can be performed to elucidate the anti-dengue properties of geraniin metabolites, influence of DENV to colonic metabolism of geraniin and optimal dosing of oral geraniin for development into a potential anti-dengue medication.

# Group A streptococcal Enn proteins bind numerous human plasma proteins

Hannah Frost[1,2*], Gwenaelle Botquin[1], Dalila Lakhloufi[1], Martina Sanderson-Smith[3], Ailish Cleary[3], Mark Davies[4], Mark Walker[5], Andrew Steer[2], Anne Botteaux[1] and Pierre Smeesters[1,2]

*[1]Molecular Bacteriology Laboratory, Université libre de Bruxelles, Brussels, Belgium, [2]Tropical Diseases Research Group, Murdoch Children's Research Institute, Melbourne, Australia, [3]Illawarra Health and Medical Research Institute and School of Chemistry and Molecular Bioscience, University of Wollongong, Wollongong, Australia, [4]Department of Microbiology and Immunology, University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, Australia, [5]Australian Infectious Diseases Research Centre and School of Chemistry and Molecular Biosciences, University of Queensland, St Lucia, Australia*

* = presenting author

Background: The Group A Streptococcus (GAS) has developed a myriad of immune evasion techniques to allow effective colonisation and infection of humans and the establishment of diverse diseases in various tissues. The binding of GAS M proteins to host proteins to induce phagocytosis resistance by immune masking and the inhibition of the complement system has been characterised to a structural level in many instances, for example binding of the complement inhibitor C4BP. However not all M proteins carry the same binding capacities and frequently M protein interactions do not account for observed whole cell binding of host proteins to GAS. The carriage of genes for the M-like protein Enn in 85% of strains, suggests a potential important role for these proteins in survival and virulence, though their binding capacities remain poorly characterized to date.

Methods: Binding motifs previously published for M proteins for albumin, fibrinogen and the Fc of immunoglobulins IgG and IgA, and hypothetical motifs for C4BP and Factor H were used to predict potential binding sites in Enn proteins. A selection of 9 Enn proteins with different predicted binding capacities were produced recombinantly.

Results: Binding of C4BP from human serum was observed by pull-down assays for 7 Enn proteins and was negative for the 2 other Enn proteins. We have mapped binding to the N-terminus of Enn for one of the C4BP binding proteins and determined essential residues required for C4BP binding using targeted mutagenesis. Preliminary investigation of further binding partners indicates Enn proteins bind albumin via the same residues as M proteins, bind either IgG in their central domain or IgA, and in some cases are able to bind fibrinogen and factor H.

Conclusions: This work suggests that Enn proteins may also play a significant role in binding of C4BP and other plasma proteins at the GAS surface. The impact of this interaction on virulence and vaccination requires further investigation.

# Investigating the cargo selection mechanism of the *Plasmodium* translocon of exported proteins

Mikha Gabriela[1,2], Dene Littler[3], Cas Boshoven[1], Brad Sleebs[4], Benjamin K. Dickerman[1], Paul R. Sanders[1], Tania F. de Koning-Ward[2], Brendan S. Crabb[1,3,5], and Paul R Gilson[1]

[1]*Burnet Institute, Melbourne* [2]*Deakin University, Waurn Ponds*

[3]*Monash University, Melbourne* [4]*Walter and Eliza Hall Institute, Melbourne*

[5]*University of Melbourne, Melbourne*

* = presenting author

To evade immune system and rapidly grow inside the erythrocyte, *Plasmodium* parasite extensively remodel its host-cell by exporting hundreds of effector proteins. These proteins are secreted out from the parasite cell into the lumen of parasitophorous vacuole, a membranous compartment where the intracellular parasite reside. In contrast to the vacuole-resident proteins which remain in the vacuole, effector proteins are further translocated across the parasitophorous vacuole membrane into the host cell by the *Plasmodium* translocon of exported proteins (PTEX), a protein-conducting channel consisting of a pore-forming protein EXP2, structural protein PTEX150, and an ATP-dependent unfoldase HSP101. It is currently unclear how PTEX selectively exports the effector proteins. Current hypothesis postulates that the N-terminal export motif of the effector proteins act as a discriminatory signal for PTEX. To test this, we characterised the substrate binding domain of HSP101 (HSP101-NTD) from two *Plasmodium* species and found that PfHSP101-NTD did not specifically interact with a model protein containing the N-terminal sequence of exported protein Pf3D7_0113300 *in vitro*, despite several possible binding pockets found on the surface of the HSP101-NTD. To validate this finding, we episomally expressed various Pf3D7_0113300 chimera with mutated export motif in parasite cells to assess their ability to be recruited to the native PTEX complex. We observed one Hyp1 PEXEL mutant that did not undergo export motif maturation and was retained in the parasite' endoplasmic reticulum (ER). Interestingly, despite not being secreted to the parasitophorous vacuole membrane where the translocon resides, the mutant interacted with a subpopulation of PTEX-free HSP101 often localized in the parasite ER. Collectively, these findings suggest that HSP101 first recruit effector proteins independent of export motif maturation in the parasite and escort them to the rest of PTEX complex in the parasitophorous vacuole membrane.

# The *Salmonella* Effector SseK3 Targets Small Rab GTPases

Jiyao Gan[1,2*], Nichollas Scott[2], Joshua Newson[2], Rachelia R. Wibawa[1,2], Tania Wong Fok Lung[2], Georgina L. Pollock[1], Garrett Z Ng[3], Ian van Driel[3], Jaclyn S Pearson[1,4], Elizabeth L Hartland[1,4] and Cristina Giogha[1,4]

*[1]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Victoria, Australia, [2]Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Victoria, Australia,[3]Department of Biochemistry and Molecular Biology, The University of Melbourne at the Bio21 Molecular Science & Biotechnology Institute, Victoria, Australia, [4]Department of Molecular and Translational Science, Monash University, Clayton, Victoria, Australia*

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

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

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

**Using combination immune checkpoint blockade to reinvigorate exhausted T cells in people living with HIV (PLWH) on antiretroviral therapy (ART)**

Celine Gubser[1*], Christine Durand[2], Ashanti Dantaranayana[1], Ajantha Solomon, Carolin Tumpach[1], Axel Kallies[1], Thomas A Rasmussen[1], Sharon R Lewin[1]

*[1]Peter Doherty Institute of immunity and infection, University of Melbourne, AU*
*[2] John Hopkins University, Baltimore, USA*

ART has dramatically improved life expectancy for PLWH but it needs to be taken lifelong and immune dysfunction persists with elevated expression of immune checkpoints (IC) including programmed death (PD-1) and cytotoxic T lymphocyte Antigen (CTLA-4). In individuals with cancer, immune checkpoint blockade (ICB) augments tumour-directed T cell responses resulting in significant clinical cures. Recently, a subset of T cells expressing the transcription regulator T cell factor 1 (TCF1) and termed precursor exhausted T cells (TPEX) were shown to be responsible for the proliferative burst and increased effector functions of CD8 T cells after ICB. Given these results, there is substantial interest in understanding whether blockade of PD-1 and/or CTLA-4 can drive recovery of HIV-specific T cell cytolytic function in PLWH on suppressive ART. In a phase one clinical trial, where PLWH on ART with cancer received ICB (anti-CTLA-4 and anti-PD1 or anti-PD1 alone), we quantified HIV-specific T cell function prior to and on at least 2 time points during treatment. We stimulated peripheral blood mononuclear cells (PBMC) from six participants who received anti-PD1 alone and in one case anti-CTLA-4 in combination with anti-PD1 with pools of overlapping HIV peptides (Gag and Nef) and measured the percentage of TNFa and IFNg producing CD8 T cells to identify polyfunctional T cells. We identified two participants who had a >2 fold increase in the frequency of HIV-specific cytokine-producing CD8 T cells following ICB, which we defined as a ‘’responder’’. Next, we determined the frequency of TPEX cells (defined as CD8+PD1+TCF1+GzmB-CD45RA-) at baseline in these six participants and found that TPEX frequency was highest in the two treatment responders (3.1% and 1.7%) compared to non-responders (mean <0.9%). Taken together we identified two participants who had an increase in polyfunctional HIV specific CD8 T cells following ICB. These same participants had a high frequency of TPEX cells at baseline, suggesting that TPEX frequency predicts an effective response to ICB. ICB can enhance HIV-specific T cell function and should be further explored as a component of cure strategies.

# Pulmonary inflammation alters the lung disposition of a PEGylated liposome based inhalable nanomedicine

Shadabul Haque[1,2*], Orlagh Feeney[1,2], Els Meeusen[3], Ben Boyd[1,2], Michelle McIntosh[1], Colin Pouton[1], Michael Whittaker[1,2], Lisa Kaminskas[1,4]

* = presenting author

[1]Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Pde, Parkville, VIC 3052, Australia
[2]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, Melbourne, VIC 3052, Australia
[3]School of Health & Life Sciences, Federation University, VIC 3842, Australia
[4]School of Biomedical Sciences, University of Queensland, St Lucia, 4072, QLD, Australia

**Purpose**: Worldwide focus on the development and clinical translation of inhalable nanomedicines is gaining increasing momentum, with a dozen inhalable liposome-based formulations currently in various stages of clinical trials for indications such as cystic fibrosis, asthma, and lung cancers. Although these nanomedicines are intended for the treatment of respiratory diseases that are commonly associated with varying degrees of local lung inflammation, we still have limited understanding about a) how pulmonary inflammation impacts on the lung clearance pathways and kinetics of the loaded drug and the liposome after inhaled delivery, and b) how the inhaled delivery of a relatively biocompatible nanomaterial impacts on the severity of underlying lung inflammation. It should be noted that both FDA and EMEA have raised concerns about the long-term safety and tolerability of inhalable liposomal formulations (e.g. Arikayce® and Linhaliq®) during clinical evaluation.

**Method**: The lung disposition and clearance kinetics of a ciprofloxacin loaded PEGylated liposome in healthy rats and rats with stable bleomycin-induced lung inflammation after intratracheal instillation were evaluated by following the drug (via LC-MS) as well as the liposome (by following structural $^{3}$H-phosphatidylcholine). In addition, changes in several markers of lung inflammation after pulmonary delivery of the liposome were also evaluated and compared.

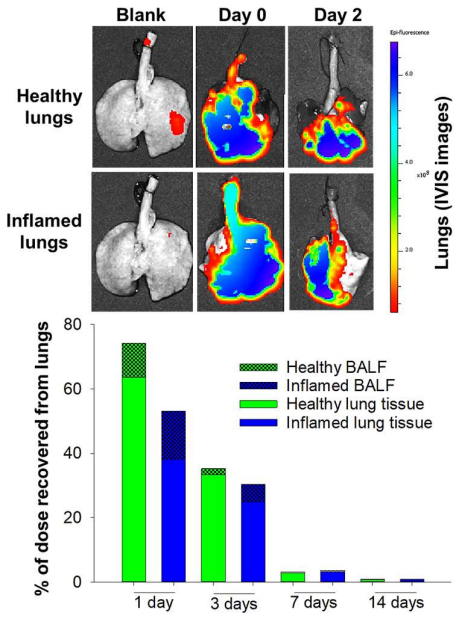



Figure 1. Lung distribution of fluorescent-labelled liposomes & proportion of the lung administered dose recovered from lungs over 2 weeks.

**Major findings**: Mucociliary clearance of liposomes was significantly compromised in rats with lung inflammation. Significant differences in lung disposition of the liposomes between healthy and inflamed lungs were also observed, with inflamed lungs exhibiting greater liposome retention in the bronchoalveolar lavage fluid and less association with lung tissue than in healthy lungs (Fig 1). Interestingly, although no significant differences were observed in the plasma pharmacokinetics of the loaded drug after pulmonary delivery, the liposome (by following $^{3}$H-labelled phosphatidylcholine) exhibited greater systemic bioavailability and more prolonged absorption (and therefore greater long term systemic exposure) from inflamed lungs when compared to healthy lungs. Finally, the pulmonary administration of liposomes to inflamed lungs led to progressive, and significant, increases in the pro-inflammatory cytokines over 2 weeks, an effect which was not evident after liposome delivery to healthy lungs. These results shed light on the possible impact of severe lung inflammation on the response of the lungs to, and pulmonary clearance of inhaled phosphatidylcholine liposome-based nanomedicines.

# Evaluation of immune longevity through quantification and immunophenotyping of SARS-CoV2-specific memory B cells

Gemma E. Hartley[1*], Stephanie Stojanovic[2], Emily S.J. Edwards[1], Pei M. Aui[1], Nirupama Varese[1,2], James McMahon[3], P. Mark Hogarth[1,3], Robyn E. O'Hehir[1,2] and Menno C. van Zelm[1,2]

*[1] Department of Immunology and Pathology, Monash University, Melbourne, VIC, Australia*

*[2] Allergy, Asthma and Clinical Immunology, Alfred Health, and Department of Allergy, Immunology & Respiratory Medicine, Central Clinical School, Monash University, Melbourne, VIC, Australia [3] Department of Infectious Diseases, Alfred Health and Monash University, Melbourne, VIC, Australia*

*[4] Centre for Biomedical Research, Burnet Institute, Melbourne, VIC, Australia*

**Background:** The antibody response to SARS-CoV2, the virus that causes Coronavirus Disease-2019 (COVID-19), is predominantly directed against the nucleocapsid (NCP) and spike proteins. Virus-specific IgG is detected from 7-9 days following symptom-onset, but in convalescence serum IgG levels decline suggesting that immunity may only be short-lived. Antibodies are the product of end-stage effector cells, many of which are short-lived, causing the rapid decline. The other arm of functional immunity lies in long-lived resting memory B (Bmem) cells, which we hypothesize are better markers of long-term immunity.

**Objective:** To identify the longevity and immunophenotype of SARS-CoV2-specific Bmem cells in COVID-19 convalescent patients.

**Methods:** Recombinant NCP and spike receptor binding domain (S1RBD) proteins were produced for ELISA based serology and biotinylated to produce fluorescent protein tetramers for flow cytometry. SARS-CoV2-specific B mem were identified by flow cytometry using the fluorescent tetramers in combination with a panel of 13 mAbs. Cells were obtained from 20 convalescent patients (1-2 months post positive PCR) and repeat samples were taken from six individuals at 4-6 months.

**Results:** The recombinant NCP and S1RBD proteins were specifically recognized by serum IgG of all patients, and not in 36 uninfected controls. NCP and S1RBD IgG were significantly lower at 4-6 months than at 1-2 months post-infection. NCP- and S1RBD-specific Bmem cells were detected in all patients, at 0.008-0.1% of total B cells corresponding to 1.25-170 cells/ml of blood. NCP- and S1RBD-specific Bmem cells predominantly expressed IgG1 with very low numbers of $IgG2^+$ and $IgG4^+$ cells, and IgG+ cell numbers trended to increase with time post-infection. A higher frequency of S1RBD-specific IgG+ Bmem was positive for CD27 than NCP-specific IgG+ Bmem and total IgG+ Bmem at 1-2 months post infection.

**Conclusion:** We here developed and validated a comprehensive flow cytometry panel to evaluate SARS-CoV2-specific Bmem cells. Detailed profiling of Bmem cells in COVID-19 convalescent patient peripheral blood revealed consistent responses to NCP and S1B with stable cell numbers and a predominant IgG1 response. This new assay with insights from our convalescent patients is a unique resource for evaluation of SARS-CoV2 humoral immunity in convalescence and in vaccination studies.

**Word count:** 348 (350 max)

# Characterising a novel Type VI DNase effector and immunity protein pair from *Acinetobacter baumannii*

Brooke K. Hayes[1*], Amy Wright[1], Jessica Lewis[1], Nyssa Drinkwater[1], Marina Harper[1], John D. Boyce[1] and Sheena McGowan[1].

[1] *Infection and Immunity Program, Monash Biomedicine Discovery Institute and Department of Microbiology Department, Monash University, Melbourne, Australia.*

* = presenting author

The opportunistic pathogen *Acinetobacter baumannii* is commonly associated with hospital-acquired bacteremia and respiratory infection. Antibiotic resistance is rapidly emerging in *A. baumannii*, posing such a global health risk that the World Health Organisation has listed it as a priority 1 critical organism where new antibiotic agents are urgently required. Many strains of *A. baumannii* express a type VI secretion system (T6SS). This molecular machine delivers antibacterial toxins directly into competitor bacteria; providing T6SS+ cells with a competitive advantage. Most strains deliver multiple T6SS toxins, and to prevent self-toxicity T6SS+ cells express cognate immunity proteins that directly bind and neutralise the effectors. One such effector-immunity pair from *A. baumannii* strain AB307 is the Type VI DNase effector 16 (Tde16) and its cognate DNase immunity protein (Tdi16). To confirm the function and activity of Tde16, attempts were made to express and purify the predicted Tde16 DNase domain in a bacterial expression system; however, protein toxicity resulted in death of the *E. coli* expression strain. Therefore, guided by homology to other DNases, a double alanine mutant at the predicted active site was constructed to produce $Tde16_{AAA}$. $Tde16_{AAA}$ was non-toxic when expressed in *E. coli* and showed no degradative activity against DNA. Purification of $Tde16_{AAA}$ confirmed the protein was monomeric and capable of binding DNA. Purification of Tdi16 showed that the two proteins directly interact with a binding stoichiometry of 1:1 and that presence of Tdi16 disrupts $Tde16_{AAA}$ DNA binding. Further investigations into the DNA binding properties of $Tde16_{AAA}$ are being conducted, as well as crystal trials with the aim to solve the structure of $Tde16_{AAA}$ alone and in combination with Tdi16. Exploring effector/immunity interactions may allow for manipulation and treatment of *A. baumannii* infections in future, and the effectors alone may also represent novel antimicrobial agents.

# Defining novel components of the innate immune response to unedited endogenous dsRNA.

Jacki Heraud-Farlow[1,2,]*, Scott Taylor[1], Alistair Chalk[1,2], Carl Walkley[1,2,3]

*[1]St.Vincent's Institute of Medical Research, Fitzroy,Victoria, Australia.; [2]Department of Medicine, St. Vincent's Hospital, University of Melbourne, Fitzroy,Victoria, Australia; [3]Mary MacKillop Institute for Health Research, Australian Catholic University, Melbourne, Victoria Australia*

* = presenting author

Adenosine-to-inosine (A-to-I) editing of double-stranded RNA (dsRNA) by ADAR proteins is a highly prevalent form of RNA base modification that is essential to distinguish between self and non-self dsRNA. The primary physiological function of ADAR1 is to edit long dsRNA structures in endogenous RNAs, resulting in a change in secondary structure. Non-edited endogenous dsRNA would otherwise be recognised as non-self/viral dsRNA by the innate immune system. In the absence of editing the cytosolic dsRNA sensor MDA5 oligomerises on endogenous dsRNAs and signals to the mitochondrial protein MAVS leading to the ongoing production of interferon-stimulated genes (ISGs) and a permanent antiviral state that is extremely detrimental. Causative mutations have been identified in both *Adar1* and *Ifih1* (MDA5) in individuals with the rare auto-inflammatory disease, Aicardi-Goutieres syndrome (AGS).

We have developed a cell culture model for Adar1-editing deficiency which recapitulates the *in vivo* biology in order to perform a genome-wide CRISPR screen for genes which modify the phenotype. I will present data describing the screening approach and the results from the screen where we have identified novel genes required for the detection of endogenous dsRNA.

# Modulation of host immune responses by ST34 *Salmonella* typhimurium

Sahampath Hettiarachchi

*Monash University -Department of Microbiology, Hudson Institute of Medical Research-CIIID*

*Salmonella* is a Gram negative, motile pathogen that infects the gut. It is classified into serovars based on its somatic and flagellar antigens. Non-typhoidal *Salmonella* (NTS) serovars are globally distributed infecting 93.8 million individuals per year. This is due to the large number of animal reservoirs in addition to poverty and a lack of hygiene in developing countries. NTS mainly causes self-limiting gastroenteritis but in certain subgroups, NTS is also capable of causing invasive bacteremia which is treated with antibiotic therapy. There are currently no recommended therapies to combat non-typhoidal disease directly. *Salmonella* 4,[5],12:i:- ST34 is a monophasic variant of *S.* Typhimurium, lacking expression of phase 2 flagella. Additionally, *Salmonella* 4,[5],12:i:- ST34 monophasic strains have also shown antibiotic resistance to many different drugs, some of which are last-line antibiotics. Over the past three decades these strains have drastically increased in prevalence worldwide. The exact reasons as to this are largely unknown. This will be the first study to produce extensive data on the host cell signalling pathways that are affected by ST34 *Salmonella* 4,[5],12:i:-. Our preliminary data suggests that loss of the second flagella antigen (FljB) in ST34 monophasic *Salmonella* 4,[5],12:i:- allows the bacteria to evade the immune system. Hence, we predict that ST34 *Salmonella* 4,[5],12:i:- will elicit an altered host immune response in comparison to biphasic *S.* Typhimurium strains. The results will give insight into the mechanisms of host signalling to intracellular pathogens in general, together with assisting in the future development of therapeutics for NTS.

# Lymphoma and metastatic melanoma induce phenotypic changes in fibroblastic reticular cells in secondary lymphoid organs

Thomas Hueneburg*[1], Yannick Alexandre[1], Sapna Devi[1], Simone Park[1], Scott Mueller[1]
*[1]Affiliation, [2]Affiliation*

[1] Department of Microbiology & Immunology, The University of Melbourne, The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia

Mounting of immune responses against pathogens involves the interaction of various cells including stromal cells that construct the lymphoid organs. Recently, stromal cells in the bone marrow have been found to be involved in accelerating tumour spread to distant organs. Here, we are used two different approaches, a B16 melanoma model and a Eμ-Myc B cell lymphoma model to examine if stromal cells in lymph nodes (LNs) contribute to tumour progression. In mice with B16 murine melanoma cells we identified macroscopic metastasis in LNs. These LNs had increased numbers of stromal cells, particularly the fibroblastic reticular cell (FRC) subpopulation when quantified via flow cytometry. In addition, inflammatory and cancer associated marker were altered in these subpopulations. Furthermore, confocal imaging further highlighted that melanoma cells metastasised into lymph nodes via an increased network of lymphatic vessel and intercalated into the FRC network. In the lymphoma model we observed an enlargement of the spleen and lymph nodes, which is a common characteristic of this disease. We also found increased numbers of FRC within both organs as well as a substantial loss of lymphocytes in the spleen. Our study provides evidence that tumour invasion drives cellular changes in the stromal microarchitecture in lymph nodes and the spleen that may accelerate tumour progression and a possibly contribute to immune suppression.

# Influences on surgical antimicrobial prophylaxis decision making by surgical craft groups, anaesthetists, pharmacists and nurses in public and private hospitals.

Courtney Ierano[1,2]*, Karin Thursky[1,2,3], Trisha Peel[1,4], Arjun Rajkhowa[1,2], Caroline Marshall[1,2,5,6] and Darshini Ayton[7]

[1] *NHMRC Centre of Research Excellence: National Centre for Antimicrobial Stewardship (NCAS), Peter Doherty Research Institute for Infection and Immunity Melbourne, Australia*

[2] *University of Melbourne, Faculty of Medicine, Dentistry and Health Sciences, Department of Medicine, Royal Melbourne Hospital, Parkville, Australia*

[3] *Department of Infectious Diseases, Peter MacCallum Cancer Centre, Melbourne, Australia*

[4] *Department of Infectious Diseases, Alfred Health/Monash University, Melbourne, Australia*

[5] *Infection Prevention and Surveillance Service, Royal Melbourne Hospital, Parkville, Australia*

[6] *Victorian Infectious Diseases Service (VIDS), Royal Melbourne Hospital, Parkville, Australia*

[7] *Monash University, Department of Epidemiology and Preventive Medicine, Melbourne, Australia*

**Best Fit category:** Other- Antimicrobial Stewardship

**Second Best Fit category:** Public Health

**Introduction:** Surgical antimicrobial prophylaxis (SAP) is a leading indication for antibiotic use in the hospital setting, with demonstrated high rates of inappropriateness. Decision-making for SAP is complex and multifactorial. A greater understanding of these factors is needed to inform the design of targeted antimicrobial stewardship interventions and strategies to support the optimization of SAP and its impacts on patient care.

**Methods:** A qualitative case study exploring the phenomenon of SAP decision-making. Focus groups were conducted with surgeons, anaesthetists, theatre nurses and pharmacists across one private and two public hospitals in Australia. Thematic analysis was guided by the Theoretical Domains Framework (TDF) and the Capabilities, Opportunities, Motivators-Behaviour (COM-B) model.

**Results:** Fourteen focus groups and one paired interview were completed. Ten of the fourteen TDF domains were identified as relevant. Thematic analysis revealed six significant themes mapped to the COM-B model, and subthemes mapped to the relevant TDF domains in a combined framework. Key themes identified were: 1) Low priority for surgical antimicrobial prophylaxis prescribing skills; 2) Prescriber autonomy takes precedence over guideline compliance; 3) Social codes of prescribing reinforce established practices; 4) Need for improved communication, documentation and collection of data for action; 5) Fears and perceptions of risk hinder appropriate SAP prescribing; and 6) Lack of clarity regarding roles and accountability.

**Conclusions:** SAP prescribing is a complex process that involves multiple professions across the pre-, intra- and post-operative surgical settings. The utilisation of behaviour change frameworks to identify barriers and enablers to optimal SAP prescribing supports future development of theory-informed antimicrobial stewardship interventions. Interventions should aim to increase surgeon engagement, enhance the prioritisation of and accountability for SAP, and address the underlying social factors involved in SAP decision-making, such as professional hierarchy and varied perceptions or risks and fears.

**Reference:** Ierano C, Thursky K, Peel T, Rajkhowa A, Marshall C, et al. (2019) Influences on surgical antimicrobial prophylaxis decision making by surgical craft groups, anaesthetists, pharmacists and nurses in public and private hospitals. PLOS ONE 14(11): e0225011. https://doi.org/10.1371/journal.pone.0225011

# Epigenetic profiling of B cells in food allergy

Samira Imran[1]*, Melanie Neeland[1], Kirsten Perrett[1,2], Boris Novakovic[1], Richard Saffery[1], SchoolNuts Investigator Team

*[1]Murdoch Children's Research Institute, and Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Flemington Road, Parkville, Victoria 3052, Australia*

*[2]Department of Allergy and Immunology, Royal Children's Hospital, Melbourne, Australia*

IgE-mediated food allergy (FA) rates have rapidly increased in the last few decades. Allergy to more than one food (multi-food allergyor MFA) can be up to 30% in those with food allergy, with risk of severe allergic reactions highest in adolescents. The molecular profiles of B-cells in food allergic or MFA individuals has been investigated minimally.

We profiled B-cell populations from adolescents aged 10-14 years (SchoolNuts, n=27), generating genome-wide methylation data from >850,000 sites from a cross-sectional cohort of 10 peanut-allergic adolescents (single-food allergic or SFA), 8 peanut-allergic adolescents sensitised to one or more foods (MFA) and 9 non-allergic controls (NA). We observed 144 differentially methylated probes (DMPs) in allergic individuals compared to controls. Eleven of these probes were associated with differentially methylated region near vtRNA-2, a meta-stable epigenetic locus. FA compared to NA groups separately showed increased numbers of DMPs (SFA vs NA – 192 DMPS; MFA vs NA – 335 DMPs) suggesting the presence of group-specific methylation patterns. An additional 318 DMPs are revealed when comparing SFA to MFA individuals. In SFA individuals, probes near genes associated with myeloid cell function and differentiation (CD1d, PSMD5 and DCSTAMP) show differential methylation. In MFA individuals we identified differential methylation in probes near genes involved with antigen presentation (HLA-DQB2, HLA-DRB1), immune regulation (MAP3K1, MAPK9) and cytokine response (CCL25, IFNAR2).

Our results show unique epigenetic profiles between NA, SFA and MFA individuals which may provide insight into the molecular basis of severe food allergy and pathways underlying co-sensitization to multiple food allergens.

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

David J Delgado-Diaz[1,2], Brianna Jesaveluk[1,2]*, David Tyssen[1], Adam Johnson[1], Joshua A Hayward[1,2], Raffi Gugasyan[1,3], Anna C Hearps[1,4] and Gilda Tachedjian[1,2,5]

*[1]Disease Elimination Program and Life Sciences Discipline, Burnet Institute, Melbourne, VIC, Australia, [2]Department of Microbiology, Monash University, Clayton, VIC, Australia, [3]Department of Immunology, Monash University, Melbourne, VIC, Australia, [4]Department of Infectious Diseases, Monash University, Melbourne, VIC, Australia, [5]Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, VIC, Australia.*

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

The effect of L- and D-LA on epithelial barrier integrity was analysed by culturing cervicovaginal epithelial (Ect) cells in a transwell system. Ect cells were treated apically for 1 h with either 0.3% L-LA or D-LA (at pH 3.9 or pH 7.0), or acidity alone control (pH 3.9, HCl adjusted). Transepithelial electrical resistance (TEER) across the cell monolayer was determined prior to and 24 h post-treatment. Expression of junctional molecule mRNA after L or D-LA treatment was determined by RNASeq and qRT-PCR, and protein levels were determined by Western blot.

Treatment of Ect cells with L- or D-LA significantly increased TEER by 1.5 fold ($n= 4$; $p<0.05$), in contrast to treatment with neutralized L- or D-LA (pH 7.0), or the pH 3.9 control. RNASeq and gene ontology enrichment analysis were consistent with the TEER functional data demonstrating that L- and D-LA caused significant differential expression (FDR<0.05) of at least 11 genes associated with intracellular junctions and barrier function, including claudin-1 (CLDN1, Log2 fold change L-LA 1.12/ D-LA 1.17), claudin-4 (CLDN4, Log2FC 1.47/1.63) and occludin (OCLN, Log2FC 0.49/0.55), with no differential gene expression between isoforms. These findings were confirmed by qRT-PCR. In addition, tight junction protein levels (such as CLDN1 and TJP2), were significantly increased by L-LA treatment (CLDN1 FC = 1.56, TJP2 FC = 1.42) but not with the pH 3.9 control ($n= 5$; $p<0.05$).

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

# Antimicrobial genes are packaged, protected and transferred by bacterial membrane vesicles produced by pathogenic bacteria.

Ella L. Johnston[1,2*], Emily Gulliver[3] and Steve Petrovski[1], Samuel Forster[3], Maria Kaparakis-Liaskos[1,2]

*[1]Department of Physiology, Anatomy and Microbiology, La Trobe University, Bundoora, Victoria, Australia, [2]Research Centre for Extracellular Vesicles, School of Molecular Sciences, La Trobe University, Bundoora, Australia, [3]Centre for Innate Immunity and Infectious Diseases, Hudson Institute for Medical Research, Victoria, Australia*

* = presenting author

Bacterial acquisition of antimicrobial resistance genes (ARGs) via horizontal gene transfer (HGT) has contributed to the rise of superbugs globally. Although there are multiple known mechanisms used by bacteria to transfer ARGs, a less explored mechanism involves bacterial membrane vesicles (BMVs). BMVs are released by all bacteria as part of their normal growth and are rich in DNA. In this study, we examined the ability of BMVs produced by opportunistic pathogens and the human gut microbiota to contribute to the spread of ARGs.

We found that BMVs released by *Pseudomonas aeruginosa* grown in laboratory conditions contained plasmid DNA encoding for ARGs. This DNA was protected from degradation by DNases. BMVs containing plasmid DNA could mediate HGT, resulting in antibiotic-resistant transformants. By comparison, plasmid DNA alone was less efficient in the transformation of recipient bacteria. BMVs isolated from *P. aeruginosa* grown as a biofilm packaged more plasmid ARGs compared to BMVs isolated from bacteria grown in laboratory conditions. The transformation efficiency of biofilm-derived BMVs is currently being determined.

Furthermore, as the transfer of ARGs via BMVs within mixed microbial cultures, such as the human gut microbiota has not been examined, we next investigated the ability of BMVs produced by the human gut microbiota to contain ARGs. The human gut microbiome is a reservoir for ARGs, and it is proposed that commensal organisms of the gut can transfer ARGs to pathogenic or opportunistic organisms. As BMVs have already been demonstrated to contribute to the dissemination of ARGs, we hypothesize that BMVs will also contribute to HGT in a mixed microbiota culture.

To investigate the ability of microbiota-derived BMVs to contain ARGs and mediate HGT, we purified BMVs from a mixed microbiota culture consisting of 95 isolates obtained from the gut of healthy individuals. We are currently sequencing the DNA within microbiota-derived BMVs to determine their ARG composition, and we are investigating their ability to mediate HGT. Collectively, these studies will advance our limited knowledge regarding the contribution of BMVs in the transfer of ARGs to facilitate pathogenesis and their role in a physiological setting such as the human microbiota.

# Using serology to explore heterogeneity in malaria transmission in Southeast Myanmar

Ellen A. Kearney*[1,2], Katherine O'Flaherty[1,2], Win Han Oo[1], Julia C. Cutts[1], Paul A. Agius[1,3,4], Merryn Roe[1,3], Win Htike[1], Kyaw Zayar Aung[1], Aung Thi[5], Nyi Nyi Zaw[1], Htin Kyaw Thu[1], Wai Yan Min Htay[1], Julie A. Simpson[2], James G. Beeson[1,6,7], Naanki Pasricha[1], Freya J. I. Fowkes[1,2,3,]

*[1]Burnet Institute, Melbourne, Australia and Yangon, Myanmar*
*[2]Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Australia*
*[3]Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia*
*[4] Judith Lumley Centre, La Trobe University, Melbourne, Australia*
*[5] Department of Public Health, Myanmar Ministry of Health and Sport*
*[6] Department of Medicine, University of Melbourne, Melbourne, Australia*
*[7] Department of Microbiology and Central Clinical School, Monash University, Melbourne, Australia*

* = presenting author

The emergence and rapid spread of artemisinin-resistant *Plasmodium falciparum* throughout the Greater Mekong Subregion has seen the countries in the region pledge to accelerate malaria elimination efforts and to eliminate malaria from the region by 2030. As the malaria burden declines, transmission becomes increasingly heterogeneous and results in the formation of discrete foci. Being able to identify these hot spots of residual malaria transmission to better guide interventions is an essential facet of achieving malaria elimination. Serological surveillance has been proposed as a useful tool with which to explore and define this heterogeneity, at both a macro- and micro-level.

In 2015 a longitudinal study of 114 villages from 7 townships in Southeast Myanmar was performed. The prevalence of *Plasmodium* spp. infection detected by rapid-diagnostic test was low (0.18%; 15/8,318), while qPCR increased detection by approximately 22-fold (2.47%; 200/8,094). The IgG response to a panel of transmission and blood-stage antigens was characterised, and macro- and micro-heterogeneity explored. While anti-malarial IgG seropositivity was similar across regions (p=0.280), there was significant variation at the township (p=0.013) and village (p<0.001) levels. It was found that seropositivity for anti-malarial IgG was associated with a reduction in the odds of a qPCR-detectable *Plasmodium* spp. infection (aOR:0.45; 95%CI: 0.26, 0.76; p=0.003). Further spatial analyses are being undertaken to explore potential influences of heterogeneity in qPCR-detectable malaria cases and anti-malarial IgG levels. This data will help identify residual hot spots of malaria transmission and inform appropriate interventions.

# A New Class of Antimalarials with an Unknown Mechanism of Action

Jomo K. Kigotho,[1*] Shane M. Devine,[1] Darren J. Creek,[2]
Raymond S. Norton[1] and Peter J. Scammells[1]

*[1]Medicinal Chemistry and [2]Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Australia*

* = presenting author

Malaria poses a major global health challenge as resistance develops to current treatments, requiring new antimalarials with alternate mechanisms of action. A fragment screen at MIPS identified 2-arylbenzimidazoles as potential antimalarial lead compounds,[1] while a contemporaneous high-throughput screen by AstraZeneca found a structurally similar compound containing a 2-aminobenzimidazole (ABI) to be a potent inhibitor ($IC_{50}$ <100 nM) of both drug-sensitive and drug-resistant strains of *Plasmodium falciparum* suggesting a possibly novel mechanism of action.[2]

We have explored the structure-activity relationship of ABIs and found that the 2-amino and phenol moieties were crucial to antiplasmodial activity.[3] Substitution around the phenol has been explored, with a number of substituents improving potency while methyl and methoxy derivatives were twice as potent as the parent compound.[3] Substitution around the benzimidazole was yet to be explored and various derivatives at differing positions of the benzo ring have now been selectively synthesised. Compounds were assessed for antiplasmodial activity in a 72-hour, SYBR green, growth inhibition assay against *Pf* 3D7. Methyl substitution led to a number of derivatives with $IC_{50}$ values below 20 nM, while disubstitution of the benzimidazole led to an $IC_{50}$ of 5 nM, representing a 10-fold improvement on the non-substituted parent compound. Currently this compound is being assessed both *in vivo* and *in vitro* for drug-likeness with alternate benzimidazole substitutions to be explored in future to further improve the physicochemical and pharmacokinetic properties.

1. Lim, S. S.; Debono, C. O.; MacRaild, C. A., et al., *Aust. J. Chem.* **2013,** *66*, 1530-1536.
2. Hameed, S.; Chinnapattu, M.; Shanbag, G., et al., *J. Med. Chem.* **2014,** *57*, 5702-5713.
3. Devine, S. M.; Challis, M. P.; Kigotho, J. K., et al., *Eur. J. Med. Chem.* **2020**, in preparation.

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

M Koutsakos[1*^], L Rowntree[1^], L Hensen[1^], B Chua[1,2^], C. van de Sandt[1,3^], J Habel[1], W Zhang[1], X Jia[1], L Kedzierski[1], T Ashhurst[4], G Putri[4], F Marsh-Wakefield[4], M Read[4], D Edwards[4], B Clemens[1], CWong[1], F Mordant[1], J Juno[1], F Amanat[5], J Audsley[6], N Holmes[7,8], CM Hughes[9], M Catton[10], J Denholm[11], S Tong[11,12], D Doolan[13], T Kotsimbos[14,15] , D Jackson[1,2], F Krammer[5], D Godfrey[1], A Chung[1], N King[4], S Lewin[6,14,15], A Wheatley[1], S. Kent[1] , K Subbarao[1,16], J McMahon[9,14,15], I Thevarajan[6,11], O Nguyen[1#], A Cheng[14,15#] & K Kedzierska[1,2#]

*[1]University of Melbourne, Peter Doherty Institute, [2]Hokkaido University, [3]University of Amsterdam, [4]Centenary Institute and University of Sydney, [5]Icahn School of Medicine at Mount Sinai, [6]Peter Doherty Institute, [7]Austin Hospital, [8]University of Melbourne, [9]Monash Medical Centre, Monash Health, [10]Victorian Infectious Diseases Reference Laboratory, [11]Victorian Infectious Diseases Services, [12]Charles Darwin University,[13]James Cook University,[14]Monash University,[15]Alfred Hospital, [16]World Health Organization Collaborating Centre for Reference and Research on Influenza,*

* = presenting author, ^co-first, #co-senior

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

# Tuesday

# 13 October

## 7:00-8:15pm

# Arts in Science

**Click here to go back to Program table**

**Self and Non-Self: A lifetime commitment to art and biology**

Ms Marta de Menezes, Artist

*Cultivamos Cultura*

Experimenting in their own bodies the immune response of rejection, artist Marta de Menezes and Immunologist Luis Graca depart from the experience of intimate collaboration, as a form of binding and differentiation. With some exceptions, the endeavour of scientists and artists have been historically pursued in different environments. In recent years an intersection between the two cultures – science and art – became increasingly common. As part of this trend, research laboratories started to be used by artists as studios for art production. There are, however, significant differences between the approach of artists and scientists when working in the laboratory. The differences and similarities between art and science, two activities based on creativity, will be discussed.

**Creative Collisions: Art + Science at Science Gallery Melbourne**

Dr Ryan Jefferies, Creative Director

*Science Gallery Melbourne, The University of Melbourne*

Science Gallery Melbourne inspires and transforms curious minds through the collision of art and science. Part of the acclaimed Global Science Gallery Network, and embedded in the University of Melbourne, the Gallery opens in 2021 as a dynamic new model for engaging 15-25 year olds through science, art and innovation. Learn about Science Gallery Melbourne's exciting new plans and ways to get involved!

# Wednesday 14 October

# Wednesday
# 14 October

## 1:10 - 2:30pm

# E-Poster Session III

Click here to go back to program table

bioproduction

gibco

# Optimized for Tregs by Design


## Improved activation and expansion of regulatory T cells for clinical research

Gibco™ CTS™ Dynabeads™ Treg Xpander provides an optimized activation signal for activation and expansion of regulatory T cells (Tregs). It yields high fold expansion of suppressive Tregs with a stable Treg phenotype, which is essential for clinical research.

### Key features:

- Scalable technology for robust activation and expansion of regulatory T cells
- A relevant number of regulatory T cells with >800-fold expansion after two stimulations
- Aseptically manufactured and ready for clinical research
- Maintained Treg phenotype (FOXP3) and suppressive function
- Drug master file (DMF) available
- Conformity with cGMP guidelines

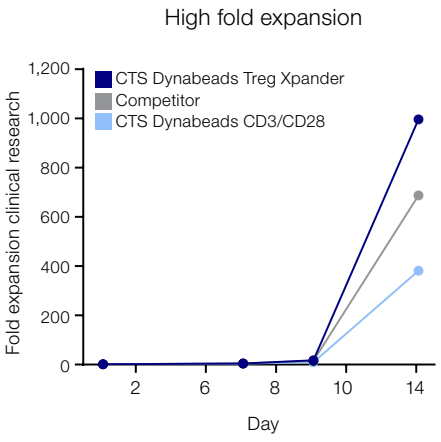



**Figure 1. Optimized activation signal expands Tregs to relevant numbers.** Flow-sorted regulatory T cells were expanded with CTS Dynabeads Treg Xpander, Gibco™ CTS™ Dynabeads™ CD3/CD28 beads, or other supplier's technology for 14 days with restimulation at day 9.

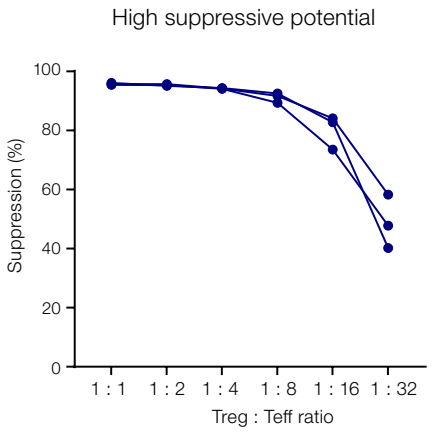



**Figure 2. Optimized activation signal expands Tregs with high suppressive potential.** Magnetically isolated regulatory T cells were expanded three times with CTS Dynabeads Treg Xpander for 14 days. Expanded Tregs are highly suppressive.

Find out more at **thermofisher.com/treg**

ThermoFisher SCIENTIFIC

**For Research Use or Non-Commercial Manufacturing of Cell-Based Products for Clinical Research. Caution: Not intended for direct administration into humans or animals.** © 2019 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. **COL19064 0519**

# Characterization of a new *Coxiella burnetii* effector that blocks cell death at host mitochondria

Robson Kriiger Loterio[1*], Yi Wei Lee[3], Leonardo Lima dos Santos[1], Jéssica Chiaratto[2], Nichollas Scott[3], Gustavo Henrique Goldman[2], Hayley Joy Newton[3] & Dario Simões Zamboni[4]

1. School of Medicine of Ribeirão Preto, Program of Basic and Applied Immunology, University of São Paulo, Brazil.
2. School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Brazil.
3. Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Australia.
4. School of Medicine of Ribeirão Preto, Department of Cellular and Molecular Biology, University of São Paulo, Brazil.
* Presenting author.

*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. The virulence of *C. burnetii* depends on the translocation of bacterial proteins (called effectors) into the host cytoplasm through the Dot/Icm type 4 secretion system (T4SS). Collectively these effectors facilitate the formation of a spacious vacuole that supports bacterial replication. Similar to *C. burnetii*, *Legionella pneumophila* also relies on a Dot/Icm T4SS and previous studies have demonstrated that *Legionella* can translocate *C. burnetii* effectors through this T4SS. *L. pneumophila* activates intracellular pathways in macrophages that are not activated during *C. burnetii* infection. Therefore, we have used *L. pneumophila* to express *C. burnetii* effectors and monitor their impact on pathways normally activated during *L. pneumophila.* A library of *L. pneumophila* Δ*flaA* expressing 80 different *C. burnetii* effectors was constructed and screened for impact on host cell death, cytokine production and virulence in *Galleria mellonella*. Three *C. burnetii* effectors that may be involved in the manipulation of macrophage cell death were identified. The overexpression of MceX (Mitochondrial *Coxiella* protein effector X) by *L. pneumophila* led to decreased pyroptosis and increased cytokine production in BMDMs. During *in vitro* infections, overexpression of MceX by *L. pneumophila* or by *C. burnetii* in BMDMs or HeLa cells, respectively, showed increased replication of both bacteria. Finally, coinfection assays and rotenone-induced cell death have demonstrated that the overexpression of MceX delays and decreases host cell death. When ectopically expressed in epithelial cells, MceX specifically localizes to mitochondria and preliminary proteomic analysis indicates that MceX influences the abundance of specific mitochondrial proteins. Thus, we aim to further understand the possible pathway modulated by MceX. This study will provide insight into the evasive mechanisms used by intracellular pathogens to control the human host cell.

# Superinfection exclusion can prevent Chikungunya infection in *Aedes* mosquitoes

Mathilde Laureti*, Julio Rodriguez[1], Prasad Paradkar[2], and John Fazakerley[1]

[1]*Peter Doherty Institute, Melbourne*

[2]*Australian Centre for Disease Preparedness, Geelong*

* = presenting author

The rise of arthropod-borne virus (arbovirus) emergence such as alphaviruses, is currently a major public and veterinary health issue. Chikungunya virus (CHIKV) especially has become a health burden over the recent years by infecting millions of people across the world. While no vaccine or treatment are currently available for CHIKV infection, and insecticides and other mosquito control tools are failing us, new fighting strategies are needed to control mosquito spreading. Superinfection exclusion is a promising phenomenon already present in nature that could be used to limit arbovirus transmission. This cellular phenomenon can prevent replication of a superinfecting virus in cells already infected with a similar or closely related virus. This phenomenon has been observed for years in plants, insects and mammalian cells, but little is still known about the mechanism behind exclusion.

In this study, we are investigating the phenomenon of superinfection exclusion of the highly pathogenic alphavirus CHIKV by the apathogenic Semliki Forest virus (SFV), another alphavirus closely related to CHIKV, in *Aedes* mosquito cells and *Aedes aegypti* mosquitoes in order to identify and understand the mechanism behind the exclusion phenomenon. This knowledge will inform on new mosquito control strategies in order to reduce the burden of mosquito-transmitted diseases.

# Sleep disturbance and its impact on children with primary ciliary dyskinesia

Rachel Leong[1*], A/Prof Philip Robinson[1,2,3] and Dr Moya Vandeleur[2]

*[1]The University of Melbourne, [2]Department of Respiratory and Sleep Medicine, Royal Children's Hospital, Melbourne, Victoria, [3]Murdoch Children's Research Institute.*

* = presenting author

**Background:** Children with primary ciliary dyskinesia (PCD) are more likely to experience sleep disturbance than healthy peers. Reduced sleep quality in this group is likely related to suppurative upper airway disease causing obstructive sleep apnoea, and nocturnal cough secondary to progressive lower airway disease. Studies of both healthy children and those with chronic illness consistently demonstrate that poor sleep quality significantly impacts on behaviour, mental health and quality of life. We aimed to investigate the relationship between sleep quality, mood and health-related quality of life (HRQOL) in children with PCD (which has not been previously reported).

**Methods:** Children (0–19 years) with PCD and their parents completed subjective sleep evaluation (Obstructive Sleep Apnea-18; OSA-18, Sleep Disturbance Scale for Children; SDSC, Pediatric Daytime Sleepiness Scale; PDSS). Age-appropriate questionnaires assessed mood (Children's Depression Inventory; CDI) and PCD specific HRQOL (QOL-PCD). Demographic information and pulmonary function test (PFT) data were also collected.

**Results:** 19 children (6.8 ± 4.5 years old) were recruited from the Royal Children's Hospital statewide PCD service (~70% of the total clinic population). Parents reported a high prevalence of sleep disturbance in their children (31.6% had clinically significant elevated scores on the OSA-18, 68.8% on the SDSC) and 46.2% of children self-reported clinically significant daytime sleepiness with elevated PDSS scores. There were statistically significant and strong correlations between sleep quality and mood and HRQOL. There was no correlation between pulmonary function (most recent FEV1) and sleep quality.

**Conclusions:** Children with PCD demonstrate a high prevalence of subjective sleep disturbance and impaired sleep quality is associated with lower mood and HRQOL. Further research that includes objective assessment of sleep is warranted to better understand the causes for sleep disturbance and treatment outcomes in this group. Our findings support the implementation of a sleep-screening program for all children with PCD.

# Neuroimmune interaction of the sympathetic nervous system and antiviral immune responses

Joon Keit Loi[1,2*], Sapna Devi[1,2], Erica Sloan[3] and Scott N Mueller[1,2]

[1] *Department of Microbiology and Immunology, University of Melbourne, at The Peter Doherty Institute for Infection and Immunity, Parkville, Vic, Australia*

[2] *ARC Centre of Excellence in Advanced Molecular Imaging, The University of Melbourne, Parkville, VIC 3010, Australia*

[3] *Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Vic, Australia*

The regulation of the immune responses is important in maintaining good health. Interactions between the nervous and immune systems are increasingly studied and widely appreciated to be influential in orchestrating immune responses. T cells express adrenergic receptors (AR) that enable them to respond to neurotransmitters produced by the sympathetic nervous system (SNS), noradrenaline (NA) and adrenaline, inducing downstream signalling and modulating cell functions, although whether this is stimulatory or inhibitory in T cell antiviral responses is not well defined. We examined the role of adrenoceptor signals via chemical sympathectomy prior to acute and chronic lymphocytic choriomeningitis virus (LCMV) infection. We found no alteration to the magnitude of antigen specific CD4 and CD8 T cell responses after ablation of sympathetic nerves. However, viral clearance in the spleen and blood was impaired. Notably, LCMV infection resulted in a significant reduction in sympathetic innervation and a decrease in catecholamine levels in the spleen as early as 3 days post infection. These results suggest that virus infection impacts sympathetic innervation of lymphoid organs, potentially impacting immune responses through control of local neurotransmitter release.

# Outcomes in patients who are overweight or obese hospitalised with COVID-19: an international, multi-centre analysis.

Danielle K. Longmore[1,2,3,4*], Jessica Miller[1,3], Siroon Bekkering[1,4], Christoph Saner[1,5], Edin Mifsud[1,6], Yanshan Zhu[7], Richard Saffery[1,3], Alistair Nichol[8,9,10], Graham Colditz[11], Kirsty Short[7], David Burgner[1,3,4,12] on behalf of the International BMI-COVID consortium

*1.Murdoch Children's Research Institute, VIC. 2. Menzies School of Health Research, NT. 3. Melbourne University, VIC. 4. Department of Paediatrics, Royal Children's Hospital, VIC. 5. Radboud University Medical Center, the Netherlands. 6. University Hospital Inselspital, Switzerland. 7. Doherty Institute, VIC. 8. The University of Queensland, QLD. 9. Alfred Health, VIC. 10. Australian and New Zealand Intensive Care Research Centre, Monash University, VIC. 11. University College Dublin Clinical Research Centre, Ireland 12. Washington University, Missouri, United States of America. 13. Department of Paediatrics, Monash University, VIC*

Background:
Obesity together with commonly associated comorbidities, have been associated with COVID-19 severity and mortality. Single jurisdiction studies evaluate the association of obesity with in-hospital respiratory support and mortality but outcomes remain unclear.

Methods:
We retrospectively extracted data on hospitalised SARS-CoV-2 patients from 18 sites in 11 countries. Standardized definitions and analyses were used to generate site-specific estimates, modelling the odds of each outcome (supplemental oxygen/non-invasive ventilation, invasive mechanical ventilation, and in-hospital mortality) by body-mass index (BMI) category (reference, overweight, obese) adjusting for age, sex, and pre-specified co-morbidities. Site-specific estimates were combined in a meta-analysis.

Results:
Among 7244 patients, those with obesity were more likely to require oxygen/non-invasive ventilation (random effects adjusted odds ratio (AOR) 1.75; 95%CI, 1.33-2.30; p<0.001) and invasive mechanical ventilation (AOR 1.73; 95%CI, 1.29-2.32; p<0.001). Similar but less pronounced effects were observed in those who were overweight. The association between obesity and in-hospital mortality was not statistically significant (AOR 1.23; 95%CI, 0.92-1.64; p=0.17). Among the co-variables, males had increased risk of all outcomes, and age >65 years was associated with increased oxygen/non-invasive ventilation requirement and in-hospital mortality. Cardiovascular disease and diabetes were associated with COVID-19 severity, independent of BMI.

Conclusions:
Overweight and obesity were independently associated with respiratory support in COVID-19 patients. These findings inform individual-level risk stratification, management and health system planning for these high-risk groups.

# Transcriptional regulation of CCL17 production by Dexamethasone

Tanya Lupancu* [1], Ming- Chin Lee [1] and Adrian Achuthan [1]

[1] *Department of Medicine, The University of Melbourne, Parkville, Victoria, Australia*

* Tanya Lupancu

Rheumatoid arthritis (RA) is a complex, autoimmune disease perpetuated by various inflammatory mediators. Granulocyte macrophage-colony stimulating factor (GM-CSF) is a pro-inflammatory cytokine that is highly upregulated in RA patients (RA) and its inhibition is targeted in several clinical trials. We have previously shown that GM-CSF upregulates CCL17 production, a pain inducing mediator, via IRF4 transcription factor and JMJD3 demethylase in human monocytes and mouse macrophages.
Dexamethasone (Dex) is a potent immunosuppressive agent use to treat inflammation in RA patients but its long-term use leads to adverse side effects. Moreover, the molecular mechanisms of Dex action on immune cells is unknown.

We report here that GM-CSF-induced CCL17 expression is inhibited by Dex in human monocytes and mouse macrophages. Dex mediated its suppressive effect by targeting JMJD3 activity, which in turn suppressed IRF4 levels and CCL17 expression. Significantly, an inflammatory arthritis mouse model showed that Dex administration could ameliorate GM-CSF driven inflammatory pain and arthritis and suppress CCL17 expression. Having delineated the suppressive mode of action by Dex on GM-CSF activity, new therapies that target the mediators of this pathway may provide more efficacious and tolerable treatment for RA patients.

# Prevention Of Asplenic Pneumococcal Infection (PAPI): Protecting Asplenic Children And Adults Against Pneumococcal Disease And Identifying Optimal Immunisation Regimens

Sarah Luu[1,4*], Ian Woolley[1-4], Penelope Jones[3,4], Denis Spelman[3,4], Anne Balloch[5], Paul Licciardi[5], Paul Monagle[5-7], Chris Barnes[6], Mimi LK Tang[5-7], Claire-Anne Siegrist[8] and Jim Buttery[1,2,9]

*[1]Monash University, [2]Monash Medical Centre, [3]The Alfred Hospital, [4]Spleen Australia [5]Murdoch Children's Research Institute, [6]Royal Children's Hospital, [7]University of Melbourne,[8]University Geneva, [9]Monash Children's Hospital*

**Introduction**:

Individuals with asplenia are at increased risk of sepsis from encapsulated organisms. Vaccination may offer protection against such infections. The optimal post-splenectomy vaccination schedule remains uncertain.

**Methods:**

We compared the immunogenicity of different pneumococcal immunisation regimens in individuals with asplenia. Subjects naïve to PPV23 were randomised to (early) 6-week or (late) 6-month PPV23 vaccination post-PCV10 (10-valent pneumococcal conjugate vaccine) vaccination. Subjects previously vaccinated with PPV23 (≥3 years prior) were randomised to PCV10 and 6-week PPV23, or 6-week PPV23 revaccination. Serum levels of serotype-specific IgG were measured by WHO ELISA. Functional activity of antibodies was measured by multiplexed opsonophagocytic assay (OPA). Differences in quantitative response (antibody concentrations) and functional response (OPA titres) were compared post-immunisation. A p-value of ≤0.01 was considered statistically significant.

**Results:**

122 individuals were recruited (mean age 46 years, 48 female) and randomised to early (n=26) or late (n=24) PPV23 vaccination post-PCV10 if PPV23 naïve, or PCV10 and PPV23 (n=36), or 6-week PPV23 revaccination (n=36) if PPV23 experienced. Amongst PPV23 exposed individuals, five had 'protective' antibody levels against all tested serotypes at the initial visit, and 20 had 'protective' activity against all tested serotypes. Quantitative and functional responses were similar at 6-weeks after early and late PPV23 vaccination post-PCV10. Vaccination with PCV10 and PPV23 in PPV23 experienced subjects resulted in increased quantitative response to only serotype 4 (p=0.002) and similar functional responses compared with PPV23 alone. Responses were similar for PPV23 naive and experienced individuals. There was no evidence of immune tolerance with repeated polysaccharide vaccination.

**Conclusion:**

There is little evidence in this study that conjugated vaccines should be used after primary PPV23 vaccination in individuals with asplenia. Prior PPV23 does not appear to be associated with any long-term hypo-responsiveness.

# Antibiotic exposure across pregnancy and early life, and maternal and infant metabolomic and lipidomic profile

Toby Mansell[1]*, Fiona Collier[1,2,3], Anne-Louise Ponsonby[1,4,5], Peter Vuillermin[1,2,3], Peter J Meikle[6], Satvika Burugupalli[6], Richard Saffery[1,4] and David Burgner[1,4,7], on behalf of the Barwon Infant Study Investigator Team

*[1]Murdoch Children's Research Institute [2]Deakin University [3]Child Health Research Unit, Barwon Health [4]Department of Paediatrics, The University of Melbourne [5]The Florey Institute of Neuroscience and Mental Health [6]Metabolomics Laboratory, Baker Heart and Diabetes Institute [7]Department of Paediatrics, Monash University*

**Background:** Antibiotics affect the gastrointestinal microbiome, with potential impact on cardiometabolic health. Prenatal and childhood antibiotic exposure have been linked to childhood obesity and may be related to adverse preclinical cardiometabolic phenotypes. There are currently very limited data on the relationship between antibiotic exposure and the metabolome/lipidome. Pregnancy and infancy are critical periods for later health outcomes and perturbations during these periods may have persistent health consequences. We investigated whether prenatal and postnatal antibiotic exposure was associated with distinct metabolomic and lipidomic profiles in pregnancy and early infant life.

**Methods:** Self/parent-reported antibiotic exposure data from 1027 mother-infant dyads in the Barwon Infant Study across pregnancy and the first 12 months postnatally were used. Serum (maternal at 28 weeks gestation, infant at birth) or plasma (12 month infant) metabolome was quantified by nuclear magnetic resonance (NMR) (72 metabolites) and the lipidome quantified by liquid chromatography and mass spectrometry (LC/MS) (799 lipid species). Linear regression models investigated the association of antibiotic exposure either during pregnancy or postnatally, on maternal and metabolomic and lipidomic measures. Adjustment for maternal and infant age and weight, pregnancy complications and lifestyle factors was considered. P-values were adjusted for multiple comparisons within platforms.

**Results:** There was no evidence that any antibiotic exposure during pregnancy was associated with altered metabolomic or lipidomic measures at 28 weeks gestation, nor at birth. Recent antibiotic use (within preceding 3 months) was associated with higher glycoprotein acetyls (GlycA, a marker of inflammation) and lower apolipoprotein A1 and high-density lipoprotein (HDL) cholesterol, but not with other metabolomic or lipidomic measures in 12-month infant plasma.

**Conclusions:** Although, recent postnatal antibiotic exposure is associated with differences in metabolomic measures previously reported in acute infections in older populations, we were unable to identify analogous patterns in pregnant women. By contrast, recent antibiotic exposure in infants was associated with potentially important differences in the metabolome.

# The Immunomodulatory and Anti-Inflammatory Effects of the Dietary Compound, L-Sulforaphane

Nadia Mazarakis[1, 2*], Jeremy Anderson[2,3], Zheng Quan Toh[2,3], Rachel A Higgins[2], Lien Anh Ha Do[2,3], Rodney B Luwor[4], Kenneth J Snibson[1], Tom C Karagiannis[5] and Paul V Licciardi[2,3]

*[1] Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Parkville, Victoria 3010, Australia, [2] New Vaccines, Murdoch Children's Research Institute, Melbourne, Victoria 3052, Australia, [3] Department of Paediatrics, University of Melbourne, Parkville, 3052, VIC, Australia, [4] Department of Surgery, Royal Melbourne Hospital, University of Melbourne, Melbourne, Victoria, 3052 Australia, [5] Epigenomic Medicine Program, Department of Diabetes, Central Clinical School, Monash University, Alfred Centre, Melbourne, Victoria 3004, Australia*

The dietary polyphenol L-sulforaphane (LSF), a compound in cruciferous vegetables, has important biological properties including chromatin modifying, antioxidant, and anti-microbial effects. However, the anti-inflammatory effects of LSF are less characterised. In this study, we investigated the anti-inflammatory effects of LSF in healthy human peripheral blood mononuclear cells (PBMCs) from healthy adult volunteers (N=14) using flow cytometry and multiplex assays. Pre-treatment of PBMCs with LSF (10µM and 50µM) for 24h reduced IL-6, TNF-$\alpha$, IL-1$\beta$ and MCP-1 production with or without 24h stimulation of bacterial (lipopolysaccharide) and viral (imiquimod) toll-like receptors (TLRs) ($p$<0.01). Furthermore, a decrease in $CD14^+$ monocytes expression accompanied by an increase in $lineage^-/HLA\text{-}DR^+/CD11b^{low\text{-}high}/CD11c^{high}$ dendritic cells (DCs) was observed following LSF treatment in a dose-dependent manner ($p$<0.01). To further evaluate these effects on monocytes, we used a luciferase reporter assay on a monocyte cell line (THP-1) and found LSF increased the antioxidant response element (ARE) activity at 6h and 24h ($p$<0.001). This activation of the ARE complex may be a key player in its anti-inflammatory and immunomodulatory effects. Taken together, our findings as well those from others, highlight the potential therapeutic utility of LSF. Further investigation into the anti-inflammatory effects of LSF, particularly its ability to enhance antigen presentation is warranted.

# Insights into mechanism of action of Interleukin-1 receptor associated kinase-3 (IRAK3)

Trang Nguyen[1], Anna Axell[1], Bree Mellberg[1], Ilona Turek[1], Helen Irving[1]

[1]La Trobe Institute for Molecular Science, La Trobe University, Bendigo, VIC 3550

* = presenting author

IRAK3 is known as a critical negative regulator of innate immunity, and involved in many diseases such as sepsis and asthma. Thus, IRAK3 is potentially a diagnostic biomarker or immunotherapy agent. IRAK3 down-regulates inflammatory responses and is required for endotoxin tolerance in which the production of inflammatory cytokines is diminished upon constant exposure or re-challenge of endotoxins. However, mechanisms of IRAK3 actions are not fully understood, and a variety of different patterns of IRAK3 expression and actions in inflammatory responses are reported depending on cell type, kind of stimuli and stimulation duration. Recent studies showed IRAK3 contains a guanylate cyclase centre that can generate cyclic guanosine monophosphate (cGMP) [1]. Mutations in the catalytic site modify IRAK3 function in transiently transfected HEK293 cells. Therefore, we explored how cGMP produced by IRAK3 modulates NF-κB activity and cytokine production in monocytic cell lines (THP-1 and THP-1 BLUE) during endotoxin challenge. A decrease of NF-κB expression and cytokine (IL-6 and TNF-α) production was observed in the presence of membrane permeable cGMP (8-bromo cGMP) at 0.1 nM to 10 μM concentrations and lipopolysaccharide. Using CRISPR/Cas9 we have generated IRAK3 knock-down THP-1 cell lines to further investigate IRAK3 effect and its association with cGMP in modulating inflammation. Therefore, guanylate cyclase centre of IRAK3 can may regulate the inhibitory effect of IRAK3 on inflammation.

1. Freihat L, Wheeler J, Wong A, Turek I, Manallack D, Irving H. IRAK3 modulates downstream innate immune signalling through its guanylate cyclase activity. Sci Rep. 2019;9(1):15468.

# CD1a-restricted T cells: a subset of “unconventional” T cells like no other

Catriona V Nguyen-Robertson[1,2*], Scott JJ Reddiex[2], Willem Van Der Byl[3], Michael NT Souter[1], Janice MH Cheng[1,4], Adam P Uldrich[1], Jamie Rossjohn[5], Ildiko Van Rhijn[6], Spencer J Williams[4], D Branch Moody[6], Fabio Luciani[3], Dale I Godfrey[1], Daniel G Pellicci[2]

*[1]Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, The University of Melbourne, [2]Cellular Immunology Group, Murdoch Children’s Research Institute, [3]Kirby Institute, University of New South Wales, [4]School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, [5]Department of Biochemistry and Molecular Biology, Monash University, [6]Brigham and Women's Hospital Division of Rheumatology, Immunology and Allergy and Harvard Medical School*

* = presenting author

In contrast to conventional T cells that recognise peptide antigens presented by MHC molecules, a subset of “unconventional” T cells recognise lipid antigens presented by MHC-like CD1 family members: CD1a, CD1b, CD1c and CD1d. CD1a-restricted T cells is one such group that comprise a significant proportion of the peripheral T cell pool yet, compared to CD1d-restricted, natural killer T (NKT cells), relatively little is known about their role in the immune system.

We produced CD1a tetramers to investigate the phenotype and function of human CD1a-restricted T cells directly *ex vivo*. We isolated CD1a-restricted T cells that recognise non-self-lipid antigens, particularly dideoxymycobactin (DDM), a lipid antigen derived from *Mycobacterium tuberculosis*. DDM was a potent activator of these T cells, suggestive of a role for these cells in mycobacterial immunity. We also isolated CD1a-restricted T cells that react to self-lipid antigens, and some reactive to both self-lipids and DDM, suggesting that some CD1a-restricted T cells display a degree of autoreactivity.

Additionally, we 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. Experiments with CD1a mutant cell lines revealed that TCRs that incorporate different variable genes can bind across the entire binding cleft of CD1a, which is likely to increase the diversity of lipid antigens that can be recognised by CD1a-restricted T cells.

Phenotypic analyses and RNA-sequencing of CD1a-restricted T cells revealed that they are distinct from other innate-like T cells, such as NKT cells and CD1b-restricted T cells, distinguishing them as a unique population of unconventional T cells. They did not express innate-like markers such as CD161, IL-18R, and PLZF, but instead appeared similar to conventional $CD4^+$ T cells. Collectively, these studies represent an important step forward in characterising CD1a-restricted T cells and further understanding their role in infection and autoimmune responses.

# HIGH SEROPREVALENCE OF VIRAL HEPATITIS AMONG ANIMAL HANDLERS IN ABEOKUTA, NIGERIA

**Adeolu Oluremi.,[12] * Tawakalitu Ajadi[2] Opaleye Oluyinka.[2,] Alli Oyebode Terry.[3] Ogbolu David.**

[1]Department of Medical Laboratory Science, Babcock University, Ilishan Remo, Nigeria
[2]Department of Medical Microbiology and Parasitology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria [3]Department of Medical Laboratory Science, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

Viral hepatitis is a deadly disease which can manifest as acute, chronic, hepatocarcinoma and liver failure. Information about hepatitis is scarce among animal handlers. Due to Federal Government of Nigeria diversification programmes, many people are now involved in animal farming which can make them susceptible to viral hepatitis. This study aimed at determining the prevalence of Hepatitis B, C and E viruses among animal handlers in Abeokuta, south western Nigerian. A total of 156 subjects were recruited for the study. Information was fetched from subjects using interviewer-administered questionnaire. Blood samples were collected via venepuncture and tested for HCV, HBV and HEV using ELISA technique. Results were analysed using SPSS software version 21.0 and p-value $\leq 0.05$ was considered significant. The prevalence of HCV, HBV and HEV were 46 (29.5 %), 20 (12.8 %) and 4 (2.6 % ) respectively while 6 (3.8%), 1 (0.6% ) and 1 (0.6% ) had co-infection of HBV-HCV, HBV-HEV and HCV- HEV respectively. This study concludes that there is high prevalence of hepatitis C and B viruses among animal handlers in Abeokuta, Ogun state which is of significant public health problem, warranting further attention and research.

# Fatal attractants: antibiotics that enhance neutrophil clearance of *Staphylococcus aureus* infections

Jennifer A E Payne[1,2*], Julien Tailhades[1,2], Felix Ellett[3], Xenia Kostoulias[4], Alex J Fulcher[5], Ryan Leung[1], Stephanie Louch[1], Amy Tran[1], Graham J Lieschke[6], Daniel Irima[3], Anton Y. Peleg[4,7], Max J Cryle[1,2]

[1] *Infection and Immunity Program, Monash Biomedicine Discovery Institute, Department of Biochemistry and Molecular Biology and ARC Centre of Excellence in Advanced Molecular Imaging, Monash University, Clayton, Victoria, 3800 Australia.*

[2] *EMBL Australia, Monash University, Clayton, Victoria, 3800 Australia.*

[3] *BioMEMS Resource Center, Center for Engineering in Medicine and Surgical Services, Massachusetts General Hospital, Shriners Hospital for Children, and Harvard Medical School, Charlestown, MA 02129, USA.*

[4] *Infection and Immunity Program, Monash Biomedicine Discovery Institute and Department of Microbiology, Monash University, Clayton Campus, Melbourne, Victoria, Australia and*

[5] *Monash Micro Imaging, Monash University, Clayton, Victoria, 3800, Australia*

[6] *Australian Regenerative Medicine Institute, Monash University, Clayton, Victoria, 3800, Australia*

[7] *Department of Infectious Diseases, The Alfred Hospital and Central Clinical School, Monash University, Melbourne, Victoria, 3004, Australia.*

* = presenting author

*S. aureus* is a deadly pathogen due to its abilities to readily develop antibiotic resistance and evade our immune system. Antibiotic resistance in *S. aureus* is associated with reduced levels of neutrophil recruitment, which is a vital step in triggering an immune response to resolve the infection. In this work, we have developed enhanced antibiotic agents that act as dual-function antibiotic-chemoattractant, enabling augmented neutrophil recruitment to *S. aureus* along with direct killing. Our agents exploit formylated peptides as chemoattractant for neutrophil recruitment, which is combined with the targeted binding of vancomycin to bacteria that generates a chemokine gradient for neutrophil recruitment. Using the combination of *in vitro* assays, infection-on-a-chip and *in vivo* mouse models, we have determined that these antibiotics improve the recruitment, engulfment and killing of *S. aureus* by neutrophils. Our approach offers a new paradigm in antibiotic development to overcome the threat of bacterial resistance in the clinic.

# Comparison of Two Pre-clinical Animal Models of Non-Alcoholic Steatohepatitis

Cheng Peng[1,2,3*], Siobhan Finlayson[3], Minh Deo[2], Hooi Hooi Ng[3,4], Alastair G Stewart[1], Rebecca H Ritchie[1,2,3], Chengxue Qin[1,2,3].

*[1]Dept of Pharmacol Ther, Univ of Melbourne, VIC; [2]Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, VIC; [3]Baker Heart & Diabetes Institute, Melbourne, VIC; [4]Dept of Human and Molecular Genetics, Florida Int'l Univ, Miami, FL, United States.*

* = presenting author

Non-alcoholic steatohepatitis (NASH) is characterised by liver steatosis, inflammation, hepatocyte ballooning and fibrosis driven by metabolic disruption. Currently there is a clear unmet clinical need, with a lack of clinically-relevant pre-clinical animal models that recapitulate all key features of human NASH. We hypothesised that the mouse model composed of streptozotocin (STZ) (3x55mg/kg/day, i.p.) in C57BL/6 male mice (6wks old) followed by 20wks of high fat diet (HFD) (42% fat) maybe a more clinically-relevant model than the published methionine-choline-deficient (MCD) diet-induced NASH model for evaluating the efficacy of novel drug-candidates against NASH. NASH was induced in the MCD-diet model by feeding C57BL/6 male mice (8wks old) with MCD diet for 4wks. At study end, systemic and liver characteristics were examined. Liver injury was significantly higher in disease than sham mice (plasma alanine aminotransferase: 159±10 MCD vs 33±4 sham, $p<0.0001$) and (85±16 STZ+HFD vs 20±2 sham, $p<0.001$). Overall the MCD model showed a more severe phenotype in liver histology scored by a pathologist, presenting inflammation (2.3±0.2 vs 0.0±0.0, $p<0.001$) and steatosis (3.0±0.0 vs 0.0±0.0, $p<0.0001$) compared to the sham mice. The MCD model displayed significant weight loss (19.6±0.2 vs 20.6±0.3, $p<0.0001$) and there was no sign of hepatocyte ballooning when compared to the sham mice. The STZ+HFD model also showed steatosis (2.0±0.3 vs 0.1±0.1, $p<0.0001$), mild inflammation (0.8±0.3 vs 0.3±0.1) and ballooning was evident in some of the STZ+HFD-treated animals. Fibrosis quantified using picrosirius-red staining demonstrated a significant increase in the STZ+HFD model (1.6±0.5 vs 0.4±0.2, $p<0.05$) when compared to its sham group, but not in the MCD model. This study demonstrated that the STZ+HFD model may be a more suitable model for evaluating drug efficacy for an increasing incidence of NASH.

# Transcriptomic analysis of naïve CD4+ T cells in peanut allergic adolescents

Stephen Peng*[1,2], Samira Imiran[1,2], Melanie R. Neeland[1,2], Boris Novakovic[1,2], Richard Saffery[1,2]

[1] Murdoch Children's Research Institute, Parkville, VIC, Australia. [2] Department of Pediatrics, The University of Melbourne, Parkville, VIC, Australia.

**Background:** IgE mediated food allergy is a major public health concern in western countries due to its rising prevalence over the last decade. Among all types of food allergies, peanut allergy, as the one that is least likely to be naturally resolved when children grow up, has contributed disproportionally to hospital food related anaphylaxis admission rate of preadolescent age group (5-14 years old).

**Aim:** The purpose of this study is to investigate if peanut allergic adolescents demonstrate significant alteration in naïve CD4+ T cells (nCD4 T cells) gene expression relative to non-allergic controls based on transcriptomic profiles. Furthermore, peanut allergic only adolescents will be compared against multi-allergic (peanut allergy with other food allergies) adolescents to determine if there are significant differential gene expressions of nCD4 T cells between the two groups.

**Hypothesis:** It is hypothesized that peanut allergy is associated with distinct gene expression changes in naïve CD4+ T cells in peanut allergic adolescents.

# The role of citrullination on defensin function in innate defence and cancer

Minuri Ratnayake[1*],Guneet Bindra[1],Scott Williams[1],Fung Lay[1] and Mark Hulett[1]

*[1]Department of Biochemistry and Genetics, La Trobe Institute for Molecular Sciences, La Trobe University, Melbourne VIC 3086*

* = presenting author

Defensins, a subclass of cationic antimicrobial peptides, are small cysteine-rich peptides that play a significant role in eukaryotic immune defence and are emerging as promising therapeutic agents. Defensins from various species including plants (NaD1) and humans (HBD-2 and HBD-3) exhibit membrane permeabilisation activity against pathogenic microbes and cancer cells. This activity is attributed to the binding of defensins to membrane phospholipids such as phosphoinositides (PIPs), an interaction mediated by key arginine residues such as arginine 40 and arginine 22 for NaD1 and HBD-2, respectively. Citrullination is an important post-translational modification process in humans, particularly at sites of inflammation and the tumour microenvironment, where arginine residues are modified to neutral citrulline that can modulate protein function and counteract host defences. However, the regulatory action of citrullination on defensin function remains to be investigated. In this study citrullination of NaD1, HBD-2 and HBD-3, were examined using human peptidyl arginine deaminase 2 enzyme (PAD2). Of these 3 defensins, NaD1 and HBD-3 were demonstrated to be citrullinated using Western blot analysis and mass spectrometry. Protein lipid overlay assays and transmission electron microscopy interaction of citrullinated NaD1 with $PI(4,5)P_2$ suggests the importance of other residues in addition to arginine in phosphoinositide binding. Citrullinated NaD1 had substantial reduction in activity against human fungal pathogen *Candida albicans* strains (ATCC10231 and ATCC90028) as well as cervical (HeLa), prostate (PC3) and human histiocytic lymphoma (U937) cancer cell lines when compared to native NaD1. Despite not interacting with arginine for its membranolytic activity, citrullinated HBD-3 appears to reduce the activity of *Candida albicans* as well as HeLa and PC3 cancer cell lines. This study provides new insights into the citrullination of defensins and its potential regulatory effects on defensin-mediated lipid binding and membranolytic ability, as well as raising potential issues in the use of defensins as therapeutic agents.

# Identifying novel host resistance factors in *Toxoplasma gondii* infection

Ushma Ruparel[1, 2*], Marcel Doerflinger[1, 2] and Chris Tonkin[1, 2]

[1]*The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia*
[2]*Department of Medical Biology, The University of Melbourne, Melbourne, Victoria, Australia*

* = presenting author

*Toxoplasma gondii*, the causative agent of toxoplasmosis, is responsible for substantial disease burden worldwide. Drugs against acute infection are limited by toxicity whilst chronic forms are completely drug resistant. Host cell invasion is critical for the establishment of infection due to the obligate intracellular lifecycle of the *T. gondii*, and this parasite relies heavily on manipulation of host signalling pathways to facilitate intracellular survival. Therefore, a promising avenue for the development of new treatments for *T. gondii* infection is the targeting of host cell pathways that the parasite requires and modulates, without harming the host. However, pursuit of these targets is hampered by lack of insight into the molecular mechanisms of such interactions. Presented is the use of CRISPR/Cas9 gene-editing technology in pooled genome-wide screens to identify host-cell factors that contribute to resistance to *T. gondii* infection. Firstly, two screening approaches differing in selection pressure were tested in order to determine their comparative strengths. Subsequently, Ptger1, a host factor involved in prostaglandin E2 signalling, was chosen for phenotypic validation using CRISPR/Cas9 genetic disruption. Pharmacological intervention was also used to analyse the function of Ptger1 in *T. gondii* infection, and it was discovered that competitive antagonism of this receptor increased infection levels. These results provide a basis for further investigation of host factors that contribute to resistance to *T. gondii* infection, which may be harnessed for therapeutic gain in the treatment of toxoplasmosis.

# Investigating the role of macrophages in dying cell fragmentation

Jascinta Santavanond[1*], Rochelle Tixeira[1], Georgia Atkin-Smith[1,2], Lanzhou Jiang[1], Cristina Keightley[3] and Ivan Poon[1]

*[1]Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Victoria 3086, Australia, [2]Inflammation Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, 3052, Australia, [3]Department of Pharmacy and Biomedical Sciences, La Trobe University, Bendigo, Victoria 3552, Australia.*

* = presenting author

Apoptosis and the disassembly of apoptotic cells into apoptotic bodies (ApoBDs) is essential for efficient clearance by phagocytes. Deficiency in clearance can lead to the release of pro-inflammatory molecules, and consequently the onset of inflammatory diseases. Currently, the only known regulators of apoptotic cell disassembly (rho-associated kinase 1, Pannexin 1 channels and Plexin B2 receptors) are intrinsic factors, however the involvement of extrinsic factors in this process is not fully understood. To investigate this, a mouse and zebrafish model was established to monitor apoptosis and the formation of ApoBDs *in vivo*. The first involves inducing apoptosis in 5 weeks old C57bl/6 mice with dexamethasone or x-ray irradiation. Quantitative analysis by flow cytometry revealed that when apoptosis occurred *in vivo*, a large number of thymic ApoBDs was formed, and a large number of macrophages was detected. Interestingly, when apoptosis occurred *ex vivo*, the number of ApoBDs formed and macrophages present significantly decreased, suggesting a correlation between ApoBD formation and macrophage infiltration to the site of cell death. To further investigate apoptotic cell disassembly *in vivo*, developmental and drug induced apoptosis in 24 hpf secAnnexins5:mVenus zebrafish embryos was monitored by time-lapse imaging over 10 h. Qualitative analysis by confocal microscopy demonstrated that apoptosis occurs globally in both settings and a number of ApoBD sized fragments and membrane protrusions were identified. To further visualise the interaction between apoptotic cells and macrophages, secAnnexin5:mVenus fish will be crossed with mpeg:mcherry fish and monitored by confocal microscopy. Collectively, this data suggests that macrophages have the ability to infiltrate to the site of cell death to aid ApoBD formation, a process which is necessary to increase engulfment efficiency and to prevent the onset of inflammatory diseases.

# Wednesday
# 14 October

## 1:10 - 2:30pm

# E-Poster Session IV

Click here to go back to program table

scientifix

# Promotions

## BUY 2 GET 3RD FREE

### MACHEREY-NAGEL CLASSICS

**Stock Up on Essentials**

- Plasmid Preps
- Gel & PCR Clean-Up
- RNA
- DNA

VIEW PROMOTION


## 30% OFF

### PROTEIN PURIFICATION

**His- & GST-tagged Protein Purification**

- Cobalt TALON
- Ni-NTA
- Glutathione
- Resins & Columns

VIEW PROMOTION

## 40% OFF

### NEW LIGATION-FREE CLONING

**Evaluation Offer**

Seamless cloning of any PCR fragment or multiple fragments.

>95% cloning accuracy.

No restriction digest, ligation, phosphorylation or blunt-end polishing required.

VIEW PROMOTION

## 25% OFF

### LENTIVIRUS, ADENO, AAV, RETROVIRUS SYSTEMS & TOOLS

**Tools for all serotypes**

- Packaging Systems & Cell Lines
- Titration Kits
- Purification Kits
- Concentration Reagents
- Transduction Enhancers

VIEW PROMOTION

See All Our Current Promotions: **scientifix.com.au/promotions**

Please contact Scientifix for more product information, pricing or to request a quote
For customer service, call 1800 007 900 or email info@scientifix.com.au
More information visit www.scientifix.com.au

# High burden of reproductive tract infections and other poor sexual and reproductive health indicators in pregnant women in East New Britain Province of Papua New Guinea

Michelle J.L. Scoullar*[1], Philippe Boeuf[1], Elizabeth Peach[1], Ellen Kearney[1], Ruth Fidelis[1], Kerryanne Tokmun[1], Pele Melepia[1], Catriona S. Bradshaw[2,3], Arthur Elijah[4], Glenda Fehler[2], Peter M. Siba[5], Simon Erskine[6], Elisa Mokany[6], Elissa Kennedy[1], Alexandra J. Umbers[1], Stanley Luchters[1,7], Leanne J. Robinson[1,3,5], Nicholas C. Wong[3], Andrew Vallely[5,8], Steven G. Badmam[8], Lisa M. Vallely[8], Freya J.I. Fowkes[1,3], Chris Morgan[1], William Pomat[5], Brendan S. Crabb[1,3], James G. Beeson[1,3], and the HMHB Study Team

[1]Burnet Institute, Australia.
[2]Melbourne Sexual Health Centre, Alfred Hospital, Australia
[3]Monash University, Australia.
[4]School of Medicine and Health Sciences, University of Papua New Guinea, Papua New Guinea.
[5]Papua New Guinea Institute of Medical Research, Papua New Guinea.
[6]SpeeDx Pty Ltd, Australia.
[7]Department of Population Health, Aga Khan University, Kenya
[8]The Kirby Institute, University of New South Wales, Australia.

There is a pressing need for detailed knowledge of the range of pathogens, extent of co-infection and clinical impact of reproductive tract infections (RTIs) among pregnant women. Here, we report on RTIs (*Mycoplasma genitalium, Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Treponema pallidum subspecies pallidum,* bacterial vaginosis and vulvovaginal candidiasis) among 699 pregnant women in Papua New Guinea (PNG), with follow up at 1-, 6- and 12-months postpartum. Most pregnant women (74.1%) had at least one RTI, with a curable current sexually-transmitted infections detected in 37.7%. This first report of *M. genitalium* infection in PNG found a high prevalence (12.5%) among pregnant women, with no evidence of macrolide resistance. Prevalence of curable infections (*M. genitalium*, *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis)* decreased immediately post-partum but began to increase again by 12 months post-partum. There was remarkably little use of contraception; 98.4% report never having used barrier contraception. Most of these infections are curable when diagnosis is made available and syndromic management alone is insufficient. Ongoing studies will determine associations with adverse pregnancy outcomes. This work has implications for improving maternal and child health in PNG.

# A synergistic exacerbation between COVID-19 and tuberculosis predicted by RNA-seq comparative profiling

Dylan Sheerin*[1], Abhimanyu[2], Xuetao Wang[3], W Evan Johnson[3], Anna Coussens[1,2]

*[1] Infectious Diseases and Immune Defence Division, The Walter & Eliza Hall Institute of Medical Research, Parkville 3279, VIC, Australia [2] Wellcome Centre for Infectious Diseases in Africa, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Anzio Rd, Observatory, 7925, Western Cape, South Africa [3] Division of Computational Biomedicine and Bioinformatics Program, Boston University, Boston, MA, USA; Department of Biostatistics, Boston University, Boston, MA, USA*

* = presenting author

The spread of the novel coronavirus, SARS-CoV-2, has wreaked havoc on healthcare systems across the globe since its emergence in late 2019. Tuberculosis (TB), however, remains the leading cause of infectious death by a single pathogen, and widespread disruptions to healthcare provision caused by the COVID-19 pandemic have delayed tuberculosis (TB) diagnosis and interrupted treatment for millions living with the disease worldwide. Evidence has also emerged that latent or active TB may increase susceptibility to and severity of COVID-19[1]. This will likely exacerbate mortality and disease burden in endemic regions; as co-infection and dual presentation is occurring in these settings, understanding the immunological interaction between these diseases is important to inform the public health response, with regards to diagnosis, treatment and natural history progression. The transcriptomic responses to SARS-CoV-2 infection delineated in several studies at the single-cell and tissue level have borne striking resemblance to those underlying advanced states of TB infection, prompting us to perform a detailed comparative evaluation. Whole blood, PBMC and bronchoalveolar lavage gene signatures associated with COVID-19 clinical severity were extracted from 10 published and pre-print manuscripts and applied to RNA-seq whole blood transcriptomic data from three large TB cohorts consisting of latent, progressor and active TB cases, to calculate putative “COVID-19 risk scores” using the TBSignatureProfiler package[2]. A whole blood influenza dataset was included act as an inflammatory lung disease control. Cell- and pathway-specific signatures associated with COVID-19 disease severity were significantly enriched in active and progressor TB cases. Overlapping signatures were further scrutinised to determine commonly enriched immunological pathways. Networks of shared protein-protein interactions were generated for TB and COVID-19 whole blood signatures, several of which were of particular importance in regulating viral replication and the inflammation. Finally, an integrative analysis was performed using TB peripheral blood and COVID-19 site-of-disease single-cell RNA-seq data, identifying risk-associated immune cell macrophage phenotypes in circulation. Our results support the hypothesis that these diseases share molecular and phenotypic determinants that may complicate the derivation of diagnostic biomarkers, with co-infection posing a dual risk of enhanced TB progression and COVID-19 severity.

1 Liu, Y. *et al.* Active or latent tuberculosis increases susceptibility to COVID-19 and disease severity. *medRxiv*, 2020.2003.2010.20033795, doi:10.1101/2020.03.10.20033795 (2020).

2 Jenkins, D., Zhao, Y., Johnson, W. E., Odom, A. & Love, C. *TBSignatureProfiler: Profile RA-Seq Data Using TB Pathway Signatures*, <https://github.com/compbiomed/TBSignatureProfiler> (2020).

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

Mohini A. Shibu[1*], Olivia Carmo[1], Leann Tilley[1], Matthew W. A. Dixon[1]

*[1]Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, VIC 3010, Australia*

The malaria parasite Plasmodium falciparum modifies the host red blood cell (RBC) for survival. This remodelling is facilitated by the export of many parasite proteins into the RBC cytoplasm. Some of these proteins, including the major virulence protein PfEMP1, are exported via an intermediate trafficking structure called Maurer's clefts. The final steps in the transfer of PfEMP1 to the RBC membrane from the cleft is poorly understood. Previous electron tomography studies of the parasite have revealed tether-like structures connecting Maurer's clefts to the RBC membrane. These tethers have been hypothesized to play a role in immobilizing the Maurer's clefts at the RBC membrane, where they may function in the final steps of PfEMP1 trafficking and surface display. MAHRP2 is the only protein currently known to localize to the tether. Immunoprecipitation studies of MAHRP2-GFP revealed several new proteins. Here we report on one of these proteins termed the MAHRP2 interacting protein (MIP). MIP localizes to the Maurer's clefts and conditional knockdown studies show that MIP is required for parasite adhesion to endothelial receptors and that deletion of MIP leads to a delay in the immobilization of the Maurer's clefts to the RBC membrane.

# Genomic surveillance for antimicrobial resistance in non-typhoidal *Salmonella enterica*

Cheryll M. Sia*[1], Mary Valcanis[1], Sarah L. Baines[1], Anders G. da Silva[1], Susan A. Ballard[1], Torsten Seemann[1], Benjamin P. Howden[1], Danielle J. Ingle[1,2], Deborah A. Williamson[1]

[1] *Microbiological Diagnostic Unit Public Health Laboratory, Department of Microbiology & Immunology, The University of Melbourne at The Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia.*

[2] *Research School of Population Health, The Australian National University, Canberra, Australia*

Nontyphoidal *Salmonella* (NTS) is the second most common cause of foodborne bacterial gastroenteritis in Australia[1] with antimicrobial resistance (AMR) increasing in recent years[2]. Whole-genome sequencing (WGS) provides opportunities for *in-silico* detection of AMR determinants, which will provide enhanced surveillance of NTS. The objectives of this study were two-fold: 1) to establish the utility of WGS analyses to infer phenotypic resistance in NTS, and 2) to explore genotypic AMR patterns detected within and between different NTS serovars.

The concordance of 2490 NTS isolates with matched WGS and phenotypic susceptibility data against up to 14 clinically-relevant antimicrobials was explored. The resulting data were interrogated for known AMR determinants and plasmid replicons, using ABRicate and ARIBA against publicly available databases.

Concordance was observed between 31,466 phenotypic-genotypic combinations, with overall sensitivity and specificity rates > 98%. The most common AMR genes were *bla*$_{\text{TEM-1}}$, *sul2*, *tet*A, *strA-strB* and *floR*. Resistance to cefotaxime was low and encoded either *bla*$_{\text{CMY-2}}$ or a variant of *bla*$_{\text{CTX-M}}$. Interrogation of AMR patterns identified both known and novel profiles within different NTS lineages. Further, a shift of AMR prevalence in combination with specific plasmid replicons was detected suggesting there were emerging AMR NTS in Victoria.

Our data demonstrate a high correlation between WGS and phenotypic sensitivity testing. WGS can significantly enhance existing AMR surveillance by detecting known and emerging AMR patterns in large NTS datasets routinely produced in public health laboratories.

[1] National Notifiable Diseases Surveillance System. Notification for All Diseases by State and Territory and Year. Available: http://www9.health.gov.au/cda/source/rpt_2_sel.cfm
[2] D.A Williamson, *et al*. Increasing Antimicrobial Resistance in Nontyphoidal *Salmonella* Isolates in Australia from 1979 to 2015. Antimicrobial Agents and Chemotherapy (2018); 62(2).

# Novel 3D-compounds to fight malaria in a time of drug resistance

Liana Theodoridis[1]*, Derek Wong[1], Pallavi Sharma[2], Teresa G. Carvalho[1]

[1] *Molecular Parasitology Laboratory, Department of Physiology, Anatomy and Microbiology, La Trobe University, Bundoora, VIC, 3086, Australia*

[2] *La Trobe Institute for Molecular Science, La Trobe University, Bundoora, VIC, 3086, Australia*

* = presenting author

Malaria remains a significant global health burden, and a leading contributor to global mortality rates, particularly in Africa and South-East Asia. Despite its prevalence, an efficient vaccine has yet to be developed. The rise of antimalarial drug resistance and plateau in new therapeutic trials, create an urgent demand for new treatment options. A focus on novel antimalarial drugs with underrepresented chemical scaffolds/linkage is vital for antimalarial development and to combat drug resistance. We have synthesised a new class of 3D-spiroheterocycle compounds with chemical connectivities never previously synthesised or explored. The 3D architecture of these molecules allows them to reach biological domains otherwise inaccessible to relatively flat structures, leading to increased efficiency/efficacy. We have tested eighteen new 3D-spirocyclic compounds against human *P. falciparum* parasites *in vitro.* We have shown that two compounds induced parasite death within 24h, while their flat precursors had no effect on parasite growth. Further, we have shown that the anti-parasitic activity of both compounds is cytotoxic (i.e. irreversible). $IC_{50}$ values of both active compounds were measured in the low micromolar range, and they presented no toxicity to HeLa cell cultures. Further analysis of these novel drugs is underway to identify their specific cellular target(s). Gathering biological information will allow the synthesis of refined derivatives with higher potency that mirror major antimalarials.

Reference: **Sharma P, Prabhath MRR, Wong D, Ampem-Lassen MA, Bhat SV & Carvalho TG.** Cyanamide Click Chemistry: Synthesis of 3D-Heterospirocycles Through Iterative 1,3-Dipolar Cycloaddition Pathways. Under Review.

# Interleukin-18 is crucial to the development of 1 kidney/DOCA/salt-induced renal inflammation and elevated blood pressure

Jordyn Thomas[1*], Yeong Hann Ling[2], Prerna Sharma[3], Shalini Murali Krishnan[2], Dorota Ferens[2], Henry Diep[1], Christopher Sobey[1], Grant Drummond[1], Brooke Huuskes[1] and Antony Vinh[1]

*[1]La Trobe University, [2]Monash University, [3]Baker Heart and Diabetes Institute*

* = presenting author

Interleukin-18 (IL-18), a proinflammatory cytokine, is known to be elevated in hypertensive patients. However, whether IL-18 plays a causal role in hypertension remains unknown. This study aimed to examine whether IL-18 contributes to the development of experimental hypertension and renal inflammation. Hypertension was induced in male wildtype (WT) and IL-18$^{-/-}$ mice by uninephrectomy and treatment with deoxycorticosterone acetate (2.4 mg/d, s.c.) and saline (0.9%) drinking water (1K/DOCA/salt). After 21 days, kidneys were harvested for histopathological, mRNA and flow cytometric analyses. 1K/DOCA/salt-induced hypertension was associated with increased expression of IL-18 receptor (IL-18R) and IL-18R accessory protein on dilated tubular epithelial cells (TECs), compared to 1K/placebo mice (n=8-12). Flow cytometry and confocal microscopy also determined that IL-18R1 was upregulated on CD3+cells ($P<0.05$). Ex vivo studies demonstrated that IL-18 stimulation (10-100ng/mL) significantly augmented the proportion of interferon-γ production in enriched renal T cells from 1K/DOCA/salt-treated mice but not 1K/placebo-treated mice measured by intracellular staining ($P<0.05$). Compared to WT mice, IL-18$^{-/-}$ mice were profoundly protected from 1K/DOCA/salt-induced hypertension (tail cuff and telemetry), renal inflammation and fibrosis ($P<0.05$). Bone marrow (BM) transplant studies suggested that renal TEC, rather than macrophage-, derived IL-18 was responsible for elevations in BP. Irradiated IL-18$^{-/-}$ mice engrafted with WT BM remained protected from 1K/DOCA/salt-induced hypertension, whilst WT mice that received IL-18$^{-/-}$ BM exhibited elevations in BP. Immunolocalisation confirmed that IL-18 is predominantly localised to renal TECs during hypertension. In summary, IL-18-deficiency protected against 1K/DOCA/salt-induced renal inflammation, fibrosis and hypertension and thus targeting the IL-18 system may represent a potential therapeutic approach.

**Resolving IncL/IncM plasmid incompatibility using CRISPR-Cas9 system**

Aurelie Tsee Woon Yuen[1*], Steven Batinovic[1], Vilma Stanisich[1] & Steve Petrovski[1]

[1]: Department of Physiology, Anatomy and Microbiology La Trobe University, Bundoora, Victoria, Australia

Plasmids belonging to the incompatibility L (IncL) and M (IncM) groups were discovered in the early 1960s, but only recently received increased attention due to their association with different classes of antibiotics namely carbapenems. Additionally, both plasmid groups were found to be responsible for the worldwide spread of β-lactamase resistance genes, especially in the Mediterranean countries. Since IncL and IncM plasmids are closely related, there were some discrepancies regarding their classification. Incompatibility groupings, the first plasmid classification scheme developed, categorised plasmids based on their replication and partition controls. Caratolli et al, (2015) resolved the classification discrepancy by analysing the backbone sequences of IncL and IncM plasmids and experimentally demonstrated the compatibility between IncL and IncM plasmids. Hence, this discovery marked the clear separation between the two groups. In this study, however, we demonstrate that not all IncM and IncL plasmids are compatible. With next generation sequencing, two significant changes were observed after comparing the plasmid backbones of the IncL and IncM plasmids, notably the presence of the toxin and antitoxin (TA) system and two base changes in the plasmid copy number regulator (RNAI). Using the CRISPR-Cas9 system, we investigated the role of the TA system in plasmid incompatibility and we hypothesised that changes in the RNAI can significantly change the classification of a plasmid. Unravelling plasmid incompatibility of the IncL and IncM will provide a better understanding on plasmid divergence and differentiation.

# The Impacts of Influenza Virus NS1 Proteins on Viral Compatibility and Regulation of Innate Immune Responses

Yeu-Yang Tseng[1*], Wen-Chien Wang[2], Shan-Chia Ou[2], and Wei-Li Hsu[2]

*[1]The WHO Collaborating Centre for Reference and Research on Influenza, The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia., [2]Graduate Institute of Microbiology and Public Health, College of Veterinary Medicine, National Chung Hsing University, Taichung, Taiwan. *presenting author*

Influenza A virus nonstructural protein 1 (NS1) is essential for evading host innate immunity. NS1 inhibits interferon (IFN) responses by multiple mechanisms, including interrupting retinoic acid-inducible gene I (RIG-I) signaling by interacting with RIG-I and tripartite motif-containing protein 25 (TRIM 25). In the current study, the novel mitochondrial localization of NS1 was observed at early stage of infection. Since NS1 does not have mitochondria targeting signal, we hypothesized the interaction between NS1 and mitochondrial proteins, which was further confirmed by experiments demonstrating the potential interaction of NS1 with mitochondrial antiviral-signaling protein (MAVS) in an RIG-I-independent manner. Importantly, the association with MAVS facilitates mitochondrial localization of NS1, and thereby, significantly impedes type I IFN production mediated by MAVS. Furthermore, based on the contribution of NS1 genetic diversity in avian influenza pathogenesis, we characterize the NS1 function of virulent H5N2 strains compared with a low pathogenic avian influenza in cellular distribution, growth kinetics and induction of different cytokines. Our data indicated that differences in NS1 segments are essential for modulating anti-viral responses and viral RNA polymerase activity in a host-dependent manner.

# Development of a high throughput neutrophil chemotaxis assay to screen a transposon mutant *Acinetobacter baumannii* library to identify bacteria derived neutrophil chemotactic factors

Muhammad Ikhtear Uddin[1*], Faye C. Morris[1], Xenia Kostoulias[1], Graham Lieschke[2], Anton Y. Peleg[1, 3]

[1]*Department of Microbiology, Monash University, VIC 3800, Australia,*

[2]*Australian Regenerative Medicine Institute, Monash University, VIC 3800, Australia*

[3]*Department of Infectious Diseases, The Alfred Hospital and Central Clinical School, Monash University, VIC 3004, Australia*

* = presenting author

*Acinetobacter baumannii* infects immunocompromised individuals and those with breached anatomical barriers. Neutrophils are key defenders against *A. baumannii* infection. We have previously shown *A. baumannii paaA* mutants, secrete phenylacetate, a potent neutrophil chemo-attractant. To identify more bacteria derived chemo-attractants, we aimed to screen an *A. baumannii* transposon mutant library, in the AB5075 wild type strain. However, screening a mutant library of more than 3000 genes requires a high-throughput *ex-vivo* assay.

We have developed a high-throughput 96 well *ex-vivo* neutrophil chemotaxis assay. Neutrophils were isolated from healthy donor or buffy coat products using a neutrophil isolation kit. This neutrophil chemotaxis assay uses a transwell system performed in 96 well plate with a pore size of 3.0 µm. Neutrophils ($1x10^6$) were added to each well of the upper filter plate and chemoattractants, either bacterial resuspension ($1x10^8$), or controls (fMLP, LOS mutants) were added in lower wells. Bacteria were initially cultured in 5 mL broths and later in 96 well plates to develop the high throughput assay.

Neutrophil migration to the lower chambers were measured by a chromogenic substrate ($\lambda_{max}$ 405 nm) specific to neutrophil elastase. Robust neutrophil chemotaxis was observed towards the *A. baumannii* wild type AB5075 strain in a dose dependant manner. A multiplicity of infection (MOI) 1:100 gave least signal to noise ratio. Other factors that could potentially influence the assay, such as bacterial culture media were also tested.

We have successfully developed a high throughput 96 well neutrophil chemotaxis assay and screened 1000 mutants . Using an arbitrary cut off a 50% higher or lower migration relative to the wild type strain, we have identified 63 mutants with aberrant chemotaxis.

# Novel antigen within the RPL6 protein of *Plasmodium berghei* confers sterile immunity against malaria in mice

Ana Maria Valencia-Hernandez[1,2*], Nazanin Ghazanfari,[1] Sonia Ghilas[1], Maria N. de Menezes[1], Claerwen M. Jones[3], and Anton Cozijnsen[4], Vanessa Mollard[4], David G. Bowen[5], Nicola L. La Gruta[3], Winfried Barchet[2], Geoffrey I. McFadden[4], Mireille H. Lahoud[3], Patrick Bertolino[5], Irina Caminischi[3], William R. Heath[1] and Daniel Ferandez-Ruiz[1].

*[1] Department of Microbiology and Immunology, University of Melbourne, at the Peter Doherty Institute, [2] Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital of Bonn, Germany, [3] Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, [4] School of Biosciences, University of Melbourne, [5] Centenary Institute, The University of Sydney and AW Morrow Gastroenterology and Liver Centre*

* = presenting author

Tissue-resident memory CD8 T ($T_{RM}$) cells provide effective tissue surveillance and can respond rapidly to infection due to their strategic location. Within the liver, $T_{RM}$ cells can induce effective protection against liver-stage *Plasmodium* infection. Recently, members from our group identified PbRPL6$_{120-127}$, a H2-$K_b$ -restricted epitope within the putative 60S ribosomal protein L6 (RPL6) of *P. berghei* ANKA. Experiments were conducted to assess the suitability of PbRPL6$_{120-127}$ for the induction of endogenous liver $T_{RM}$ cells that confer sterilizing protection in B6 mice. To this end, a series of different immunization strategies targeting PbRPL6$_{120-127}$ were implemented and specific CD8 T cell responses were assessed. Results revealed that the number of naïve specific CD8 T cell precursors for PbRPL6$_{120-127}$ was very large. Specific naïve cells expand in response blood-stage infection and form memory subsets in liver and spleen after immunization with attenuated liver-stage parasites. Moreover, substantial expansion and formation of specific liver $T_{RM}$ cells was achieved by two different immunization strategies: i) Single injection with Clec9A mAb plus adjuvant and ii) Prime-and-trap, a single injection 3-component vaccine designed to prime *Plasmodium*-specific $CD8^+$ T cells in the spleen and recruit them to the liver to form $T_{RM}$ cells via recognition of locally-expressed antigen expression and adjuvant-induced inflammation (Fernandez-Ruiz et al., 2016). While mice vaccinated with Clec9A mAb developed partial protection, almost all mice vaccinated with prime-and-trap targeting PbRPL6$_{120-127}$ developed an effective and prolonged sterilizing immunity against high dose sporozoite challenges (Valencia-Hernandez et al., 2020). These findings provide insights of the highly immunogenic properties of this epitope, at least in the B6 murine model; information that can be used for the design of $T_{RM}$ cell-based subunit vaccines against *Plasmodium* infection.

Fernandez-Ruiz D, Ng WY, Holz LE, Ma JZ, Zaid A, Wong YC, et al. Liver-Resident Memory CD8+ T Cells Form a Front-Line Defense against Malaria Liver-Stage Infection. Immunity 2016;45:889–902. doi:10.1016/j.immuni.2016.08.011.

Valencia-Hernandez AM, Ng WY, Ghazanfari N, Ghilas S, de Menezes MN, Holz LE, et al. A Natural Peptide Antigen within the Plasmodium Ribosomal Protein RPL6 Confers Liver TRM Cell-Mediated Immunity against Malaria in Mice. Cell Host Microbe 2020:1–13. doi:10.1016/j.chom.2020.04.010.

# Establishing a High Throughput Sequencing Approach for Mapping Thymocyte-stromal Interactions During T Cell Development

Yi Wang[1,2*], Xin Liu[1], Seungyoul Oh[1,3] and Mark MW Chong [1,3]

*[1]St Vincent's Institute, [2]Department of Microbiology and Immunology, University of Melbourne [3]Department of Medicine, University of Melbourne*

* = presenting author

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

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

To characterize the composition of rosettes, we employed staining for various cell surface markers and analysis by flow cytometry and single cell RNA sequencing on the 10X genomics platform. This analysis revealed that rosettes contain developing thymocytes of all stages, and many types of stromal cells, including macrophages, dendritic cells, epithelial cells, B cells, granulocytes and others. This is indicative of a wide variety of different interactions captured by rosettes. However, the $CD69^{hi}$CD4+CD8+ double positive and mature CD4+ and CD8+ populations were especially enriched in rosettes. This suggests that interactions with stroma are particularly important at the later stages of T cell development.

This project provides new insights into the stromal compartment of the thymus and establishes a platform for unraveling the role of specific interactions in T cell development. Future work will apply high throughput sequencing techniques on individual rosettes to map these specific interactions. Delineating the interactions helps to complete understanding of T cell development and may thus provide novel targets for manipulating T cell development.

# *Salmonella* Typhimurium induces cIAP1 degradation to induce death in macrophages

Madeleine A. Wemyss[1,2*], Kate Lawlor[1] and Jaclyn S. Pearson[1,2]

*[1]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, [2]Department of Microbiology, Monash University*

* = presenting author

The gastrointestinal pathogen *Salmonella* 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 two specialised Type III Secretion Systems (T3SSs) which translocate effector proteins directly into the host cell cytosol. These effector proteins exert a range of pathogenic activities, including subversion of innate immune signalling and manipulation of programmed cell death processes[1]. Research from the Pearson Group (unpublished) has indicated that infection of immortalised murine macrophages (RAW264.7 cells) with wild type *S.* Typhimurium induces the degradation of cellular inhibitor of apoptosis protein 1 (cIAP1), an important host cell adaptor of tumour necrosis factor receptor 1 (TNFR1) signalling and an inhibitor of apoptotic cell death. Degradation of cIAP1 was associated with functional *Salmonella* Pathogenicity Island 1 (SPI-1) T3SS effector translocation, and was not prevented by pan-caspase, proteasomal or lysosomal inhibitors. Consistent with our understanding of cIAP1-mediated inhibition of apoptosis, we observed a strong association between loss of cIAP1 and increased cellular cytotoxicity. A cIAP1 antibody detected a low molecular weight peptide following *S.* Typhimurium infection, suggesting that a SPI-1 effector may cleave cIAP1 during infection. Current work combines several molecular and *in vitro* techniques, with the aim to elucidate the cIAP cleavage mechanism, and determine the responsible SPI-1 effector protein. Future work will assess the involvement of cIAP proteins in overall susceptibility to *Salmonella* infection *in vivo*. Together, these data provide early support for our hypothesis that cIAP1 depletion was induced by a SPI-1 effector following infection with *S.* Typhimurium in order to promote host cell death, and potentially dissemination of the bacterium.

1. Wemyss MA, Pearson JS. Host Cell Death Responses to Non-Typhoidal Salmonella Infection. *Frontiers in Immunology* (2019) 10. doi: 10.3389/fimmu.2019.01758.

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

Rachelia R Wibawa[1, 2]*, Pengfei Li[1, 2], Garrett Ng[3], Ian Van Driel[3], Shivani Pasricha[1], and Elizabeth L Hartland[1]
[1]Hudson Institute of Medical Research, Clayton VIC 3168
[2]Department of Microbiology and Immunology, The University of Melbourne, VIC 3010
[3]Bio21 Institute, Parkville, VIC 3052

*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.

These mutants were then used to identify the genomic regions important for bacterial replication *in vitro* and *in vivo.* Despite the loss of up to 42 effector-encoding genes, all mutants can replicate efficiently in THP1 macrophages. Meanwhile, in the protozoan host, at least six mutants showed a severe replication defect. Interestingly, in the mouse model, four mutants displayed an unexpected increase in bacterial burden, while one mutant showed a reduction in bacterial replication. Surprisingly, two mutants showing an increase in survival in the mouse model were unable to replicate in *Acanthamoeba castellanii*. Together, these highlight the difference in requirements to survive in different hosts. This also suggests that the large effector repertoire of the Dot/Icm T4SS effectors likely evolved to enable an intracellular lifestyle in a diverse range of hosts.

# Autoimmune epididymo-orchitis in the mouse: Indolamine deoxygenase-1 (*Ido-1*) expression and the effects of exogenous follistatin treatment

Rukmali Wijayarathna [1,2*], Hemant Khande [1], Nour Nicolas [1,4], Sneha Biniwale [1,2], Paul Gregorevic [3], Kate Loveland [1,2], Andreas Meinhardt [1,4], Monika Fijak [4], Mark Hedger [1,2]

*[(1)]Centre for Reproductive Health, Hudson Institute of Medical Research, Melbourne, Australia. [(2)]Dept. of Molecular and Translational Sciences, School of Clinical Sciences, Monash University, Melbourne, Australia. [(3)]Dept. of Physiology, School of Biomedical Sciences, University of Melbourne, Australia. [(4)]Dept of Anatomy and Cell Biology, Justus Liebig University, Giessen, Germany*

Epididymo-orchitis can cause chronic pain and infertility. The immunology underlying this condition is poorly characterised. One important factor may be *Ido-1*, a tolerogenic enzyme that is highly expressed in the caput epididymis. *Ido-1* is regulated by the pro-inflammatory and pro-fibrotic cytokine, activin A, which is antagonised in turn by its binding protein, follistatin (FST). This study examined the regional distribution of *Ido-1* expression, and the effects of exogenous FST treatment in an established murine model of experimental autoimmune epididymo-orchitis (EAEO).

Adult C57/Bl6 mice were immunised with mouse testicular homogenate in adjuvant (Complete Freund's adjuvant and Incomplete Freund's adjuvant), together with 100 ng of *Bordetella pertussis* toxin. Controls received adjuvant only, or were untreated. Prior to immunisation, some mice were injected with a non-replicative recombinant adeno-associated viral vector carrying a gene cassette for FST, which raised peripheral FST levels 5-fold, or an empty vector as control. Tissues were analysed 30 and 50 days following the first immunisation. Based on histopathology and inflammatory cytokine expression, a damage score (0 = normal, 5 = severe epididymitis/orchitis) was established.

The cauda epididymis is highly susceptible to inflammatory damage. *Ido-1*, which is expressed at relatively low levels in the normal testis and cauda epididymis, was not increased in orchitis, but increased in the cauda in proportion to the severity of inflammation. In the cauda, CD45 (leukocyte marker), F4/80 (macrophage marker) and CD80 (antigen-presentation co-receptor), were increased with increasing severity of epididymitis. Exogenous FST, which has been shown to reduce the severity of orchitis in this model, was effective at reducing expression of immune cell genes such as *Cx3cr1* and *Cd80* in the cauda during epididymitis.

These data indicate that *Ido-1* expression is selectively increased in the cauda epididymis during EAEO. This may be due to differences in the immunological environment and functional role of *Ido-1* within the testis and different epididymal regions. Exogenous FST reduces inflammation and the subsequent increase in *Ido-1* expression in the cauda epididymis, indicating its therapeutic potential for treatment of this disease.

# Development and Homeostasis of Unconventional T cells

Calvin Xu[1*], Dr. Hui-Fern Koay[1] and Prof. Dale Godfrey[2]

*[1]Department of Microbiology and Immunology, Peter Doherty Institute of Infection and Immunity, The University of Melbourne, Parkville, VIC 3010, Australia*

* = presenting author

Unconventional T cells, including NKT, MAIT, and γδ T cells, are abundant T cell populations that recognise non-peptide antigens presented by non-classical MHC-like molecules, and develop in the thymus alongside their conventional peptide-reactive, classical MHC-restricted counterparts. Following their unique developmental pathway, unconventional T cells gain an 'effector-like' phenotype at the steady-state. This results in their ability to rapidly produce a large quantity of various cytokines upon antigen recognition leading to their implication in modulating a number of host immune responses such as to infection, cancer, and autoimmunity. NKT cells are well-studied and recognise glycolipid antigens, like α-GalCer, presented by the CD1d molecule. Furthermore, MAIT cells recognise derivatives of vitamin B metabolites, such as 5-OP-RU, presented by MR1. Lastly, γδTCR-expressing γδ T cells can recognise a variety of understudied or unknown antigens. Despite their collective abundance, the frequencies of unconventional T cell subsets can vary widely between individuals, the cause of which remains elusive. However, recent evidence has suggested that NKT, MAIT, and γδ T cells may reside within a shared developmental or homeostatic niche, potentially competing for common growth factors, and may be regulated by similar genetic and/or environmental factors. Thus, we aim to further investigate this shared niche, and have confirmed previous findings in CD1d-deficient mice, which lack NKT cells but exhibit increased MAIT cells. We also demonstrate drastically increased MAIT cells in both TCRδ-deficient and newly generated CD1d/TCRδ-doubly deficient mice. Accordingly, increased MAIT cells in these mouse models appear to be phenotypically and functionally similar and may have expanded to partially compensate for the loss of other unconventional T cell subsets. Thus, our work provides insight into factors affecting unconventional T cell frequencies and cautions the use of CD1d- or TCRδ-deficient mice for studying the specific immunological functions of NKT and γδ T cells, respectively.

# Untangling the roles of T follicular helper (Tfh)-germinal center (GC) response to helminth infection

Aidil Zaini[1,2*], Kim Good-Jacoson[1,2] and Colby Zaph[1,2]

[1]*Infection and Immunity Program, Monash Biomedicine Discovery Institute and* [2]*Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia, 3800*

* = presenting author

CD4 T follicular helper (Tfh) cells are an important component of germinal centers (GCs), whose overarching goal is to produce an effective humoral immunity. However, the precise roles for Tfh and GC B cells during helminth infections via antibody-mediated mechanisms and regulatory functions of the protective type 2 immune responses, remain highly controversial. Here, we demonstrate that diminished Tfh-GC B cell response is associated with chronic infection of the helminth parasite *Trichuris muris* (*Tm*). We show that a high-dose (HD) of parasite eggs in mice that leads to an acute infection and worm clearance, results in a significant increase in Tfh and GC B cells, which is typified by the selection of parasite-specific IgG1 class switching. In contrast, a low-dose (LD) infection that results in a chronic infection fails to induce a potent Tfh-GC response, despite production of high levels of parasite-specific IgG2c. Strikingly, blockade of Tfh-GC interactions via anti-CD40L treatment during HD infection promotes chronic infection. Additionally, we found that Tfh cells are a main source of IL-4 that co-produce IL-21, all of which is to collectively sustain the GC reactions in the mesenteric lymph nodes during HD infection. Overall, these data provide cellular insights into the roles of Tfh-GC response during helminth infection and identify a potent Tfh-GC response as a protective component of the Th2 cell-dependent immunity to helminth infection.

# Unravelling immunity towards seasonal influenza vaccine in haematopoietic stem cell transplant recipients

Wuji Zhang[1*^], Oanh Nguyen[1^], Adam Wheatley[1], Malet Aban[2], Maria Auladell[1], Robyn Esterbauer[1], Timon Damelang[1], Louise Rowntree[1], Stephen Turner[1], Stephen Kent[1], Bruce Wines[3], Mark Hogarth[3], Amy Chung[1], Aeron Hurt[2], Tom Kotsimbos[4], Allen Cheng[4], Marios Koutsakos[1#] and Katherine Kedzierska[1#]

[1]*Department of Microbiology and Immunology, University of Melbourne, Peter Doherty Institute, Melbourne, Australia.*

[2]*World Health Organisation Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia.*

[3]*Burnet Institute, Prahran, Australia.*

[4]*Department of Infectious Diseases, Alfred Hospital, Prahran, Australia; [^#]equal contributions*

* = presenting author

Seasonal influenza infections cause significant morbidity and mortality, with ~500,000 deaths annually. Current vaccination regimens are the best method to combat annual influenza disease, although efficacy varies across years and can be low in high-risk groups. Haematopoietic stem cell transplant (HSCT) recipients are at high risk of severe influenza infection and show impaired immune responses towards inactivated influenza vaccine (IIV), especially within six months post-HSCT. We investigated humoral immunity to the trivalent IIV in a cohort of HSCT recipients (n=18) in comparison to healthy controls (n=14). IIV significantly increased hemagglutination inhibition (HAI) titres in HSCT recipients, similar to healthy controls. A systems serology approach revealed increased levels of IgG1 and IgG3 antibodies towards influenza-specific haemagglutinin (HA) head, but not to neuraminidase, nucleoprotein or HA stem. IIV also increased the frequency of total, IgG class-switched and activated-memory ($CD21^{lo}CD27^{+}$) influenza-specific B-cells, determined by recombinant HA-probes and flow cytometry. For those HSCT recipients who did not respond to the first dose, the second IIV dose did not improve humoral responses, although some donors reached a seroprotective HAI titre (≥40). Strikingly, selected HSCT recipients had profoundly higher antibody responses towards the A/H3N2 vaccine strain compared to healthy controls, and even showed cross-reactivity to antigenically-drifted A/H3N2 strains. Such superior responses were associated with a greater time-interval after HSCT and multivariant analyses revealed the importance of pre-existing immune memory. Overall, our study demonstrated efficient but variable immune responses to IIV across HSCT recipients. The findings provide insights into influenza vaccination strategies targeted to immunocompromised high-risk groups and the efficacy of two IIV doses.

Words 350 (limit 350)

# The development and functional fitness of type 1 conventional dendritic cells require the transcription factor DC-SCRIPT

Shengbo Zhang[1,2,*], Hannah Coughlan[1,2], Andrew J. Kueh[1,2], Mitra Ashayeripanah[3], Simona Seizova[1,2], Daniel Brown[1,2], Wang Cao[1,2], Elise Clayer[1,2], Angela D'Amico[1,2], Chris Tonkin[1,2], Yifan Zhan[1,2], Jose A. Villadangos[3], Gordon K. Smyth[1,2], Stephen L. Nutt[1,2] and Michaël Chopin[1,2]

*[1] The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia. [2] Department of Medical Biology, University of Melbourne, Parkville, Victoria 3010, Australia. [3] Department of Microbiology and Immunology, The University of Melbourne, at The Peter Doherty Institute of Infection and Immunity, Melbourne, VIC, Australia; Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, VIC*

,* = presenting author

The functional diversification of dendritic cells (DCs) is a key step in establishing a protective immune response. Despite the importance of this lineage diversity, its genetic basis is not fully understood. DC-SCRIPT, a poorly known transcription factor expressed in mature conventional DCs (cDCs) and their committed progenitors bone marrow progenitors but not in plasmacytoid DCs (pDCs). Mice lacking DC-SCRIPT showed substantially impaired development of IRF8-dependent conventional DC1 (cDC1), while cDC2 differentiated normally. The residual DC-SCRIPT-deficient cDC1s were impaired in antigen cross presentation function, which could be in part explained by the direct control exerted by DC-SCRIPT on IL12-p40 production. Genome wide mapping of DC-SCRIPT binding and gene expression analyses revealed a key role for DC-SCRIPT in maintaining cDC1 identity through the direct regulation of cDC1s signature genes, including *Irf8*. In summary our study reveals DC-SCRIPT as a critical component of the gene regulatory program shaping the unique functional attributes of cDC1s.

# Antimicrobial peptides and their antibiofilm effects

**Maryam Ghahri[1*], Mrinal Bhave[1], Enzo Palombo[1]**

**[1]Department of Biochemistry and Biotechnology, Faculty of Science, Engineering and Technology, Swinburne University of Technology**

Emergence of resistance among pathogens has made a serious challenge to effective treatment of infectious diseases by conventional antibiotics (Shagaghi et al. 2016). The development of resistance can be seen in nearly against all antibiotics (Anderson 2003). Many studies have confirmed the wide -range activity of the antimicrobial peptides (AMPs) against different microorganisms from bacteria to fungi. Four small, cationic, tryptophane rich AMPs including PuroA (FPVTWRWWKWWKG-NH2), W7 (WRWWKWW-$NH_2$), GN-2 (RWKRWWRWI-$NH_2$), and P1 (RKRWWRWWKWWKR-$NH_2$) were tested to evaluate their antimicrobial properties against clinically important pathogens viz *Pseudomonas aeruginosa, Staphylococcus aureus, Acinetobacter baumannii, Listeria monocytogenes, Klebsiella pneumonia*, and *Escherichia coli.* All peptide showed antimicrobial activity especially W7, GN-2, and P1. Furthermore, the MIC concentration of PuroA, W7, GN-2, and P1, inhibited the initial biomass attachment of biofilm of E. coli by 66.17%, 69.85%, 64.1%, and 38.9% respectively. The metabolic activity of the biofilms was also declined by employing the peptides. In addition, W7 showed having activity in reduction of cell surface hydrophobicity of *S. aureus* that consequently decrease the ability of the cells to form biofilm, also this peptide and PuroA showed a great activity to inhibit the slime layer production by *S. aureus*, which is an indicator of biofilm formation. W7 peptide, likewise, gave rise to arrest the Quorum-sensing that revealed by decrease in violacein production by *Chromobacterium violaceusm*. Meanwhile, W7 stimulated E. coli cells to elongate that contribute to activation of *sfiA* gene, which in turn causes E. coli filamentation. The results suggested the potential of these AMPs in combating with resilient form of bacteria such as biofilm that have made a serious problem in treatment of infectious diseases globally.

## References

1) Anderson, D I 2003, 'Presence of antibiotic resistant bacteria', *Current opinion in Microbiology*, vol.6, pp. 452-456.
2) Shagaghi, N., Alfred, R.L., Clayton, A.H., Palombo, E.A. and Bhave, M., 2016. Anti-biofilm and sporicidal activity of peptides based on wheat puroindoline and barley hordoindoline proteins. *Journal of Peptide Science*, 22(7), pp.492-500.

# Thursday 15 October

# Thursday
# 15 October
# Keynote speaker II

**Click here to go back to Program table**

John Morris GROUP

www.johnmorrisgroup.com

For over 65 years **John Morris Group** has been the industry leader in the supply, installation & servicing of precision instruments throughout the South Pacific.

## Laboratory Instruments and Consumables

### Pipettes & Tips


### Viscometers/Rheometers

### Centrifuges

### Ovens and Incubators

### Osmometers

### Water Baths

### Digestion & Extraction

### Peristaltic Pumps

### Spectroscopy

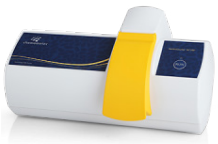
### Balances & Scales

### Hotplates & Stirrers

### pH/DO/Conductivity Meters

### Fridges/Freezers

### Cell Counting



### Microwave Chemistry

ADVANCED INSTRUMENTS | CEM | Cole-Parmer scientific experts | GILSON | Julabo | LabFriend | LAMY RHEOLOGY INSTRUMENTS | memmert Experts in Thermostatics | SIGMA

Our nationwide service & repair facilities deliver fast technical support, ensuring our customers' equipment is always running at optimum performance. Our factory trained engineers install customers' equipment, provide training and offer technical advice.

1300 501 555 info@johnmorrisgroup.com

A Family Tradition of Helping You Succeed with Technology

**Victorian Infection and Immunity Network**

**Young Investigator Symposium**

**15 October 2020**

# Profiling protective humoral immunity against infectious diseases using Systems Serology

Dr Amy Chung, Laboratory Head

*University of Melbourne, Doherty Institute*

Antibodies are a vital component of the immune response required for protection and control of infectious diseases. Beyond neutralization, antibodies can mediate an array of functions by instructing the innate immune system with their Fc region on how to attack pathogens. “Systems Serology” combines experimental and computational technologies to tease out the most important humoral immune responses required for protection or control of infection. This work has helped identify key “humoral fingerprints” associated with protection against HIV and Mycobacterium Tuberculosis and is now being applied to other infectious diseases including Malaria and COVID19.

# Thursday
# 15 October

## 10:40-11:10am

# Science Bites II

Click here to go back to program table

STEMCELL™
TECHNOLOGIES

# BREAKTHROUGHS TAKE TIME. ISOLATING THE RIGHT CELLS SHOULDN'T.

**How much time does it take you to isolate cells? What if there was a smarter, more efficient way to isolate immune cells without compromising your results?**

**LEARN MORE ›**


Copyright © 2020 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, and EasySep are trademarks of STEMCELL Technologies Canada Inc. All other trademarks are the property of their respective holders.

STEMCELL TECHNOLOGIES INC.'S QUALITY MANAGEMENT SYSTEM IS CERTIFIED TO ISO 13485. PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED.

# Allergen-specific memory B cell numbers and their transcription profile are affected within 16 weeks of sublingual immunotherapy for ryegrass pollen allergy

Craig I. McKenzie[1,2*], Nirupama Varese[1,2], Pei M. Aui[1], Bruce D. Wines[3], P. Mark Hogarth[3], Mark Hew[4], Jennifer M. Rolland[1,2], Robyn E. O'Hehir[1,2,4] and Menno C. van Zelm[1,4]

*[1]Department of Immunology and Pathology, Central Clinical School, Monash University; [2]Department of Allergy, Immunology and Respiratory Medicine, Central Clinical School, Monash University; [3]Immune Therapies Group, Burnet Institute; [4]Allergy, Asthma and Clinical Immunology, Alfred Health.*

* = presenting author

**INTRODUCTION:** Ryegrass pollen (RGP) is the most prevalent cause for seasonal allergic rhinitis and asthma in Australia. Preseasonal sublingual allergen-specific immunotherapy (SLIT) for 16 weeks reduced symptoms with 30% over placebo. Treatment is associated with increased serum specific IgG4, but the nature of this response, and effects on allergen-specific memory B (Bmem) cells are still poorly understood.

**AIM:** Examine the effects of 16 weeks of SLIT on allergen-specific memory B cells in patients with RGP allergy using newly-developed fluorescent allergen tetramers.

**METHODS:** Recombinant RGP allergen Lol p 1 was produced for ELISA-based serology or as fluorescent protein tetramer for flow cytometry. RGP-specific Bmem were identified by flow cytometry using the fluorescent tetramer in combination with a panel of 8 mAbs. Blood was obtained from 30 RGP patients at t=0 and t=16 weeks, 15 of which had received SLIT and 15 standard-of-case only. From 4 patients, Lol p 1-specific Bmem were sorted pre- and post-SIT for single-cell transcriptomics.

**RESULTS:** Serum Lol p 1-specific IgE and IgG4 were increased after SLIT and not in untreated patients. Of the 8 subsets of allergen-specific Bmem cells, 5 were decreased in number following SLIT, i.e. CD27- IgA, IgG and IgE subsets, and the CD27+IgM and CD27+IgE subsets. No changes to allergen-specific Bmem numbers were seen in untreated controls. Transcriptional profiling revealed downregulation of 27 genes and upregulation of 93 genes, the latter included *IGHE*, *ITGB1*, *PPP1R18*, *PARM1*, *STAT5B* and *FCER2*. Transcriptomics analysis of 183 cells pre- and 322 cells post-SLIT showed that the frequency of *IGHE*-expressing Bmem were increased following SLIT, and these highly expressed *FCER2* (encoding CD23) and pro-plasma cell transcription factor *ZTBT20*.

**CONCLUSION:** Preseasonal SLIT drives allergen-specific IgE and IgG4 production with a concomitant decrease in allergen-specific Bmem. Those Bmem are skewed towards *IGHE* expression and a pre-plasma cell transcriptional profile. Plasma cell differentiation and production of high-affinity antibodies can block allergen engagement on effectors cells, such as mast cells and basophils. These insights highlight transcriptomics as a powerful method for investigating rare allergen-specific B cells. Future studies with longer follow up will be needed to determine long-term and lasting effects of treatments.

# Mode of birth and risk of infection-related hospitalisation in childhood: A population cohort study of 7.17 million births from four countries

Jessica E. Miller*[1,2], Raphael Goldacre[3], Hannah C. Moore[5], Justin Zeltzer[6], Marian Knight[4], Carole Morris[7], Sian Nowell[7], Rachael Wood[7], Kim W. Carter[8], Parveen Fathima[5], Nicholas de Klerk[5], Tobias Strunk[9], Jiong Li[10], Natasha Nassar[6], Lars H. Pedersen[11], David P. Burgner[1,2,12]

[1]*Murdoch Children's Research Institute, The Royal Children's Hospital, Parkville, Victoria, Australia*
[2]*University of Melbourne, Department of Paediatrics, Parkville, Victoria, Australia*
[3]*University of Oxford, Big Data Institute, Nuffield Department of Population Health, Oxford, United Kingdom*
[4]*University of Oxford, National Perinatal Epidemiology Unit, Nuffield Department of Population Health, Oxford, United Kingdom*
[5]*Wesfarmers Centre of Vaccines and Infectious Diseases, Telethon Kids Institute, University of Western Australia, Perth, Australia*
[6]*University of Sydney, Child Population and Translational Health Research, Children's Hospital at Westmead Clinical School, Sydney, Australia*
[7]*NHS National Services Scotland, Information Services Division, Edinburgh, United Kingdom*
[8]*Telethon Kids Institute, University of Western Australia, Perth, Australia*
[9]*Centre for Neonatal Research and Education, University of Western Australia, Perth, Australia*
[10]*Aarhus University, Department of Clinical Epidemiology, Aarhus, Denmark*
[11]*Aarhus University, Department of Clinical Medicine, Aarhus, Denmark*
[12]*Monash University, Department of Paediatrics, Clayton, Victoria, Australia*

**Background** The proportion of births via caesarean section (CS) varies worldwide and in many countries exceeds WHO-recommended rates. Long-term health outcomes for children born by CS are poorly understood, but limited data suggest CS is associated with increased infection-related hospitalisation. We investigated the relationship between mode of birth and childhood infection-related hospitalisation in high-income countries with varying CS rates.

**Methods** We conducted a multi-country population-based cohort study of all recorded singleton live births from January 1 1996–December 31 2015, using record-linked birth and hospitalisation data from Denmark, Scotland, England, and Australia (New South Wales and Western Australia). Birth years within the date range varied by site but data were available from at least 2001-2010 for each site. Mode of birth was categorised as vaginal or CS (emergency/elective). Infection-related hospitalisations occurring after the birth-related discharge date were identified in children until five years of age by primary/secondary ICD-10 diagnosis codes. Analysis used Cox regression models, adjusting for maternal factors, birth parameters, and socio-economic status, with results pooled using meta-analysis.

**Results** 7,174,787 live recorded births were included. 1,681,966 (23%, range by jurisdiction 17-29%) were by CS, of which 727,755 (43%, range 38-57%) were elective. 1,502,537 offspring (21%) had at least one infection-related hospitalisation. Risk was greater among CS-born versus vaginally-born children (hazard ratio HR 1.10, 95% CI 1.09-1.12), and slightly higher following elective than emergency CS (HR 1.13, 95% CI 1.12-1.13 versus HR 1.09, 95% CI 1.06-1.12). Increased risks persisted to five years and were highest for respiratory, gastrointestinal, and viral infections. Findings were comparable in pre-specified sub-analyses of children born to mothers at low obstetric risk and unchanged in sensitivity analyses. Limitations include site-specific and longitudinal variations in clinical practice and in the definition and availability of some data.

**Conclusions** We observed a consistent association between CS births and infection-related hospitalisation in early childhood. Notwithstanding the limitations of observational data, the associations may reflect differences in early microbial exposure by mode of birth. Our findings may inform efforts to reduce elective CS rates that are not clinically indicated.

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

Doerflinger M[1*], Deng Y[1], Whitney P[2], Salvamoser R[1], Engel S[2], Pellegrini M[1], Strasser A[1], Bedoui S[2] and Herold M[1].

[1]The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; [2]Peter Doherty Institute, Melbourne, Australia

* = presenting author

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

To explore this systematically, we ablated key components of all relevant cell death pathways genetically both *in vitro* and *in vivo*. Deletion of the critical pyroptosis inducers caspase-1/11/12 or its effector GsdmD in bone marrow derived macrophages (BMDMs) caused a delay in their death upon *Salmonella* infection. Interestingly, *Salmonella*-induced cell death in Caspase-1/11/12- or GsdmD-deficient BMDMs showed morphological and biochemical features of apoptosis, such as cleavage of the effector caspases-3 and -7 and their substrate PARP. Generation of Caspase-1/11/12/8/RipK3 deficient immortalized (i)BMDMs caused complete protection from *Salmonella*-induced cell killing. Surprisingly, GsdmD/Casp8/RipK3 deficient iBMDM cells were less protected from *Salmonella*-induced cell death. Molecular analysis revealed that Caspase-1 can act in a surprisingly flexible way to deploy all apoptotic effectors when pyroptosis is disabled. Remarkably, when we examined the relative roles of pyroptosis, apoptosis and necroptosis *in vivo* during *Salmonella* infection, we found that mice lacking Caspase-1/11/12/8/RipK3 could no longer control this infection, as evidenced by abnormally high bacterial loads in the spleen and liver.

Collectively, these findings demonstrate substantial functional overlap between caspase-1 mediated pyroptosis and caspase-8 mediated apoptosis in assuring effective immune defense against intracellular bacterial infections.

# Characterizing the molecular basis of *Klebsiella pneumoniae* zinc tolerance

Eve Maunders[1*], Aimee Tan[1], Stephanie L. Neville[1] and Christopher A. McDevitt[1]

[1]*Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Victoria, Australia*

* = presenting author

Zinc is a first-row transition metal ion that is essential for a diverse range of biological functions in bacteria, though can mediate significant toxicity if present in excess. As such, bacteria tightly regulate intracellular zinc abundance to protect crucial metalloproteins and cellular processes from inappropriate zinc binding, i.e. mismetallation, which can result in perturbed function. *Klebsiella pneumoniae* is an important nosocomial Gram-negative pathogen and WHO priority organism for urgent antimicrobial development. *K. pneumoniae* is responsible for a diverse range of human diseases including respiratory tract, urinary tract and bloodstream infections. Zinc acquisition and homeostasis is an essential process for infection and virulence and, accordingly, interference with these pathways may provide new or complementary antimicrobial strategies.

The molecular mechanisms that facilitate zinc homeostasis in *K. pneumoniae* remain poorly defined. Here, we report the mechanisms that contribute to *K. pneumoniae* AJ218 resistance to extracellular zinc stress. This was addressed by transcriptomic profiling, which revealed 134 up-regulated genes and 103 down-regulated genes ($log_2$ fold change of >1 and >-1, respectively) when grown in zinc-replete conditions. Notably, the most highly up-regulated gene encoded a putative P-type ATPase (4.5 $log_2$ fold change) with homology to *E. coli* ZntA, an important zinc-export protein critical in protecting the cell against the toxic effects of excess zinc. To determine the role of ZntA in *K. pneumoniae*, we generated an isogenic deletion strain, which rendered AJ218 highly susceptible to exogenous zinc stress, manifesting as a severely impaired growth phenotype. Whole cell metal ion content of the *zntA* mutant by inductively coupled plasma-mass spectrometry showed that zinc stress resulted in significantly greater zinc accumulation (3.4 fold), concordant with dysregulation of iron and manganese homeostasis. Quantitative PCR analyses of the *zntA* mutant identified a number of modulated iron and manganese transport systems whilst alternative putative zinc transport systems, *zitB* and *YiiP*, remained unaffected. Collectively, this work shows, for the first time, the fundamental role of ZntA in *K. pneumoniae* zinc tolerance and provides a foundation for further studies on zinc homeostasis and future development of novel antimicrobials targeting this pathway.

# TCR repertoire and transcriptome differs between optimal HLA-A*02:01- and high-risk HLA-A*24:02-restricted CD8$^+$ T cell immunity against influenza A virus

So Young Chang[1*], Luca Hensen[1], Carolien E Van de Sandt[1], Simone Rizzetto[2], Fabio Luciani[2], Thi HO Nguyen[1#], Marios Koutsakos[1#] and Katherine Kedzierska[1#]

*[1]Department of Microbiology and Immunology, University of Melbourne, at the Peter Doherty Institute for Infection and Immunity, Parkville, Victoria, Australia, [2]School of Medical Sciences and The Kirby Institute, UNSW, Sydney, New South Wales, Australia: [#]equal contribution*

* = presenting author

Influenza viruses circulate annually and cause significant morbidity and mortality during seasonal epidemics. CD8$^+$ T cells provide broad protective immunity against influenza viruses. The quality of CD8$^+$ T cell response against viral infections and its protective capacity can be influenced by Major histocompatibility complex (MHC) class I polymorphisms, binding affinity of T cell receptor (TCR)/peptide-MHC-I complex, functional avidity, and the nature of the TCRαβ repertoire. Here, we assessed at the single cell level, the TCRαβ repertoire and transcriptome of CD8$^+$ T cells against an influenza-specific HLA-A*24:02-PB1$_{498\text{-}505}$ (A24/PB1$_{498}$) epitope, restricted by a human leukocyte antigen (HLA) allele frequently found in Indigenous Australians and associated with severe influenza disease during the pH1N1 outbreak. Using *ex-vivo* peptide-HLA tetramer-associated magnetic enrichment (TAME), single-cell multiplex-nested RT-PCR for paired TCRαβ repertoires, and TCRdist analysis of PBMCs from HLA-A*24:02 healthy donors, we assessed the quality of A24/PB1$_{498}^+$CD8$^+$ T cells compared to the HLA-*02:01-M1$_{58}$ epitope, which is the most well-defined and immunodominant human influenza epitope restricted to the most common HLA-allele in Caucasians. We found that the TCRαβ repertoire of A24/PB1$_{498}^+$CD8$^+$ T cells was biased in usage of TRBV9 (42.5%) with variable TRAV segment usage and highly diverse TCRαβ clonotypes. To further understand global qualitative differences between optimal A2/M1$_{58}$ and high-risk A24/PB1$_{498}$ epitopes, we performed single-cell mRNA sequencing of peptide-HLA tetramer-specific CD8$^+$ T cells *ex vivo* and showed that gene expression levels of cytotoxic molecules such as granzyme A, granzyme B and CCL5 were lower in A24/PB1$_{498}^+$CD8$^+$ T cells than in A2/M1$_{58}^+$CD8$^+$ T cells. Overall, our findings provide new insights into the mortality-associated HLA-A*24:02 allomorph and suggest strategies to develop universal T cell-mediated vaccines and immunotherapies.

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

Thorey Jonsdottir[1,2*], Natalie Counihan[3], Benjamin Dickerman[1], Smitha Sudhakar[3], Joyanta Modak[3], Betty Kouskousis[1,4], Mikha Gabriela[1,3], Paul Sanders[1], Haley Bullen[1], Tania de Koning-Ward[3], Brendan Crabb[1,2,5] and Paul Gilson[1]

*[1]Burnet Institute, [2]Peter Doherty Institute, [3]Deakin University, [4]Monash Micro Imaging, [5]Monash University*

* = presenting author

Every day >1000 people die of malaria, most of who are children under the age of five. The emergence of drug resistance in *Plasmodium falciparum*, the causative agent of severe malaria, is alarming and we need new antimalarial drugs. A crucial part of this process is to understand the basic biology of the parasite and elucidate targetable proteins/pathways. The ability of *Plasmodium falciparum* to survive within the erythrocyte is dependent on the new permeability pathways (NPPs) which provide import of vital nutrients, making them an attractive drug target. The NPPs have been affiliated with the activity of three parasite proteins: RhopH1, RhopH2 and RhopH3. We previously showed that RhopH2 was associated with 30 additional proteins predicted to be exported into the RBC. We therefore hypothesised that some of these exported proteins might be important for NPP function, as protein export is a prerequisite for NPP activity. Here we studied 14/30 proteins and their potential NPP-related functions. Reciprocal binding experiments indicated only four proteins, all PHISTb proteins, were able to strongly bind RhopH2. However, conditional knockdown of the four proteins revealed that individually none were required for NPP activity. This raised the question as to whether 1) knockdown was insufficient, 2) if the proteins were functionally redundant or 3) the proteins have roles unrelated to the NPPs. With respect to the last point, only two of the PHISTb proteins associated with RhopH2 and not RhopH1 or RhopH3, suggesting these proteins might have a different role. Although the 14 proteins studied do not appear to be essential for NPP function, this study greatly expands our current knowledge of exported proteins. We reveal for the first time the location of six new proteins, hypothesised to be exported, one of which was retained within the parasite vacuole.

# Is ovarian infection the missing piece in the puzzle of *Chlamydia*-associated infertility?

Urooza Sarma[1*], Alison Carey[2], Kenneth Beagley[2] and Karla Hutt[1]

[1]*Hutt Laboratory, Department of Anatomy and Developmental Biology, Monash Biomedicine Discovery Institute, Monash University, Melbourne, Australia*

[2]*School of Biomedical Sciences and Institute of Health & Biomedical Innovation, Queensland University of Technology, Herston, Queensland, Australia*

* = presenting author

*Chlamydia trachomatis* is the most prevalent bacterial sexually transmitted infection in the world and the leading cause of infertility in women. To date, infertility in Chlamydia-infected women has been solely attributed to pelvic inflammatory disease and scarring of the Fallopian tubes. However, fertility is also decreased in women without tubal damage, suggesting that addititional reproductive pathologies exist. In this study, we investigates the hypothesis that *Chlamydia* can infect and damage the ovary. BALB/c mice (7 weeks-old) were sham infected or vaginally inoculated with 5 x $10^4$ IFU of mouse-specific *Chlamydia muridarum* (n=3-8/group/time point). At 6 days (during the acute phase of infection), *Chlamydia* was detected within macrophages and supporting cells of ovarian follicles using qPCR and immunofluorescence. FACS analyses revealed that ovarian infection was accompanied by significant local recruitment of neutrophils, NK cells, macrophages and CD4+ T cells. Furthermore, intra-ovarian mRNA levels of pro-inflammatory cytokines, CXCL16, IL6 and IFNγ, were significantly elevated. Strikingly, primordial follicles were dramatically depleted in infected animals compared to controls (Sham: 3620 vs. Infected: 1865; $p<0.05$). At 35 days, active infection was cleared from the lower reproductive tract, but *Chlamydia* was still detectable in the ovary. Immune cells such as macrophages and CD8+ T cells, and the expression of pro-inflammatory cytokines such as TNFα and IL1α were elevated in ovaries from infected animals compared to controls. This prolonged inflammatory response was associated with increased ovarian fibrosis in ovaries collected 100 days after infection, along with poorer oocyte *in vitro* maturation and *in vitro* fertilization success rates. Collectively, these data demonstrate that *Chlamydia* can penetrate the ovary and induce a sustained immune response, resulting in depletion of the ovarian follicular reserves and reduced oocyte quality. Our data suggests that the ovary is a mucosal hotspot for chlamydial infections, long after it is cleared from the lower reproductive tract. This in turn can cause permanent damage to the ovary, which may underlie some cases of unexplained infertility and poor IVF outcomes in women.

# Anti-pneumococcal defences are altered in house-dust mite aeroallergen challenged mice

Angelica Papanicolaou[1*], Hao Wang[1], Christian Aloe[1], Jonathan McQualter[1]
Catherine Satzke[2-4], Ross Vlahos[1] and Steven Bozinovski[1]

[1]*School of Health and Biomedical Sciences, RMIT University, Bundoora, AUSTRALIA*

[2]*Murdoch Children's Research Institute, Parkville, Victoria, AUSTRALIA*

[3]*Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, AUSTRALIA*

[4]*Department of Paediatrics, Royal Children's Hospital, Parkville, Victoria, AUSTRALIA*

**Introduction/Aim:**

Asthmatics are highly susceptible to developing lower respiratory tract infections caused by *Streptococcus pneumoniae* (the pneumococcus, Spn). Alveolar macrophages play a crucial role in clearing pneumococci from the lungs, however key macrophage functions appear to be altered in asthmatics. This study aimed to investigate how innate immune responses to pneumococcal lung infections were impacted by prior house dust mite (HDM) aeroallergen sensitization.

**Methods:**

7-week old female BALB/c mice were exposed to saline (SAL, 35μl) or challenged with HDM (25μg in 35μl SAL) aeroallergen intranasally 3 times per week over a 3-week period. Following HDM exposure, Spn (EF3030, $1x10^6$ CFU in 35μl PBS) was instilled intranasally and mice were euthanized 48 hours, 4-days and 7-days post infection. Pneumococcal loads were assessed in the bronchoalveolar lavage fluid (BALF). Isolated alveolar macrophages were evaluated for their capacity to phagocytose Spn and were used to assess markers of macrophage phenotype (RT-qPCR).

**Results:**

Mean pneumococcal loads in the BALF were increased in HDM-exposed mice 48 hours (125±44 (SEM) CFU in SAL vs 228247±60326 CFU in HDM), 4-days (517±498 CFU in SAL vs 22238±10004 in HDM) and 7-days (2860±2755 CFU in SAL vs 3875±2797 in HDM; n=7-10, $p<0.05$) post infection. High levels of expression of typical alternatively activated macrophage (AAM) markers Arg1, IL-10, FIZZ1 and YM1 were observed in isolated alveolar macrophages from HDM-exposed animals. In addition, isolated alveolar macrophages obtained from HDM-challenged mice had a reduced phagocytic capacity for Spn.

**Conclusion:**

HDM-aeroallergen challenge suppresses pneumococcal lung clearance, possibly due to reduced alveolar macrophage phagocytosis of Spn. Increased expression of Arg1, IL-10, FIZZ1 and YM1 indicates the presence of a predominant AAM phenotype that may not adequately control Spn in the lungs.

# Technical Workshop II
# BMG LabTech

**Click here to go back to Program table**

BMG LABTECH

*The Microplate Reader Company*

# High throughput microplate-based methods to accelerate your virology research

**Your assay? We have it covered!**

From ELISA assays for measuring antibody titers, binding & virus neutralization, & real-time live cell assays, to studying the molecular mechanisms of viral replication, to high throughput anti-viral compound screening...

## ... BMG LABTECH microplate readers can significantly increase your research throughput


Detection Modes*

UV/vis | FI | Lum | TRF | TR-FRET | AS | FP

* Availability depends on reader specifications

### Customer Stories

### Studying the molecular mechanism of viral replication in real-time

**Alfredo Castello-Palomares: Principal Investigator, Oxford University** talks about identifying and characterising cellular RNA-binding proteins required for viruses to spread using HIV as a model.

**Topics:** RNA-binding assays, protein-RNA interaction, real time monitoring of viral protein production over 72 hours

*"We have compared many devices and found [the CLARIOstar Plus] to be the most sensitive."*

Read BMG LABTECH's virology blog articles

For further information, an instrument demonstration or quotation request, click here.

W: www.bmglabtech.com
E: australia@bmglabtech.com.au
T: (03) 5973 4744

## Technical Workshop II

# Walk-away solutions to streamline your bacterial growth assays.

Justin Robin, Area Manager

*BMG LABTECH*

The use of microplates in formats up to -384 well has provided the means to measure multiple conditions simply and easily in BMG LABTECH microplate readers. You can do away with tedious cuvettes and spectrophotometer! The ability to replicate growth requirements using accurate temperature and independent atmospheric control helps to keep your cells happy. In addition, pre-programmed shaking enables long-term growth studies requiring little user interaction - other than to load the plate and analyse the data. For high-throughput requirements, robotic integration can be supported.

Multi-mode microplate readers offer the capacity to read in absorbance, fluorescence, and luminescence in combination, and additional reagent injection provides further flexibility in experiments assessing gene expression or cell activity in relation to growth.

In this workshop, we will discuss BMG LABTECH's instrument capabilities in this context, including the measurement of bacterial growth, quorum sensing inhibition, gene expression and data analysis.

# Thursday
# 15 October

## 11:25am-12:25pm

# Oral Presentations II

Click here to go back to program table

# Crux Biolabs

**Crux Biolabs specializes in customizing and validating protein, antibody & cell assays. Our expert immunology services are flexible, timely & cost-effective.**

**TIMELY**
Efficient Data Turnaround

**ECONOMICAL**
Australian R&D Advantage

**BESPOKE**
Custom Test Development

**RELIABLE**
Accreditation & Quality Focus

Crux Biolabs has experience developing & validating assays for clinical & pre-clinical studies for vaccine & therapeutic companies.

We are a lean company with efficient processes & low overheads.

We pride ourselves on being flexible & consultative in our approach to help advance clinical research.

*"Working with Crux Biolabs was a seamless, easy and straight forward process. We were very pleased with the data output as well as expertise in assay set up and design."*

*Alex Suchowerksa PhD*
*Neuroscience Researcher, Nyrada Inc.*

# Quantification of membrane vesicles for investigation of their immunological properties

Natalie Bitto[1,2], Lauren Zavan[1,2], Ella Johnston[1,2], Neil O'Brien-Simpson[3], Timothy P. Stinear[4], Maria Kaparakis-Liaskos[1,2]

*[1]Department of Physiology, Anatomy and Microbiology, La Trobe University, Bundoora, Victoria 3086, Australia. [2]Research Centre for Extracellular Vesicles, School of Molecular Sciences, La Trobe University, Bundoora, Victoria 3086, Australia. [3]Oral Health Cooperative Research Centre, Melbourne Dental School, The University of Melbourne, Victoria, Australia. [4]Department of Microbiology and Immunology, University of Melbourne, Parkville, Victoria 3010, Australia.*

Bacterial membrane vesicles (BMVs) are ubiquitously produced by bacteria and can facilitate immune modulation by delivering biologically functional molecules to host cells and activating pathogen recognition receptors (PRRs). The first step in the characterisation and analysis of BMVs is their quantification. This critical step forms the basis of downstream analyses and therefore has the potential to affect the observed biological functions of BMVs. Currently, the most widely used method of BMV quantification is to determine vesicle protein concentration by protein assays, as protein content is thought to correlate with BMV quantity. However, our recent findings have shown that the protein content of BMVs varies throughout bacterial growth and is also affected by vesicle size. This suggests that vesicle protein concentration varies independently of vesicle number and is therefore not an accurate measure of BMV quantity. An alternative method to measuring vesicle protein concentration is the direct detection of vesicles through nanoparticle counting, however this is not widely used for BMV quantification.

This study aims to investigate how the fundamental biological effects of BMVs is biased by the quantification method used to enumerate BMVs. We quantified BMVs from Gram-positive and Gram-negative species using protein assay and Nanoparticle Tracking Analysis (NTA). Using vesicle number to normalise the concentration of protein, DNA and RNA between bacterial species, strains and growth stages, we demonstrated the dynamic nature of vesicle contents and show that vesicle protein concentration does not always correlate with vesicle number. Furthermore, we performed immunological assays based on vesicle protein and particle number to demonstrate that the ability of BMVs to signal via PRRs, activate NF-κB, and induce chemokine production differed significantly when BMVs were quantified by protein or particle number. Our data has implications on the investigation of the immunogenic properties of BMVs and their roles in bacterial pathogenesis. Moreover, we bring to light the need for a standardised approach to BMV quantification to bring consistency and reproducibility to the field of BMVs and enable comparisons between studies.

# Identification of neoepitopes from tumour biopsies: how low can you go?

Sri H. Ramarathinam[1*], Pouya Faridi[1], Angela Peng[2], Pacman Szeto[2], Nicholas C. Wong[3], Andreas Behren[4], Andreas Huhmer[5], Mark Shackleton[2,6], Anthony W. Purcell[1]

[1.] *Department of Biochemistry and Molecular Biology, Infection and Immunity Program, Biomedicine Discovery Institute, Monash University, Melbourne, Victoria*
[2.] *Central Clinical School, Department of Medicine, Monash University, Australia*
[3.] *Monash Bioinformatics Platform, Australian Centre For Blood Diseases, Central Clinical School, Monash University, Melbourne, Victoria, Australia.*
[4.] *Tumour Immunology Laboratory (TIL), Olivia Newton-John Cancer Research Institute, Victoria, Australia*
[5.] *ThermoFisher Scientific, San Jose, CA, USA*
[6.] *Alfred Health, Melbourne, Victoria, Australia*

* = presenting author

**Background:**

Peptides presented by Human Leukocyte antigen (HLA) class I and II molecules form an important component of the adaptive immune response against viruses, bacteria and tumours. Identifying HLA-bound peptides is therefore crucial to understand the specificity of T-cell responses in cancer and infectious disease. Due to sample limitations, it is often difficult or impossible to confirm epitope presentation in clinical biopsy material. To develop truly personal medicine, target epitopes need to be directly identified or validated in patient tumours at the peptide level to guide treatment.

**Methodology:**

We have developed a microscale HLA immunoprecipitation protocol which when combined with a tandem mass tag (TMT)-based barcoding approach was able to identify HLA-bound peptides. HLA-complexes were affinity purified from either cells or biopsy and the peptide cargo was tagged using TMT barcodes and analysed using high-resolution mass spectrometry.

**Results:**

We demonstrate the clinical utility of this approach by confirming the presentation of around 1000 peptides from a challenging melanoma biopsy (~1-20mg in total), including the detection of a potential neoepitope and several known melanoma epitopes.

**Conclusion:**

This strategy will be useful for studying peptidome subsets in other clinical samples where the amount of tissue available via sampling is limiting, including biopsies taken for autoimmune diseases and infections, or to sample rare cell types in tissue/cell samples, such as different antigen-presenting cell subsets. The approach can directly isolate antigens that are already presented by HLA molecules, thereby increasing the precision of personalised approaches to cancer treatment.

# Uncovering mammary gland-resident macrophages by 3D and intravital imaging

Caleb A. Dawson[1,2]*, B. Pal[1,2], F. Vaillant[1,2], L. C. Gandolfo[1,3], Z. Liu[4], C. Bleriot[5], F. Ginhoux[4,5], G. K. Smyth[1,3], G. J. Lindeman[1,6,7], S. N. Mueller[8,9], A. C. Rios[1,2] and J. E. Visvader[1,2]

*[1]The Walter and Eliza Hall Institute of Medical Research. [2]Department of Medical Biology, The University of Melbourne. [3]School of Mathematics and Statistics, The University of Melbourne. [4]Department of Immunology and Microbiology, Shanghai Jiao Tong University School of Medicine. [5]Singapore Immunology Network, Agency for Science, Technology and Research (A*STAR). [6]Department of Medicine, The University of Melbourne. [7]Parkville Familial Cancer Centre and Department of Medical Oncology, The Royal Melbourne Hospital and Peter MacCallum Cancer Centre. [8]The Peter Doherty Institute for Infection and Immunity. [9]The Australian Research Council Centre of Excellence in Advanced Molecular Imaging*

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

# A Vδ3+ subset of MR1 reactive γδ T cells recognise the side of the MR1 molecule

Michael Rice[1*], Wael Awad[1], Jérôme Le Nours[1,2], Richard Berry[1,2], Benjamin S. Gully[1,2] & Jamie Rossjohn[1,2,3]

[1]Infection and Immunity Program and Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria 3800, Australia. [2]Australian Research Council Centre of Excellence in Advanced Molecular Imaging, Monash University, Clayton, Victoria 3800, Australia. [3]Institute of Infection and Immunity, Cardiff University School of Medicine, Heath Park, Cardiff CF14 4XN, UK.

* = presenting author

PUBLISH CONSENT WITHHELD

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

Marina Zupan[1*], Zhenyao Luo[2,3,4] Aimee Tan[1], Victoria Pederick[5], Evelyne Deplazes[6,7], Katherine Ganio[1], Timothy Stinear[1], Stephanie Neville[1], Bostjan Kobe[2,3,4], Christopher McDevitt[1]

*[1]Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Victoria, Australia, [2]School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland, Australia, [3]Australian Infectious Diseases Research Centre, The University of Queensland, Brisbane, Queensland, Australia, [4]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, Australia, [5]Research Centre for Infectious Diseases, School of Biological Sciences, The University of Adelaide, Adelaide, South Australia, Australia, [6]School of Life Sciences, University of Technology Sydney, Sydney, NSW, Australia, [7]School of Pharmacy and Biomedical Sciences, Curtin Health Innovation Research Institute, Curtin University, Western Australia, Australia*

* = presenting author

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

# Liver resident CD4 T cell in malaria infection

Matthias H. Enders[1,4*], Lynette Beattie[1], Anton Cozsijnsen[2], Vanessa Mollard[2], Irina Caminschi[3,] Geoffrey I. McFadden[2], Elvira Mass[4], Daniel Ferndanez-Ruiz[1], William R. Heath[1]

*1 Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, University of Melbourne, Victoria 3000, Australia,*

*2 School of BioSciences, University of Melbourne, Victoria 3000, Australia ,*

*3 Infection & Immunity Program, Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC 3800, Australia,*

*4 LIMES-Institute, University of Bonn, 53115 Bonn, Germany*

* = presenting author

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

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

# Friday 16 October

# Friday
# 16 October
# Keynote speaker III

**Click here to go back to Program table**

## Antibody-mediated targeting of factor XII: from bench to clinic

Dr Con Panousis, Senior Director - Head of Molecular Biology

*CSL Limited*

FXIIa-mediated activation of the contact system can result in both proinflammatory and procoagulant activities via the kallikrein kinin-system and the intrinsic coagulation pathway, respectively. CSL312, a potent FXIIa antagonist antibody is currently being investigated in a phase 2 clinical study in patients with hereditary angioedema. In this talk, the preclinical evaluation of anti-FXII antibodies in thrombotic and inflammatory indications, and early clinical investigations, will be presented.

# Friday
# 16 October

## 10:40-11:10am

# Science Bites III

Click here to go back to program table

# Neural regulation of leukocyte trafficking and immunity

Sapna Devi[1,2*], Yannick O. Alexandre[1], Joon Keit Loi[1,2], Nazanin Ghazanfari[1], Laura K. Mackay[1], William R. Heath[1,2], Erica K. Sloan[3] and Scott N. Mueller[1,2]

*1Department of Microbiology and Immunology, The University of Melbourne, The Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia.*

*2The Australian Research Council Centre of Excellence in Advanced Molecular Imaging, The University of Melbourne, Melbourne, Victoria, Australia.*

*3Drug Discovery Biology Theme, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria, 3052 Australia.*

Patrolling lymphocytes are crucial for immunosurveillance and for eliciting efficient immune responses towards pathogens. T cells express receptors that enable them to respond to catecholaminergic neurotransmitters including those of the sympathetic nervous system (SNS) including noradrenaline (NA) and adrenaline. These neurotransmitters bind to α and β adrenoreceptors (AR) inducing downstream signaling and modulating cell functions. Signaling through βAR on T cells may alter immune functions, although whether this is stimulatory or inhibitory during the context of immune responses to virus infection remains unclear. Using intravital multiphoton microscopy to visualise immune cells in tissues, we discovered catecholamines can acutely alter lymphocyte dynamics and impact the ability of immune cells to navigate in tissues. This was found to alter the induction of immune responses and protection from disease. These studies will provide new insight into how we can target neural pathways to therapeutically augment immune responses and improve disease outcomes.

# Polymyxins bind to the cell surface of unculturable *Acinetobacter baumannii* and cause unique dependent resistance

Yan Zhu[1#*], Jing Lu[1#], Mei-Ling Han[1#], Xukai Jiang[1#], Mohammad A. K. Azad[1], Nitin A. Patil[1], Yu-Wei Lin[1], Jinxin Zhao[1], Yang Hu[1], Heidi H. Yu[1], Ke Chen[1], John D. Boyce[1], Rhys A. Dunstan[1], Trevor Lithgow[1], Christopher K. Barlow[1], Weifeng Li[2], Elena K. Schneider-Futschik[3], Jiping Wang[1], Bin Gong[2], Bjorn Sommer[4], Darren J. Creek[1], Jing Fu[1], Lushan Wang[2], Falk Schreiber[4], Tony Velkov[3], Jian Li[1].

*[1] Monash University, Australia, [2] Shandong University, China, [3] University of Melbourne, Australia, [4] University of Konstanz, Germany.*

[#] = equal contribution

* = presenting author

Multidrug-resistant *Acinetobacter baumannii* is a top-priority pathogen globally and polymyxins are a last-line therapy. Polymyxin dependence in *A. baumannii* (i.e. non-culturable on agar without polymyxins) is a unique and highly-resistant phenotype with a significant potential to cause treatment failure in patients. The present study discovered that a polymyxin-dependent *A. baumannii* strain possessed mutations in both *lpxC* (lipopolysaccharide biosynthesis) and *katG* (reactive oxygen species scavenging) genes. We discovered significantly remodeled cell envelope and remarkably abundant phosphatidylglycerol in the outer membrane (OM). Molecular dynamics simulations and quantitative membrane lipidomics revealed that polymyxin-dependent growth emerged only when the lipopolysaccharide-deficient OM distinctively remodeled with >35% phosphatidylglycerol, and with 'patch' binding on the OM by the rigid polymyxin molecules containing strong intramolecular hydrogen bonding. Rather than damaging the OM, polymyxins bound to the phosphatidylglycerol-rich OM and strengthened the membrane integrity, thereby protecting bacteria from external reactive oxygen species. Dependent growth was observed exclusively with polymyxin analogues, but not LL-37, poly-L-arginine, or poly-L-lysine, indicating a critical role of the specific amino acid sequence of polymyxins in forming unique structures for patch-binding to bacterial OM. Polymyxin dependence is a novel antibiotic resistance mechanism and the current findings highlight the risk of 'invisible' polymyxin-dependent isolates in the evolution of resistance.

# SELECTIVELY TARGETING THE NLRP3 INFLAMMASOME TO ATTENUATE DIABETES-ASSOCIATED ATHEROSCLEROSIS

Arpeeta Sharma[1,2*], So Young Judy Choi[1], Daniel Simpson[3], James E. Vince[3], Rebecca M. Ritchie[4], Judy De Haan[1,5,6]

*[1]Oxidative Stress, Baker Heart and Diabetes Institute, Australia*
*[2]Department of Diabetes, Central Clinical School, Monash University, Australia*
*[3]The Walter and Eliza Hall Institute of Medical Research, Department of Medical Biology, University of Melbourne, Australia*
*[4]Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Australia*
*[5]Department of Immunology and Pathology, Central Clinical School, Monash University, Australia*
*[6]Department of Physiology, Anatomy and Microbiology, La Trobe University, Australia*

Diabetes is associated with an increased risk of atherosclerosis, driven by low-grade persistent inflammation. Particularly, activation of the NLRP3-inflammasome and subsequent secretion of the inflammatory cytokine, interleukin-1β (IL-1β), is closely linked to the severity of the atherosclerosis process. We investigated whether inhibiting the NLRP3-inflammasome, through the use of a specific small molecule inhibitor, MCC950, could reduce inflammation, improve endothelial function and attenuate diabetes-associated atherosclerosis.

Eight-week old ApoE knockout ($ApoE^{-/-}$) mice were rendered diabetic with streptozotocin. At 18-week of age, non-diabetic and diabetic mice were injected with either MCC950 (5.0mg/kg) or vehicle (3% DMSO) three-times a week for a period of 10-weeks. At termination, atherosclerotic plaque, endothelial function, oxidative stress and inflammation were analysed. Inflammasome activity was assessed in mouse bone marrow derived macrophages (BMDMs) and PMA-differentiated human THP-1 cells that were cultured in high glucose (25mM) and were stimulated with LPS (0.1µg/ml) as well as in control and diabetic human aortic smooth muscle cells (HAoSMCs). The NLRP3 inhibitor, MCC950, was added to primed and/or activated BMDMs and HAoSMC prior to the second stimulus (ATP; 1mM). Inflammatory gene expression (qRT-PCR) and ELISA of NLRP3-inflammasome components were assessed.

Diabetes led to a ~4-fold increase in atherosclerosis in diabetic $ApoE^{-/-}$ mice, which was significantly attenuated with MCC950 treatment (~49% reduction, $p<0.001$). This was associated with reduced macrophage abundance, oxidative stress and attenuation of inflammatory markers (TNF-α, MCP-1, ICAM-1 and IL-1β). Vascular function, as assessed by phenylephrine-induced contraction was improved in diabetic vessels treated with MCC950. Additionally, MCC950 treatment decreased systemic inflammation and oxidative stress. In LPS and/or high glucose-treated BMDMs and PMA-differentiated THP-1 cells, as well as in control and diabetic HAoSMCs, MCC950 significantly attenuated caspase-1 and IL-1β secretion, while gene expression of NLRP3 components were unaffected. Moreover monocyte adhesion to HAoSMCs was attenuated with MCC950 treatment.

This study demonstrated that the NLRP3-inflammasome inhibitor MCC950 reduces endothelial dysfunction and atherosclerosis, by attenuating inflammation and oxidative stress in the diabetic setting. In the era of targeted therapeutics, specific NLRP3 inhibition by MCC950, may represent a novel way to improve diabetes-associated atherosclerosis.

# β-adrenergic regulation of macrophage immunometabolism

Amanda Peterson[1*], Erica Sloan[1, 2] and Darren Creek[1]

*[1]Monash Institute of Pharmaceutical Science, Monash University, Parkville, Victoria 3052, Australia; [2]Division of Cancer Surgery, Peter MacCallum Cancer Centre, Melbourne, Victoria 3000, Australia*

Macrophages play a vital role in the immune system and are responsive to environmental cues which control their function. Generally, these have been classified as either pro-inflammatory or anti-inflammatory. These functional phenotypes have been correlated with metabolic changes, where pro-inflammatory macrophages are highly glycolytic and anti-inflammatory macrophages predominantly use oxidative phosphorylation. The release of catecholamines from sympathetic nervous system activation orchestrate cross-talk between the neuro-endocrine and immune system. There is evidence to suggest an anti-inflammatory role for β-adrenergic (βAR) signalling in the immune system. The metabolic phenotype of macrophages exposed to βAR signalling has not been defined. To address this, an untargeted metabolomics approach was used to explore the effects of β-agonism on the metabolome of lipopolysaccharide (LPS)-treated macrophages.

The addition of βAR agonist, isoprenaline to LPS-stimulated macrophages decreased levels of metabolites in glycolysis and pentose phosphate pathway by 20-50% compared to LPS-treated macrophages. $^{13}$C-glucose labelling supported the finding of LPS-induced flux of glucose from glycolysis to the pentose phosphate pathway and the addition of isoprenaline reduced levels of key products in the pentose phosphate pathway. The $^{13}$C-glucose labelling revealed a reduction of de novo biosynthesis of purines and pyrimidines. However, total pools of nucleotides were abundant in both LPS-treated groups (+/- isoprenaline), suggesting active salvage pathways. The secretion of cytokines was also measured and it was found that isoprenaline also decreased pro-inflammatory cytokine levels. Further studies are evaluating the molecular mechanisms causing β-adrenergic mediated suppression of metabolism in LPS-stimulated macrophages, and the effect altered metabolism has on function.

# A novel role of apoptotic dendritic cells as antigen presenting entities within immune settings

Amy L. Hodge[1,2*], Amy A. Baxter [1,2], Sarah Caruso [1,2], Georgia K. Atkin-Smith[1,2], Lanzhou Jiang [1,2], Jieru Deng[1], Weisan Chen[1] and Ivan K.H. Poon[1,2]

*[1]La Trobe Institute for Molecular Science, Melbourne, Australia, [2]Research Centre for Extracellular Vesicles*

* = presenting author

Apoptosis is an immunologically silent, highly regulated and controlled process essential for the maintenance of tissue homeostasis and efficient cell clearance. The apoptotic cell disassembly process is characterized by the generation of small membrane-bound extracellular vesicles, termed apoptotic bodies, which have been found to carry biomolecules (i.e., nucleic acids and proteins) and are suggested to have a pivotal role in mediating intercellular communication. The generation of apoptotic bodies has been discovered within cells of the immune system such as T cells and monocytes. However, the importance of the apoptotic cell disassembly process has not been fully elucidated in dendritic cells (DCs); professional antigen presenting cells essential for directing a properly functioning adaptive immune system through T cell tolerance and priming.

Hence, to identify the function of DC-derived apoptotic bodies in immune signalling a series of cell biological analyses via timelapse microscopy, confocal fluorescent microscopy and flow cytometry were conducted using *in vitro* and *ex vivo* models of DCs.

Upon initiation of apoptosis, time lapse microscopy and flow cytometry analyses reveal that DCs undergo the apoptotic cell disassembly process through dynamic membrane blebbing as regulated via Rho-associated kinase 1 (ROCK1), form membrane protrusions and release apoptotic bodies. Furthermore, it was identified that apoptotic DCs and DC-derived apoptotic bodies retain pivotal immune signalling molecules required for efficient antigen presentation and T cell activation, including CD40, Major Histocompatibility Complex (MHC) Class I and II, and the costimulatory molecules CD80 and CD86.

Preliminary findings suggest that Influenza A viral infected DCs undergo apoptosis and release apoptotic bodies which have the potential to directly activate $CD8^+$ T cells. Therefore, our research provides insights into a novel role of DC-derived apoptotic bodies as antigen presenting entities that harness the potential of a significant physiological role within immune regulation and disease settings.

# The epidemiology of invasive *Staphylococcus aureus* and Group A *Streptococcus* in Fiji, a prospective study from 2018-2019

Li Jun Thean[1,2*], Adam Jenney[3], Daniel Engelman[1,2,4] , Lucia Romani[5], Handan Wand[5], Jyotishna Mudaliar[1], Jessica Paka[1], Tuliana Cua[1], Sera Taole[1], Aalisha Sahukhan[6] , Mike Kama[6], Meciusela Tuicakau[6], Joseph Kado[6, 7], Natalie Carvalho[8], Margot Whitfeld[9,10], John Kaldor[5], Andrew C Steer[1,2,4]

*[1] Tropical Diseases Group, Murdoch Children's Research Institute, [2] Department of Paediatrics, University of Melbourne, [3] College of Medicine, Nursing and Health Sciences, Fiji National University, [4] Malbourne Children's Global Health, Melbourne Children's Campus, [5] Kirby Institute, UNSW, [6] Ministry of Health and Medical Services, Suva, Fiji, [7] Wesfarmers Centre for Vaccines and Infectious Diseases, Telethon Kids Institute, [8] School of population and global health, University of Melbourne, [9] Department of Dermatology, St. Vincent's Hospital, Sydney, [10] School of Medicine, UNSW*

**Background.** Invasive infections caused by Staphylococcus aureus (iSA) and Group A Streptococcus (iGAS) impose significant health burdens globally. Skin and soft tissue infections (SSTI) which are predominantly caused by these bacteria, are potential sources for invasive infection to develop. Despite this, understanding of the incidence of iSA and iGAS in settings with a high SSTI burden is limited.

**Methods.** Prospective surveillance for admissions with iSA or iAS was conducted between July 2018 to June 2019 at Labasa Hospital, the referral hospital for the Northern Division of Fiji. The Northern Division (population; 131914) has the highest community prevalence of scabies and impetigo nationally.[1]

**Results.** There were 55 admissions for iSA and 15 for iGAS, equivalent to an annual incidence of 45.2 and 12.3 per 100,000 population respectively. Highest incidence was found in the youngest and oldest age groups, peaking in those aged ≥65 years at 59.6 per 10,000 person-years for both iSA and iGAS. The iTaukei (indigenous Fijian) population demonstrated the highest ethnicity specific incidence at 71.1 per 100,000 person-years with an incidence rate ratio of 9.7, 95%CI 3.5-36.9 compared to other ethnicities. SSTIs were identified in 75% of iSA and 53.3% of iGAS cases and were the most frequent clinical focus after bacteremia. Case fatality rate was high at 10.9% for iSA and 33.3% for iGAS.

**Conclusions.** We found a very high incidence of iSA and iGAS in Fiji, which was up to three times higher than in high income countries. SSTIs were a common clinical focus for both iSA and iGAS. Both diseases demonstrated a substantial risk of death. Improved control strategies, focusing on both prevention and treatment are needed to reduce the burden of iSA and iGAS in Fiji.

1. Romani L, Koroivueta J, Steer AC, Kama M, Kaldor JM, Wand H, et al. Scabies and impetigo prevalence and risk factors in Fiji: a national survey. PLoS Negl Trop Dis. 2015;9(3):e0003452. doi: 10.1371/journal.pntd.0003452. PubMed PMID: 25738499; PubMed Central PMCID: PMCPMC4349858.

# Using Polyhydroxyalkanoate Beads as Vaccine Delivery Carriers

Devi Jenika[*1], David Wibowo[2], Bernd H. Rehm[2] and Justine Mintern[1]

*[1]Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne [2]Center for Cell Factories and Biopolymers, Griffith Institute for Drug Discovery, Griffith University*

* = presenting author

Nanoparticles have been shown to be an effective vaccine carrier capable of inducing immune response against cancer and many other pathogens. Polyhydroxyalkanoate (PHA) beads are inclusions formed by many archaea and bacteria, functioning as carbon reserve that can be used during starvation[1]. PHA beads can be genetically engineered to expressed ovalbumin (OVA)-specific peptides on the surface of the beads[1]. OVA-expressing PHA (OVA-PHA) beads can then be engulfed by dendritic cells to be processed and presented to T cells. My research has shown that OVA-PHA beads were capable of inducing the proliferation of OVA-specific OT-I and OT-II cells *in vitro.* Neither OT-I and OT-II proliferated incubated with PHA beads in absence of dendritic cells, suggesting that presentation by dendritic cells is crucial in T cell proliferation elicited by OVA-PHA beads. Additionally, OVA-PHA beads have also been shown to induce OT-I and OT-II T cell proliferation *in vivo*. To further determine if OVA-PHA beads are also capable of inducing endogenous T cell response, we performed cytotoxic T cell (CTL) assay to determine if OVA-PHA beads vaccination induces OVA-specific endogenous CTL cells against OVA-expressing target cells. It was also demonstrated that intravenous and subcutaneous vaccination of OVA-PHA beads induce approximately 50% of OVA-specific CTL cytotoxicity against target cells. To further confirm the CTL response in terms of cancer settings, we inoculated OVA-expressing melanoma cells, B16-OVA, intravenously into mice that had been vaccinated with OVA-PHA beads. The vaccination significantly reduces the number of tumor nodules in the lungs compared to mice that received no vaccination. Overall, these findings suggest that PHA beads may potentially be an effective vaccine carrier.

References

1. González-Miró, M. *et al.* Bioengineered polyester beads co-displaying protein and carbohydrate-based antigens induce protective immunity against bacterial infection. *Sci. Rep.* **8**, (2018).

Copyright © 2020 PerkinElmer, Inc. All rights reserved. PerkinElmer® is a registered trademark of PerkinElmer, Inc. All other trademarks are the property of their respective owners.


# The perfect combination of convenience and results

Drug development and disease prevention are a race against time – so researchers need the confidence in their biology to enable quick solutions.
AlphaLISA® Technology delivers a fast, cost-effective, no-wash ELISA alternative that reduces user error, prevents washing away weak interactions, and makes walkaway, overnight immunoassays possible. With **wider dynamic range and better sensitivity,** you'll get more confidence in your findings especially when coupled with our VICTOR® Nivo™ Alpha enabled-Multimode microplate reader for a complete assay solution.

## Main Advantages of AlphaLISA vs ELISA:

| | ELISA | AlphaLISA® |
|---|---|---|
| **Wash Steps** | Yes | No |
| **Time of Assay** | 6-8 Hours | 2-3 Hours |
| **Sample Volume** | 50-100µL | **1-5µL** |
| **Scalable** | No | Yes |
| **Dynamic Range** | ~2-3 Log | **~4-5 Log** |
| **Sensitivity** | 10pg/mL | 1pg/mL |
| **Signal Stability** | <15min | Up to 24 hours |

Contact your local sales representative for more information.

PerkinElmer®
*For the Better*

LASSalesAU@perkinelmer.com ☎ 1800 033 391 www.perkinelmer.com/au/category/alpha-reagents

# Friday
# 16 October

## 11:20am-12:20pm

# Oral Presentations III

Click here to go back to program table

# The importance of MAIT cells revealed through the discovery of a rare human allele that renders MR1 unable to present microbially-derived stimulatory ligand

Lauren J. Howson[1]*, Wael Awad[1,2], Anouk von Borstel[1], Hui Jing Lim[3], Hamish E.G. McWilliam[3,4], Maria L. Sandoval-Romero[1], Shamik Majumdar[5], Abdul Rezzak Hamzeh[6,7], Thomas D. Andrews[6,7], David H. McDermott[5], Philip M. Murphy[5], Jérôme Le Nours[1,2], Jeffrey Y.W. Mak[8,9], Ligong Liu[8,9], David P. Fairlie[8,9], James McCluskey[3], Jose A. Villadangos[3,4], Matthew C. Cook[6,7], Stephen J. Turner[10], Martin S. Davey[1], Samar Ojaimi[11,12] & Jamie Rossjohn[1,2,13]

*[1]Infection and Immunity Program and Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia, [2]Australian Research Council Centre of Excellence in Advanced Molecular Imaging, Monash University, Clayton, Victoria, 3800, Australia, [3]Department of Microbiology and Immunology, University of Melbourne, Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Parkville, Victoria, Australia, [4]Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria, Australia, [5]Molecular Signaling Section, Laboratory of Molecular Immunology, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, Maryland, 20892, USA, [6]Centre for Personalised Immunology, The John Curtin School of Medical Research, Australian National University, Acton, Australian Capital Territory, Australia, [7]Department of Immunology, Canberra Hospital, Canberra, Australian Capital Territory, Australia, [8]Division of Chemistry and Structural Biology, Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, Australia, [9]ARC Centre of Excellence in Advanced Molecular Imaging, The University of Queensland, Brisbane, Queensland, Australia,[10]Department of Microbiology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia, [11]Department of Infectious Diseases, Monash Health, Clayton, Victoria, Australia, [12]Centre for Inflammatory Diseases, Monash University, Clayton, Victoria, Australia,[13]Institute of Infection and Immunity, Cardiff University School of Medicine, Heath Park, Cardiff, CF14 4XN, UK*

* = presenting author

The role unconventional T cells play in protective immunity in humans is unclear. Mucosal-associated invariant T (MAIT) cells are an unconventional T cell subset restricted to the antigen-presenting molecule MR1. They are highly conserved and found in high frequency in humans. Presently, mouse models of infection and observational studies in humans have largely shaped our understanding of the role MAIT cells play in immunity. MAIT cells have been identified and characterised in various disease settings including bacterial and viral infections. However, examining the specific contribution of MAIT cells to human immunity has not been explored and we do not know whether they are integral for protective immunity, or simply bystanders with a redundant role. Here, we report the discovery of a patient homozygous for a rare Arg31His (R9H in the mature protein) mutation in MR1 who has a history of difficult-to-treat viral and bacterial infections. $MR1^{R9H}$ was unable to present the potent microbially derived MAIT cell stimulatory ligand. The $MR1^{R9H}$ crystal structure revealed that the stimulatory ligand cannot bind due to the mutation lying within, and causing structural perturbation to, the ligand-binding domain of MR1. While $MR1^{R9H}$ could bind and be up-regulated by a MAIT cell inhibitory ligand, the patient lacked circulating MAIT cells. This shows the importance of the stimulatory ligand for MAIT cell selection in humans. The patient had an expanded $\gamma\delta$ T cell population, indicating a compensatory interplay between these unconventional T cell subsets. These findings provided insight into the MAIT cell-MR1 axis and the essential niche it occupies in the human immune system.

# SUR2, a novel therapeutic target for *H. pylori* associated diseases

Sohinee Sarkar[1*], Ghazal Alipour Talesh [1] Natalia Castaño-Rodríguez[2], Hazel M Mitchell[2], Trevelyan Menheniott[1] and Philip Sutton[1,3]

*[1]Murdoch Children's Research Institute, [2] School of Biotechnology and Biomolecular Sciences, UNSW Sydney, Sydney, NSW 2052, Australia, [3] Department of Paediatrics, University of Melbourne, Parkville, Victoria 3010, Australia*

* = presenting author

**Background & Aims**: While most individuals infected with *Helicobacter pylori* (*H. pylori*) remain asymptomatic throughout their lifetime, in a significant proportion the resulting severe chronic gastritis drives development of peptic ulcers or stomach cancer. Stomach cancer has poor prognosis and is the third leading cause of cancer-related deaths globally. We have identified a new therapeutic target, a host potassium channel regulatory subunit (SUR2) with the potential to protect against *H. pylori*-associated diseases.

**Methods:** SUR2 gene (*ABCC9*) expression in human gastric biopsies was analysed by qPCR, with polymorphism screening and DNA methylation analyses performed on genomic DNA. *Helicobacter* infected mice were administered SUR2-targeting drugs, Pinacidil and Nicorandil via drinking water, with gastric tissues being analysed by histology, immunohistochemistry and qPCR, and splenic tissues by ELISA. *In vitro* studies were performed on human gastric epithelial cells, human and mouse macrophages and primary mouse splenocytes.

**Results:** *ABCC9* expression in human and mouse stomachs was down-regulated upon *H. pylori* infection. However, *ABCC9* expression increased significantly upon progression to cancer and higher gene expression negatively correlated with overall survival. Polymorphism screening revealed a SNP within the *ABCC9* gene as a strong risk factor for *H. pylori*-induced gastric cancer. The variant allele contributes a CpG motif that increases local methylation density and could cause aberrant gene expression in individuals with gastric cancer. Treatment of *Helicobacter*-infected mice with Pinacidil and Nicorandil significantly reduced atrophic gastritis. Nicorandil exerted anti-inflammatory effects on gastric epithelial cells and immune cell response to *H. pylori* infection. Nicorandil-induced opening of the SUR2 channel increased intracellular potassium, thus preventing *H. pylori*-mediated calcium influx and downstream pro-inflammatory signaling.

**Conclusions:** SUR2 is a novel host factor that plays an important role in regulating *Helicobacter* pathogenesis. By targeting this channel, this study demonstrates the first pharmaceutically-induced reduction in *Helicobacter*-associated gastritis.

# Discovering the anticipatory functions of innate cells

Cyril Seillet[1,2*], Kylie Luong[1,2], Julie Tellier[1,2], Nicolas Jacquelot[1,2], Peter Hickey[1,2], Verena C. Wimmer[1,2], Lachlan Whitehead[1,2], Kelly Rogers[1,2], Gordon K. Smyth[1,3], Alexandra L. Garnham[1,2], Matthew E. Ritchie[1,2] and Gabrielle T. Belz[1,2,4]

*[1]Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia. [2]Department of Medical Biology, University of Melbourne, Melbourne, Victoria, Australia. [3]School of Mathematics and Statistics, University of Melbourne, Melbourne, Victoria, Australia. [4]Diamantina Institute, University of Queensland, Brisbane, Queensland, Australia.*

* = presenting author

To establish effective functional barriers against commensal microorganisms and pathogens, the immunological system constantly interacts with other physiological systems to monitor and respond to cyclic variations in the diet, metabolism and microbiota in the gut. For example, upon food ingestion, the immune system needs to provide protective immunity against invasive pathogens, while at the same time tolerating food proteins and foster nutrient uptake. A dynamic interaction between ILCs and sensory circuits are essential to coordinate these processes.

Group 3 innate lymphoid cell (ILC3)-mediated production of the cytokine interleukin-22 (IL-22) is critical for the maintenance of immune homeostasis in the gastrointestinal tract. Here, we find that the function of ILC3s is not constant across the day, but instead oscillates between active phases and resting phases. Coordinate responsiveness of ILC3s in the intestine depended on the food-induced expression of the neuropeptide vasoactive intestinal peptide (VIP). VIP markedly enhanced the production of IL-22 by ILC3 and the barrier function of the epithelium. Conversely, deficiency in signalling through VIPR2 led to impaired production of IL-22 by ILC3s and increased susceptibility to inflammation-induced gut injury. Thus, intrinsic cellular rhythms acted in synergy with the cyclic patterns of food intake to drive the production of IL-22 and synchronize protection of the intestinal epithelium through a VIP–VIPR2 pathway in ILC3s.

# Investigating transcriptional correlates of naturally acquired immunity to malaria

Stephanie Studniberg[1,2*], Lisa Ioannidis[1,2], Leily Trianty[3], Yang Liao[1,4], Connie Li Wai Suen[1,2], Rintis Noviyanti[3], Alexandra Garnham[1,2], Wei Shi[1,4], Diana Hansen[1,2]

*[1]Walter and Eliza Hall Institute, [2]The University of Melbourne, [3]Eijkman Institute for Molecular Biology [4]Olivia Newton John Cancer Research Institute,*

Natural immunity to *P. falciparum* malaria is slow to develop, non-sterilizing, and only protects against the clinical symptoms of infection. Consequentially, clinically immune individuals living in malaria-endemic areas will experience asymptomatic malaria infections for the duration of their lifetime. Whereas the mechanisms defining immunity to symptomatic malaria have been thoroughly investigated, the processes underlying clinical immunity to malaria and asymptomatic malaria infections are poorly understood. To address this, we performed a transcriptional analysis of peripheral blood mononuclear cells from cross-sectional study participants living in a malaria-endemic region of Eastern Indonesia with either symptomatic or asymptomatic *P. falciparum* malaria infection, and healthy, uninfected community controls. Pathway and gene ontology analysis of differentially expressed genes revealed transcripts driving the immune response to *P. falciparum* malaria: A strong proliferative and inflammatory signature in symptomatic malaria, and immunotolerant and immunosuppressive signatures underlying asymptomatic malaria. Our results identify key immunological pathways that drive the development of symptomatic malaria and provide evidence of dysregulated immune responses suggesting that asymptomatic malaria infections inhibit the development of efficient immune processes capable of controlling the infection to subpatent levels.

# TBK1 and IKKε act redundantly to mediate NF-κB activation downstream of Stimulator of Interferon Genes (STING)

Katherine R Balka[1*], Cynthia Louis[2,5], Tahnee L Saunders[1], Amber M Smith[6], Dale J Calleja[2,5], Damian B D'Silva[2,5], Fiona Moghaddas[2,5], Maximilien Tailler[1], Kate E Lawlor[7], Yifan Zhan[3,5], Christopher J Burns[4,5], Ian P Wicks[2,5,8], Jonathan J Miner[6], Benjamin T Kile[1], Seth L Masters[2,5,9] and Dominic De Nardo[1,9]

*[1]Department of Anatomy and Developmental Biology, Monash Biomedicine Discovery Institute, Monash University, Clayton, VIC 3800, Australia. [2]Inflammation Division, [3]Immunology Division, [4]Chemical Biology Division, The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria, 3052, Australia. [5]Department of Medical Biology, The University of Melbourne, Parkville 3010, Australia. [6]Department of Medicine, Department of Molecular Microbiology, and Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA. [7]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Department of Molecular and Translational Science, Monash University, Clayton, VIC 3168, Australia. [8]Rheumatology Unit, Royal Melbourne Hospital, Parkville, Victoria, Australia 3050*

*[9]Senior authors*

* = presenting author

The cGAS-STING pathway is a critical component of the innate immune response against microbial infection. Activation of cGAS-STING is induced by the presence of microbial double stranded DNA (dsDNA) within the host cell cytosol. However, aberrant cGAS-STING activation has also been implicated in the pathogenesis of multiple autoimmune and inflammatory conditions. Therefore, blocking STING activity pharmacologically has broad therapeutic potential for inflammatory diseases. Conversely, inducing STING activation with agonists has shown great promise as a cancer therapeutic in multiple pre-clinical models.

Mechanistically, stimulation of cGAS-STING induces activation of TANK-binding kinase 1 (TBK1), which phosphorylates and activates interferon (IFN) regulatory factor 3 (IRF3) to induce the expression and production of antiviral type I IFNs (E.g. IFNβ). STING also activates the transcription factor NF-κB, which synergises with IRF3 for maximal type I IFN expression, and additionally promotes the expression of numerous pro-inflammatory cytokines. While, TBK1 has been well reported to elicit IRF3 activation, the mechanisms leading to NFκB phosphorylation downstream of STING, and particularly the involvement of TBK1 remain unclear. Using pharmacological inhibition and genetic deletion of TBK1, we demonstrate that TBK1 is not essential for driving the NFκB response downstream of STING in human and mouse myeloid cells, as well as *in vivo*. Furthermore, we show that TBK1 and the closely related kinase IκB kinase ε (IKKε, also known as IKKi) act redundantly to mediate the STING NF-κB response. In contrast to the critical dependency of the kinase activity of TBK1 for IRF3 phosphorylation and activation, STING-induced NF-κB activation is far less sensitive to TBK1/IKKε inhibition. These findings delineate the IRF3 and NF-κB pathways downstream of STING. Better understanding of STING signaling may lead to improved therapeutics for STING-dependent pathologies.

# Suboptimal SARS-CoV2-specific CD8$^+$ T-cell response associated with the prominent HLA-A*02:01 phenotype

Jennifer R Habel[1]*, Thi H O Nguyen[1], Carolien E van de Sandt[1,2], Jennifer A Juno[1], Priyanka Chaurasia[3], Kathleen Wragg[1], Marios Koutsakos[1], Luca Hensen[1], Xiaoxiao Jia[1], Brendon Chua[1], Wuji Zhang[1], Hyon-Xhi Tan[1], Katie L Flanagan[4,5,6,7], Denise L Doolan[8], Joseph Torresi[1], Weisan Chen[9], Linda M Wakim[1], Allen C Cheng[10,11], Peter C Doherty[1,12*], Jan Petersen[3,13], Jamie Rossjohn[3,13,14], Adam K Wheatley[1,15], Stephen J Kent[1,15], Louise C Rowntree[1#] and Katherine Kedzierska[1#]

*In press, PNAS*

[1]Department of Microbiology and Immunology, University of Melbourne, at the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria 3000, Australia; [2]Department of Hematopoiesis, Sanquin Research and Landsteiner Laboratory, Amsterdam UMC, University of Amsterdam, Amsterdam, Netherlands; [3]Infection and Immunity Program and Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia;[4]Department of Infectious Diseases, Launceston General Hospital, Launceston, Tasmania, Australia; [5]School of Health Sciences and School of Medicine, University of Tasmania, Launceston, Tasmania, Australia; [6]Department of Immunology and Pathology, Monash University, Commercial Road, Melbourne, Victoria, Australia; [7]School of Health and Biomedical Science, RMIT University, Melbourne, Victoria, Australia; [8]Centre for Molecular Therapeutics, Australian Institute of Tropical Health & Medicine, James Cook University, Cairns, Queensland, Australia; [9]Department of Biochemistry and Genetics, La Trobe Institute of Molecular Science, La Trobe University, Bundoora 3084, Victoria, Australia; [10]School of Public Health and Preventive Medicine, Monash University, Melbourne, Victoria, Australia; [11]Infection Prevention and Healthcare Epidemiology Unit, Alfred Health, Melbourne, Victoria, Australia; [12]Department of Immunology, St Jude Children's Research Hospital, Memphis, TN 38105, USA; [13]Australian Research Council Centre of Excellence for Advanced Molecular Imaging, Monash University, Clayton 3800, Victoria, Australia; [14]Institute of Infection and Immunity, Cardiff University School of Medicine, Heath Park, Cardiff CF14 4XN, United Kingdom; [15]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, University of Melbourne, Melbourne, Victoria, Australia;
*Presenting author
[#]Authors contributed equally to this study

An improved understanding of human T-cell-mediated immunity in COVID-19 is important if we are to optimize therapeutic and vaccine strategies. Experience with influenza shows that infection primes CD8$^+$ T-cell memory to shared peptides presented by common HLA types like HLA-A2. Following re-infection, cross-reactive CD8$^+$ T-cells enhance recovery and diminish clinical severity. Stimulating peripheral blood mononuclear cells from COVID-19 convalescent patients with overlapping peptides from SARS-CoV-2 Spike, Nucleocapsid and Membrane proteins led to the clonal expansion of SARS-CoV-2-specific CD8$^+$ and CD4$^+$ T-cells *in vitro*, with CD4$^+$ sets being typically robust. For CD8$^+$ T-cells taken directly *ex vivo*, we identified two HLA-A*02:01-restricted SARS-CoV2 epitopes, A2/S$_{269\text{-}277}$ and A2/Orf1ab$_{3183\text{-}3191}$. Using peptide-HLA tetramer enrichment, direct *ex vivo* assessment of the A2/S$_{269}^+$CD8$^+$ and A2/Orf1ab$_{3183}^+$CD8$^+$ populations indicated that the more prominent A2/S$_{269}^+$CD8$^+$ set was detected at comparable frequency (~1.3x10$^{-5}$) in acute and convalescent HLA-A*02:01$^+$ patients. But, while the numbers were higher than those found in uninfected HLA-A*02:01$^+$ donors (~2.5x10$^{-6}$), they were low when compared with frequencies for influenza-specific (A2/M1$_{58}$) and EBV-specific (A2/BMLF$_{1280}$) (~1.38x10$^{-4}$) populations. Phenotypic analysis *ex vivo* of A2/S$_{269}^+$CD8$^+$ T-cells from COVID-19 convalescents showed that A2/S$_{269}^+$CD8$^+$ T-cells were predominantly negative for the CD38, HLA-DR, PD-1 and CD71 activation markers, although the majority of total CD8$^+$ T-cells were granzyme and/or perforin-positive. Furthermore, the bias towards naïve, stem cell memory and central memory A2/S$_{269}^+$CD8$^+$ T-cells rather than effector memory populations suggests that SARS-CoV-2 infection may be compromising CD8$^+$ T-cell activation. Priming with an appropriate vaccine may have great value for optimizing protective CD8$^+$ T-cell immunity in COVID-19.

# Technical Workshop IV
# Abcam

**Click here to go back to Program table**

**Technical Workshop IV**

# Abcam overview: KnockOut (KO) validation, KO cell lines and lysates, Single Wash ELISA kits, Conjugations kits and InstantBlue® Coomassie Protein Stain

Brent Thomson, Country Manager (ANZ)

*Abcam*

As an innovator in reagents and tools, Abcam's purpose is to serve life science researchers globally to achieve their mission, faster. Providing the research community with tools and scientific support, Abcam offers highly validated antibodies and assays to address important targets in critical biological pathways. Our quick overview touches on our KnockOut (KO) validation initiative, KO cell lines and lysates, Single Wash ELISA kits, Conjugations kits and InstantBlue Coomassie Protein Stain. Find out more at www.abcam.com.