ABSTRACTS
SESSION 6

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The emerging role of lipid droplets acting as modulators for innate immune signalling

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Lipid Droplets (LDs) were initially considered simply as a cellular energy source but are now recognised as critical organelles in signalling events, transient protein sequestration and inter-organelle interactions, however, their role in innate immune pathways, and the antiviral response remains largely unknown.

Work by our lab has demonstrated LDs are upregulated during viral infection, and that this upregulation contributes to an enhanced interferon response from the infected cell, indicating for the first time that the LD contributes to an effective immune response, however the mechanism of this is unknown. Here, we describe for the first time that there are several critical key antiviral signalling molecules that localise to the LD during this response. We have optimised techniques to isolate pure lipid droplets from primary immortalised astrocyte cells before and following activation of viral RNA signalling pathways. Proteomic analysis has revealed there was 83 significantly upregulated proteins on LDs following stimulation with 10% of the significantly enriched proteins being associated with the interferon response. Of these, MX1, RIG-I, STAT1 and STAT2 were significantly upregulated on LD fractions at both 8 and 24hrs following RNA viral mimic stimulation. As many significantly upregulated proteins identified on the LD are currently labelled as cytoplasmic proteins, further work was required to validate their interaction with LDs. To confirm the localisation of these signalling proteins to the LD, a technique was designed to perform fluorescent confocal microscopy on isolated fluorescently stained lipid droplets to probe for the identified immune proteins; and this, along with western blotting, has confirmed the localisation of these proteins to LDs.

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

Variations in microRNA length: A new source of disease biomarkers?

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MicroRNAs (miRNAs) were originally posited to offer a new suite of disease biomarkers due to their ubiquitous expression in biofluids. To date, their utility in this regard has, however, been limited by a redundancy of dysregulated miRNAs in disease, and problems with miRNA normalisation. In this work, we reasoned that considering miRNA length variants could significantly increase their repertoire by 5-10 fold, to increase disease specificity and their use as biomarkers.

Previous research by our laboratory demonstrated that miRNA length variations can be observed during cell infections (Nejad et al. 2018). In the current work, we investigated whether such length variations could be used to distinguish between different cell types and cell treatments. Relying on bioinformatic analyses of public datasets, we demonstrated that variation of miRNA length can also be observed in response to various stimuli, independent of infections. Analyses of miRNA length variations between 7 blood cell subsets in a cohort of 43 patients confirmed the robustness of these variations between cell types, and across patients, supporting the rationale that these variations are related to biological function. We are currently working to confirm their utility as biomarkers of cellular responses in large cohorts of patients, while establishing a broad atlas of miRNA variations, with the objective to define disease-specific length variations. This research has the potential to revolutionise the use of miRNAs as disease biomarkers.

Nejad, C., Pillman, K. A., Siddle, K. J., Pépin, G., Änkö, M. L., McCoy, C. E., . . . Gantier, M. P. (2018). miR-222 isoforms are differentially regulated by type-I interferon. *Rna, 24*(3), 332-341. doi:10.1261/rna.064550.117

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Cyclopropyl amide antimalarials act by disrupting *Plasmodium falciparum* pyrimidine metabolism

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The spread of *P. falciparum* parasites resistant to all available antimalarial therapies is threatening the future treatment of malaria. Therefore, the discovery of new antimalarial drugs with novel mechanisms of action is vital. To aid this discovery, Medicines for Malaria Venture have started Libre, an open source drug discovery program, and have identified several new series of compounds for development. One of these series is the cyclopropyl amide aryl piperazines. These compounds display promising blood and liver stage activity and low toxicity against mammalian cells, but lack information about their mode of action, which would help guide their future development. Pulsed exposure assays showed the cyclopropyls have a slow rate of killing (>5 h) and are most active against trophozoite stage parasites. Our untargeted metabolomics studies were conducted on MACS purified mid trophozoite stage parasites that were incubated with the cyclopropyls MMV1804508 (IC50 = 225 nM) or MMV1804742 (IC50 = 835 nM) for 5 h at 5x IC50. The inactive cyclopropyl, MMV1803903, and atovaquone were also included as controls. This untargeted analysis revealed that the active cyclopropyls perturbed pyrimidine biosynthesis, resulting in a significant ($p<0.05$) increase in carbamoyl aspartate (81-fold) and dihydroorotate (25-fold) and depletion of downstream pyrimidines, such as UMP (0.5-fold) compared to untreated parasites. This metabolic signature is similar to that seen for atovaquone, a known parasite cytochrome bc1 inhibitor, indicating a similar mode of action for cyclopropyls and atovaquone. We further confirmed this mechanism in cross resistance studies with the atovaquone resistant parasite line, SB1-A6, which was completely resistant to both cyclopropyls tested (IC50 > 10 μM). Taken together, these data indicate that the cyclopropyl series act by inhibiting the parasite mitochondrial bc1 complex. Future development of this series will be guided by these findings.

Influenza infection of human macrophages

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Attachment and entry of influenza A virus (IAV) into epithelial cells, a primary target for infection in the respiratory tract, is well characterised. However, infectious entry of IAV into airway macrophages, the first immune cells to respond to respiratory pathogens, is not clearly defined. In epithelial cells and macrophages, IAV hemagglutinin (HA) mediates virion attachment to the cell surface through recognition of sialic acid expressed on glycoproteins and glycolipids. Macrophages also express pattern recognition receptors, including C-type lectin receptors (CLR), which recognise microbial carbohydrate structures. Our laboratory has shown that IAV utilises specific CLRs, such as the mannose receptor (MMR), galactose-type lectin (MGL) and Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN), for infectious entry of IAV into macrophages.

Herein, we investigated the role of CLRs and sialic acid in mediating IAV infection of primary human macrophages. We showed that monocyte-derived macrophages (MDMΦ) isolated from peripheral blood express MMR and stimulation with IL-4 resulted in upregulation of DC-SIGN, but not MGL. We observed a slight increase in IAV infection of IL-4 stimulated compared to unstimulated MDMΦ, using H1N1 and H3N2 representative strains. In addition, blockade of mannose-specific CLRs (MMR and DC-SIGN) by pre-treatment with mannan only partially reduced IAV infection. This suggests the presence of galactose-specific CLR or CLR-independent entry pathways for IAV during infection of MDMΦ. To dissect out the relative contribution of CLRs and sialic acid for IAV infectious entry into human macrophages, we engineered IAV variants with (i) varying numbers of glycosylation sites on the HA head and (ii) a preference for sialic acid expressed in either a α2,3- or α2,6-linked conformation. Modulation of receptor preference for α2,3- or α2,6-linked sialic acid did not alter the ability of the respective IAV variant to infect MDMΦ. Intriguingly, the addition of two glycosylation sites to the HA head of PR8, led to a decrease in infection of human MDMΦ. This is in contrast to findings in murine macrophages, where additional glycosylation confers increased infectivity to the PR8 strain. It is possible that glycans near the receptor binding site influence sialic acid binding strength of the HA. Future work will implement these techniques to elucidate the role of CLRs and sialic acid during IAV infection of human alveolar macrophages (AMΦ).

Single-cell analysis of αβ versus γδ T cell development

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T cells develop in the thymus, where they acquire a range of distinct functional identities. T cells are divided into the αβ or γδ lineages. Progenitors that enter the thymus remain multipotent, with the potential to differentiate into a range of cell types. T lineage identity is then fixed early in T cell development. Later, γδ T cells branch off, while αβ development continues onto additional stages. Studies over the years have defined, at a population level, many of the specific genes that have to be activated or silenced as T cell progenitors differentiate toward either lineage. However, it is still unknown whether this combination of activation/silencing actually occurs in each and every cell as they differentiate. To investigate this, single-cell RNA-sequencing (scRNA-seq) was employed to re-assemble *de novo* a model of the early stages in T cell development based on the transcriptional profiles of individual cells. >20,000 CD4-CD8- double negative (DN) and γδ thymocytes were analysed using Chromium 10x scRNA-seq over three runs. Hierarchical clustering revealed that early thymocyte populations are much more complex than the standard view of T cell development. Assembly of these thymocyte populations into developmental trajectories based on gene expression suggested that the decision to differentiate into αβ versus γδ T cells occurs at a much earlier stage than the current model and that distinct DN1 and DN2 sub-populations are specific to either the αβ or γδ developmental pathways. To confirm that specific sub-populations are restricted to either αβ or γδ lineages, they were sorted and analysed for lineage outcomes in OP9-DL1 cultures. We show that lineage is already restricted in DN1 thymocytes, which is considered the earliest stages of T cell development. Thus, γδ developmental appears to occur in a parallel with αβ development and not a branch as previously thought.

Evading the host immune response in neuronal grafts using immune-cloaked human stem cell-derived midbrain dopamine progenitors

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Clinical trials involving the transplantation of dopaminergic (DA) progenitors into the brain of Parkinson's Disease (PD) patients requires prolonged, systemic delivery of immune-suppressive drugs to prevent graft rejection. An alternative approach is use donor cells that will avoid detection by the host immune system. While the use of a patient's own stem cells or allogenic (HLA-matched) cells is optimal, it remains largely unfeasible due to high costs, necessary QC and associated labor (Lanza et al., 2019). To circumvent such conundrum, we propose to use an 'immune-cloaked' pluripotent stem cell (PSC) line – capable of evading detection by the host system. A mouse and a human 'immune-cloaked' PSC line were created by forced expression of 8 immunomodulatory genes (CCL21, PDL1, FASL, HLA-G, Serpinb9, CD47, CD200, MFGE8) that interfere with the activity of several immune cell types, including T-cells, NK-cells, antigen presenting cells and macrophages (Harding et al., 2019). Subcutaneous grafts of these mouse 'immune-cloaked' PSC were shown to generate ectopic tissues in HLA-mismatch hosts and evade long term immune detection (Harding et al., 2019). To date, only the efficiency of mouse 'immune-cloaked' cells has been demonstrated in healthy animals and therefore assessment of the therapeutic potential of human cloaked cells in healthy and disease models is needed. Moreover, while mouse cloaked cells can differentiate efficiently into cardiomyocytes in vivo (Harding et al., 2019), the capacity of human cloaked cells to generate different cell types has not been investigated. Advancing on these findings, we are now examining the capacity of human cloaked cells to (1) be differentiated into bona fide ventral midbrain DA neurons in vitro, (2) promote functional recovery following transplantation into an athymic rat model of PD (to demonstrate the functionality of the donor cells) and (3) evade immune detection in a humanised mouse model of PD (to confirm efficiency of the cloaking).

Defining the mechanisms of action of antibodies against Plasmodium vivax malaria

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Effective vaccines against both *Plasmodium falciparum* and *P. vivax* will be essential to achieve malaria elimination goals globally. There have been great strides in developing *P. falciparum* vaccines, substantially less progress has been made for developing *P. vivax* vaccines. This is partly due to the lack of knowledge of key immune functions that are naturally acquired to *P. vivax* infection. Antibodies play an essential role in malaria immunity and can target antigens to activate the complement system, mediate binding to Fcγ-receptors expressed by immune cells and promote opsonic phagocytosis. However, currently we have limited knowledge on functional activities of acquired antibodies to *P. vivax*.

Here, we quantified functional mechanisms of antibodies to *P. vivax* acquired from natural exposure. We evaluated a cohort of children (n=37; ages 5-14), resident in a malaria-endemic region of PNG, for antibody responses to 4 *P. vivax* antigens - AMA1, MSP3α and MSP1-19, and the two allelic forms of CSP. Specifically, we quantified the acquisition of IgG and cytophilic subclasses, measured the ability of antibodies to mediate complement fixation and Fcγ-receptor binding (types I, IIa and IIIa). We additionally used an established flow cytometry-based method to detect antibody-mediated opsonic phagocytosis of antigen coated beads by THP-1 monocyte cells.

Children poorly acquired antibodies with complement fixation activity to all antigens tested, whereas antibodies to MSP3α and AMA1 antigens were able to bind to Fcγ receptors. Substantial opsonic phagocytosis activity with THP-1 cells was only observed with MSP3α coated beads. Limited functional activity to the other antigens may be explained by the lower acquisition in this cohort of IgG cytophilic subclasses, IgG1 and IgG3, which were correlated with functional antibody responses. Further studies will investigate these responses in other cohorts, the neutrophil-mediated phagocytosis and other antigen targets of functional antibodies.

Our findings suggest that antibodies to *P. vivax* antigens can mediate a range of effector functions, including opsonic phagocytosis, and acquired functional antibodies target some antigens more effectively. This contributes to our knowledge of *P. vivax* immunity and will be valuable for the development of effective vaccines.

Profiling the antibody response 6 years following reduced-dose quadrivalent HPV vaccination in adolescent Fijian girls

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Prophylactic human papillomavirus (HPV) vaccines protect against persistent HPV infection, cervical pre-cancerous lesions and cancer. Although a two-dose schedule separated by 6 months is currently recommended for boys and girls under the age of 15, emerging data suggest that a single dose of the bivalent (2vHPV) or quadrivalent (4vHPV) HPV vaccine generates similarly protective antibody responses. Neutralising antibodies induced by vaccination are thought to be the primary mechanism of protection against infection. However, previous studies in animal and *in vitro* models suggest that there may be additional mechanisms of antibody-mediated protection apart from neutralisation, particularly at low antibody concentrations. This includes Fragment crystallizable-mediated effector functions such as opsonophagocytosis, cellular cytotoxicity and immune cell degranulation. At present, very little is known about the characteristics of the antibody response following reduced-dose HPV vaccination.

In this study, we will examine the antibody profile of girls who previously received 1 or 2 doses of 4vHPV 6 years earlier, compared to those who received 3 doses. In 2015, a prospective cohort study of 200 Fijian girls (aged 15-19 years at the commencement of the study) previously vaccinated with 0, 1, 2 or 3 doses was conducted, with equal numbers of girls in each dose group. Blood was taken before and 28 days following a single dose of 2vHPV. To measure antibody subclasses (total IgG, IgG2, IgG3, IgG4, IgA1, IgA2 and IgM) specific to oncogenic HPV genotypes (HPV16, 18, 31, 33, 45, 52 and 58) in serum, a multiplex microsphere-based immunoassay was employed. Preliminary analyses of a subset of the study samples (N=80; 20/group) show significant increases in total IgG, IgG1 and IgG3 to all HPV genotypes post-2vHPV. Further analyses are currently ongoing to measure HPV-specific IgM, IgA1, IgA2, IgG2 and IgG4 before stratification by dose group. Profiling the antibody response to HPV vaccination will aid our understanding of the immunological mechanisms of the prophylactic HPV vaccines and provide an explanation for the robust protection offered by single-dose vaccination.

Investigating the antimalarial activity of novel heterospirocyclic compounds in drug-resistant parasites

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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 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. We hypothesise the unique and pronounced 3D architecture of these molecules allows them to interact with biological domains otherwise inaccessible to relatively flat structures, leading to increased efficiency. We have previously shown that two spirocyclic compounds (C25 and C26) induce death of the most lethal malaria parasite *P. falciparum,* within 48hrs and present IC_{50} values in the low micromolar range. Recently, we have shown that both compounds present no toxicity in kidney- (HEK293) and hepatic- (HepG2 and Huh-7) derived humans cell lines, even at 20µM concentration, when compared to the chemotherapeutic positive control cisplatin. Further, we have shown that both compounds have a killing effect on multiple drug-resistant *P. falciparum* strains, including artemisinin-sensitive Cam3.II^{rev}, artemisinin-resistant Cam3.II^{R539T} and Cam3.II^{C580Y}, and multi-drug resistant W2mef parasite lines. Untargeted metabolomics analysis indicates that C25 perturbs pyrimidine biosynthesis by inhibition of the *de novo* pyrimidine biosynthesis pathway. Further analysis is ongoing to identify the specific target(s) of C25 and whether cross-resistance is observed with other drugs that inhibit this pathway. Parasites resistant to C25 and C26 have been generated in 3D7 and Dd2 genetic backgrounds and whole genome sequencing will be performed on the resulting resistant clones. This biological information will allow the synthesis of refined derivatives with more potent antimalarial activity.

Reference: **Sharma P, Prabhath MRR, Wong D, Ampem-Lassen MA, Bhat SV & Carvalho TG.** Synthesis of Biologically Active Heterospirocycles Through Iterative 1,3-Dipolar Cycloaddition Pathways. *The Journal of Organic Chemistry* 2020; 86(1): 1223-1230.

Multi-Pronged CAR-T Cells to eliminate Cutaneous T Cell Lymphoma

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Inflammation dependent differentiation of two distinct VAT Treg populations shape systemic metabolism

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Visceral adipose tissue (VAT) is a multifaceted organ that regulates systemic metabolism through energy storage and endocrine functions. Inflammation, induced by dietary changes or ageing, however, hamper VAT function and can lead to metabolic disease. The VAT is rich in Foxp3+ regulatory T (Treg) cells, which are recruited by inflammatory mediators and contribute to the control of VAT inflammation. Enrichment of Treg cells, however, is sexually divergent and as we have shown, imprinted by inflammatory cues and sex hormones (Vasanthakumar et al. Nat. Immunol. 2015, Vasanthakumar et al. Nature 2020). Precisely how inflammatory conditions are linked to VAT physiology and Treg cell homeostasis is, however, unclear.

Here we uncover that female and male VAT show distinct inflammatory signatures. These differences in cytokine landscape promoted the differentiation of phenotypically distinct VAT Treg cells in males and females which conform two major populations, ST2+ and ST2- VAT Treg cells. We further show that the differentiation of these VAT Treg cell populations is driven by separate molecular pathways that complement the known transcriptional regulators of VAT Treg differentiation, Blimp1 and PPARg (Cipolletta et al. Nature 2012, Vastanthakumar et al. Nature 2020). Functionally, both VAT Treg cell populations are required to control VAT inflammation and preserve organismal glucose homeostasis. Hence, this study for the first-time establishes how distinct population of Treg cells with unique molecular requirements regulate VAT inflammation and organismal metabolism in a sex-specific manner.

MAIT cells expand in the absence of NKT and γδT cells

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

We showed that NKT cell-deficient mice have increased MAIT cells and demonstrated that this increase was due to the loss of NKT cells rather than their restricting element, CD1d. MAIT cells were also markedly increased in γδT cell-deficient mice and expand further in NKT/γδT cell double-deficient mice. Expanded MAIT cells phenotypically and functionally resembled their wildtype (WT) counterparts. As MAIT cells were increased in the thymus, we analyzed *Trav1-Traj33* transcripts within developing thymocytes and showed that overall *Trav1-Traj33* transcript levels were increased 2-fold within γδT- and NKT/γδT-deficient thymocytes relative to WT controls. As γδT-deficient mice harbor a modified TCRδ locus, our findings imply that TCRα rearrangement may be altered in these mice, potentially manifesting in greater rearrangement of distal *Trav* gene segments like *Trav1* and increased intrathymic generation of MAIT cells. However, increases in peripheral MAIT cells exceeded increases in the thymus, indicating that MAIT cells may compete with peripheral NKT and γδT cells for similar homeostatic factors and expand in their absence. Accordingly, we show that adoptively transferred MAIT cells underwent more proliferation within NKT/γδT-deficient hosts relative to WT controls.

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

ABSTRACTS
SESSION 8

Increased immunopathology and perturbed immune dynamics during influenza virus and arbovirus co-infection

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Infection with more than one pathogen, in sequence or concurrently, occurs commonly in nature and can substantially affect immune responses. Reports on co-infections often note poorer health outcomes and increased pathogen burdens compared to single infections. Viral co-infections can affect individual antiviral responses, reduce protection, and enhance immunopathology. Limited information exists on the outcome of co-infection with influenza and non-respiratory viruses. As influenza is prevalent worldwide, its geographical distribution overlaps with that of many arboviruses. To explore the interaction between these two types of virus infection, we established a C57BL/6 mouse model of co-infection using Semliki Forest Virus (SFV), a neurotropic arbovirus, and Influenza A virus (IAV). Adult C57BL/6 mice were infected with IAV only (respiratory infection); SFV only (systemic infection followed by encephalitis); or sequentially co-infected on day 8 post-primary infection (either SFV→IAV or IAV→SFV). Viral, inflammatory and immunological analyses were performed on day 7 following either single infection (IAV; SFV) or co-infection (SFV→IAV; IAV→SFV). In the SFV→IAV co-infection group, we observed more severe disease. This was linked to an exacerbated lung cytokine storm and delayed viral clearance in co-infected animals, resulting in more severe lung pathology. Moreover, we found altered trafficking of immune responses, particularly IAV-specific $CD8^+$ T cells being redirected to the brain in SFV→IAV co-infection. These data provide new insights into how co-infection with viruses which cause predominantly either lung or brain disease each alter the immune response and disease outcome of the other. Improved fundamental knowledge on how viral infections interact to affect the course of immune responses, could be of a direct relevance to improved disease management programs, specialist treatments and optimisation of vaccination strategies.

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

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Airah Javorsky = presenting author

Abstract

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

References

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Influenza A virus causes maternal and fetal pathology via innate and adaptive vascular inflammation

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Introduction: Seasonal and pandemic influenza is a disease of global significance and in pregnant women, severe maternal illness and foetal complications ensue by enigmatic mechanisms. Major fetal complications develop despite a lack of vertical transmission of the influenza A virus [1,2]. Hence, to discern the mechanisms that prompt these major foetal complications, we developed specific aims targeting maternal cardiovascular function.

Aims: We aimed to determine whether influenza infection led to maternal vascular dysfunction and the accompanying foetal pathology, and to characterize the role of the innate and adaptive immune system in influenza induced vascular dysfunction.

Methods: Eight-to-twelve-week old time-mated pregnant (E12 gestation) and non-pregnant C57BL/6 female mice were intranasally infected with a moderate-pathogenic H3N2 IAV strain (HKx31; 10^4 PFU) or with PBS. Mice were culled 3 and 6 days post-infection, for tissue collection. Innate and adaptive immune inflammatory profiles of the aorta and placenta were determined by qPCR or flow cytometry. Maternal thoracic aorta vascular function was assessed via myography in response to endothelium-dependent and independent smooth muscle relaxants, acetylcholine (Ach) and sodium nitroprusside (SNP), respectively.

Results: We have demonstrated a significant and dynamic alteration in the maternal vascular landscape that underpins maternal and foetal pathology to IAV infection. IAV infection that causes only a mild local lung inflammatory response in non-pregnant female mice, resulted in contrast, in significant virus dissemination to the aorta in pregnant mice. The dissemination of virus in the aorta was associated with a significant increase in pro-inflammatory and anti-viral mediators and the influx of $Ly6C^{low}$ and $Ly6C^{high}$ monocytes, neutrophils and T cells, in what we define as a "Vascular Storm". Moreover, the vascular storm was associated with elevated levels of the adhesion molecules ICAM and VCAM, as well as pattern recognition receptor TLR7 in the vascular wall. We also report that maternal influenza infection elicited hypoxia in the placenta and foetal brain as well as placental growth retardation and intrauterine growth restriction. In contrast, IAV infection in non-pregnant mice caused no alterations in endothelial function or vascular inflammation.

Conclusion: These results indicate that IAV infection during pregnancy drives a significant cardiovascular event in pregnant mothers, which likely suppresses critical blood flow to the placenta and foetus resulting in hypoxia. This study provides a fundamental mechanistic insight and a new paradigm into how the immune system drives maternal and foetal pathologies during pregnancy to respiratory viruses such as influenza A virus, which normally do not result in transplacental infection.

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Pomalidomide as an immunomodulatory agent to enhance NK cell anti-HIV immunity

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Chronic HIV infection is characterised by dysfunction of key immune effector cells including $CD8^+$ T cells and NK cells that persist despite successful viral suppression by antiretroviral therapy (ART). Here, we investigated pomalidomide, an immunomodulatory drug licensed for the treatment of multiple myeloma and Kaposi's Sarcoma, to augment anti-HIV immune responses through enhancing NK cell cytotoxicity.

Pomalidomide was assessed at a therapeutically relevant concentration *ex vivo* in PBMC from uninfected donors and ART-suppressed people with HIV (PWH). Direct NK cytotoxicity was assessed by co-culturing pomalidomide-treated PBMC with the MHC-I devoid K562 cell line. We next established a novel assay where pomalidomide pre-treated NK cells were co-cultured with *in vitro* HIV-infected autologous $CD4^+$ T cells carrying an EGFP-reporter. The effect of pomalidomide on direct NK cell killing of productively HIV-infected $CD4^+$ T cells was quantified from the reduction in GFP^+HIV-infected cells. Antibody dependent cellular cytotoxicity (ADCC) was assessed using the 8E5 cell line, with 50% of cells containing a single defective provirus and expressing p24 and envelope. Pomalidomide pre-treated NK cells and 8E5 were cultured with anti-HIV immunoglobulin or an isotype control, with HIV-specific ADCC measured as a relative reduction in $p24^+$ 8E5 cells.

Treatment with pomalidomide in both HIV-negative donors and PWH significantly enhanced NK killing of K562 cells, in both HIV-negative donors and PWH, with 34.7% (95% CI 23.8-51.05; P=0.0002) greater cytotoxicity in PWH, relative to DMSO. Concurrently, $CD56^{bright}CD16^+$ NK cell subset was expanded in the presence of pomalidomide. Pomalidomide treatment significantly enhanced direct NK-mediated killing of *in vitro* HIV-infected autologous $CD4^+$ T cells. In contrast, pomalidomide did not enhance anti-HIV ADCC.

These results show that pomalidomide can enhance direct NK cell cytotoxicity and augment killing of HIV-infected cells, and may be employed in therapeutic strategies to eliminate or control the persisting HIV reservoir.

Snotwatch: When data go viral

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Polymerase chain reaction (PCR) testing has become the favoured viral detection testing method in recent years. Multiplex PCR data can be used to analyse viral patterns of multiple viruses over time and place.[1] Spatiotemporal data analysis, an emerging area that allows analysis of data that have both a space and a time component, is optimal for the kind of population-level data that PCR testing provides. The global response to the COVID-19 pandemic best demonstrates how PCR results can be used in conjunction with a range of geographic information systems (GIS) to track disease spread in near-real time.[2] This is one application of spatiotemporal analysis techniques.

Our project, 'Snotwatch', aims to extend the uses of spatiotemporal analysis from single variable to multi-variable. Where most previous spatiotemporal analyses consisted of surveillance studying respiratory PCR Data, we aim to analyse the relationship of viral circulation to health outcomes of interest. Looking at both the space and time component of the data, our project will create a novel statistical model for assessing *relationships* between independent datasets of respiratory virus circulation and various clinical outcome presentations using primary care and hospital encounter datasets. We will study viral incidence patterns in relation to the clinical incidence of asthma exacerbations, febrile seizures, Kawasaki disease, acute myocardial infarctions, and chilblains.

To date, we have conducted a proof-of-principle ecological study of presentations of febrile seizures and respiratory viruses detected at Monash Health hospitals and laboratories from 2010-2019. Associations were studied temporally and geo-temporally through mixed effects Poisson regression analysis. Febrile seizure incidence peaked in June-September. Temporal analysis showed febrile seizure associations with Human metapneumovirus, Influenza A, Influenza B and Respiratory syncytial virus (RSV) ($p<0.01$ for all). Geo-temporal analysis supported the association between febrile seizures and Influenza A, Influenza B and RSV (1.06, 1.03 and 1.07 risk ratio respectively, $p<0.01$). We have begun applying this same methodology to chilblain diagnoses in a general practice setting in relation to COVID-19 circulation but are yet to obtain results.

Further application, assessment and modification of this statistical model will be the focus of this project. This model has implications for predictive modelling using real-time viral data, which can subsequently inform public health policy.

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Mouse Mx1 inhibits HSV-1 at a Late stage in the Virus Replication Cycle.

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The Myxoma (Mx)-family proteins are interferon-inducible GTPases and some Mx proteins are known to be potent inhibitors of influenza A virus (IAV) and other RNA viruses. Recent evidence suggests that human Mx proteins can also modulate some herpesviruses infections however little is currently known regarding the ability of mouse Mx proteins to inhibit herpesvirus infections. Herein, we have used a doxycycline (dox)-inducible system to demonstrate that expression of mouse (m)Mx1, but not mMx2, in a murine airway epithelial cell line results in potent inhibition of IAV, as well as HSV-1 and HSV-2. Confocal microscopy confirmed dox-inducible mMx1 was expressed in the nucleus whereas inducible mMx2 localised to the cytoplasm. Subsequent studies have focussed on investigating the antiviral activity of mMx1 against HSV-1. First, primary lung fibroblasts generated from mice expressing a functional endogenous Mx1 protein were shown to mediate potent inhibition of HSV-1 replication compared to fibroblasts from control animals. Second, we generated cells expressing mMx1 mutants known to abrogate anti-IAV activity, namely T69A (abrogates GTPase activity and GTP binding) and R614E (results in relocalisation of mMx1 from nucleus to cytoplasm) and used these cells to demonstrate that restriction of IAV and HSV-1 replication was lost following expression of either mMx1 mutant. Third, we have used a labelled HSV-1 where green fluorescence protein (GFP) is driven from the gB promoter (γ1 gene, expressed in small amounts before genomic replication) and red fluorescence protein (RFP) from the gC promoter (γ2 gene, expression absolutely dependent on genomic replication) to demonstrate that mMx1 potently inhibits expression of HSV-1 late genes that are dependent on genomic replication. Finally, we demonstrated that mice with a functional endogenous mMx1 were protected from the development of sever skin lesions and had lower virus titre in the skin and dorsal root ganglia in the mouse model of cutaneous HSV-1 infection.

Illuminating the chemical space for MR1-restricted ligands: a binding assay using fluorescence polarisation

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Studies into T-cell-mediated immunity have largely been focused on understanding peptide presentation by Major Histocompatibility Complex (MHC) and their recognition by αβ T-cell receptors (αβ TCRs). By contrast, our understanding of non-peptide T-cell-mediated immunity in humans is limited. Mucosal-associated Invariant T-cells (MAIT cells) represent a significant population of T-cells in mucosal tissues and peripheral blood, and play a critical role in early immune responses to microbial infection.

MAIT cells recognise MHC class I-related (MR1) presenting small microbial vitamin B metabolites. The two major metabolites are pterin-based ligand 6-formylpterin (6-FP), a photodegradation product of folic acid (vitamin B_9); and pyrimidine-based ligand 5-OP-RU, being derived from microbial biosynthesis of riboflavin (vitamin B_2). The relative plasticity of the MR1 binding groove has recently led to the exploration of chemical space for novel MR1-restricted antigens. However, there is currently no method to quantify the affinity of a ligand bound to MR1. In addition, the current pipeline from initial hit to structural evidence is highly time- and resource-intensive.

Here, we have developed an MR1 binding assay using fluorescence polarisation (FP) technology that is able to quantify the affinity of MR1 ligands as well as provide a facile method of screening for novel ligands. We have demonstrated that the FP competitive binding assay is a suitable tool for quantifying the IC_{50} for MR1-restricted ligands in the nM to mM range, with affinity of the ligand correlating well with the number of polar interactions between MR1 and ligand. We have also determined the structure of a novel dietary antigen, ethylvanillin, bound to MR1. Through FACS, we were able to determine that ethylvanillin is an inhibitory molecule that can upregulate MR1 on the surface of cells. We have demonstrated that the FP competitive binding assay greatly accelerates the rate at which novel MR1 ligands may be identified and from these results, this developed tool will greatly contribute to both the chemical space of MR1-restricted ligands and to our knowledge on the biology of the MAIT-MR1 axis.

Lipid composition is altered in immune cells and impacts susceptibility to ferroptosis

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The cellular lipidome is comprised of thousands of individual lipid species each belonging to a number of structurally distinct lipid classes. Lipids influence numerous aspects of cellular physiology, with differences in lipid composition between cell types contributing to cell-specific functionality. Our laboratory has recently characterised the cellular lipidome of the human and mouse immune systems. One of the most striking effects we observed was a marked variance in the levels of polyunsaturated fatty acid (PUFA)-containing phospholipids (PL) between different immunes cell types. This was particularly intriguing as these lipid species have recently been identified as the key executioners of ferroptosis, a newly discovered form of cell death. Accordingly, we hypothesised that the distinct PUFA-PL composition of different immune cell types would dictate susceptibility to ferroptosis. Therefore, we treated immune cells isolated from murine bone marrow with ML210, an inhibitor of GPX4, the major ferroptosis suppressing pathway. Cell viability analysis revealed that T and B cells, cells with the highest abundance of pro-ferroptotic PUFA-PLs, were the most susceptible to ferroptosis. Importantly, ML210-induced cell death in T and B cells was prevented by treatment with inhibitors of ferroptosis. In contrast, neutrophils and monocytes, cells with the lowest abundance of pro-ferroptotic PUFA-PLs, were resistant to ferroptosis. Excitingly, supplementation with oleic acid and PE(18:0/18:1) re-modelled the cellular lipidome of T cells, protecting them from ferroptotic cell death. Meanwhile, neutrophils supplemented with arachidonic and docosahexaenoic acids demonstrated increased sensitivity towards ferroptosis. Overall, we identify that the susceptibility of immune cells to ferroptosis is governed by their cellular lipid composition. This work establishes the foundation for future *in vivo* work and promising therapeutic approaches for diseases associated with ferroptosis and immune cells.

Immune responses in the respiratory tract and blood of COVID-19 patients reveal mechanisms of disease severity

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Although the respiratory tract is the primary site of SARS-CoV-2 infection, respiratory immune responses are understudied and urgently needed to understand mechanisms underlying COVID-19 disease pathogenesis. We collected paired longitudinal blood and respiratory tract samples (endotracheal aspirate, sputum, or pleural fluid) from hospitalized COVID-19 patients and non-COVID-19 controls. Cellular, humoral and cytokine responses were analysed and correlated with clinical data. SARS-CoV-2-specific IgM, IgG and IgA antibodies were detected using ELISA and multiplex assay in both the respiratory tract and blood of COVID-19 patients, although a higher receptor binding domain (RBD)-specific IgM and IgG seroconversion level was found in respiratory specimens. SARS-CoV-2 neutralization activity in respiratory samples was detected only when high levels of RBD-specific antibodies were present. Strikingly, cytokine/chemokine levels and profiles greatly differed between respiratory samples and plasma, indicating that inflammation needs to be assessed in respiratory specimens for the accurate assessment of SARS-CoV-2 immunopathology. Diverse immune cell subsets were detected in respiratory samples, albeit dominated by neutrophils. Importantly, we also showed that dexamethasone with/without remdesivir treatment did not affect humoral responses in blood of COVID-19 patients. Overall, our study unveils stark differences in innate and adaptive immune responses between respiratory samples and blood and provides important insights into effect of drug therapy on immune responses in COVID-19 patients.

ABSTRACTS
SESSION 10

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A structural basis underpinning biased T cell receptor recognition of an immuno-dominant HLA-A2 restricted epitope from the SARS-CoV-2 spike protein

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Human Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is responsible for the ongoing worldwide COVID19 pandemic, having caused over 233 million infections to date. CD8+ T cells play a crucial role in establishing adaptive immunity against SARS-CoV-2 infection. Several CD8+ T cell epitopes with different HLA restrictions have been identified in SARS CoV-2, but the molecular basis underlying T cell receptor (TCR) recognition of SARS CoV-2 epitopes is unknown. In individuals carrying the common HLA A*201 allomorph, the spike protein derived epitope $S^{269–277}$ is the most immuno-dominant epitope. TCRs of $S^{269–277}$ responsive CD8+ T cells are characterised by biased TRAV12 gene usage. To understand the molecular mechanism behind TRAV12 bias, we expressed, refolded and purified three TRAV12-1 TCRs, hereby named NR1A, NR1C and NR1D and one TRAV12-2 TCR, named NR2F, which bound to HLA-$A2^{S269–277}$ complex with low μM affinity. We solved the crystal structure of the HLA-$A2^{S269–277}$ binary complex, and subsequently, a ternary complex NR1C TCR with HLA-$A2^{S269–277}$. We found that NR1C TCR and HLA-$A2^{S269–277}$recognition was dominated by TRAV12-1 germline-encoded residues and conserved sequence motifs located in CDR3α and CDR3β loop regions. The NR1C TCR made extensive interactions with the $S^{269–277}$ peptide, suggesting that TRAV12-1 TCRs are sensitive to substitution of epitope residues. We subsequently investigated the cross-reactivity of TRAV12 TCRs for $S^{269–277}$ variants and epitopes from other closely related coronaviruses. Surface plasmon resonance and tetramer studies showed that TRAV12 TCRs cross-react poorly with these homologous epitopes. In summary, the study defined the molecular mechanism behind the biased CD8+ T cell recognition of the immuno-dominant HLA-$A2^{S269–277}$ epitope and provided a scaffold for understanding cross-reactivity and potential viral escape in the HLA-$A2^{S269–277}$ restricted T cell response.

Discrete tissue microenvironments instruct diversity in resident memory T cell function and plasticity

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Tissue-resident memory T cells (T_{RM}) are non-recirculating cells that exist throughout the body and mediate local protection, therefore harnessing T_{RM} functionality is an advantageous strategy for bolstering durable immunity. However, the influence of local microenvironmental imprinting on T_{RM} functionality and fate in remains incomplete. Here, we chart phenotypic and transcriptional T_{RM} heterogeneity between sites and find that the different environments in which these cells differentiate dictate T_{RM} function, durability and malleability. Using organ transplantation and T_{RM} transfer experiments, we uncover TGF-β as the major driver of functional heterogeneity between epithelial and non-epithelial tissues. We found that the absence of TGF-β signaling engendered $CD103^-$ T_{RM} with increased proliferative potential, enhanced function, and trans-differentiation capacity compared to their TGF-β-responsive $CD103^+$ T_{RM} counterparts. Thus, despite common requirements for T_{RM} development, tissue adaptation of these cells confers discrete functional properties that affect T_{RM}-mediated secondary immune responses. Therefore, exploiting these mechanisms will inform new immunotherapy strategies to bolster site-specific T_{RM}-mediated immunity.

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INCREASED BASAL AND INDUCED PHOSPHOINSITOL-3-KINASE SIGNALLING IN B- AND T-CELLS OF HEALTHY ADULTS CARRYING THE PTPN22 R620W MUTATIONS

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Background: The non-synonymous common variant c.1858C>T (p.R620W) in *PTPN22* is a risk variant for autoimmunity. Furthermore, prevalence is higher in patients with predominantly antibody deficiency (PAD), a primary immunodeficiency with increased incidence of autoimmunity. PTPN22 is a phosphatase that restricts signal transduction downstream of B- and T-cell receptors. Whether this mutation enhances or impairs PTPN22 function is currently disputed. Here, we addressed this by studying phosphoinositol-3-kinase (PI3K) signalling in B- and T-cells from healthy adults with and without the c.1858C>T variant.

Methods: Peripheral blood was collected from 63 healthy adults for Sanger sequencing for *PTPN22* c.1858C>T variant identification, detailed B- and T-cell immunophenotyping, and *in vitro* stimulation of antigen receptor signalling for S6 phosphorylation.

Results: 6/63 healthy controls were heterozygous for *PTPN22* c.1858C>T, at an allele frequency of 4.8%. Heterozygous carriers had significantly higher $CD27^+IgM^+IgD^+$ B-cell numbers. Carriers expressed higher CD19 and lower CD21 levels on transitional and naïve-mature B-cells, and higher CD8 levels on cytotoxic T-cells. Additionally, their naïve B-, $CD4^+$ and $CD8^+$ T-cells exhibited higher basal phosphorylated-S6 levels. Finally, antigen receptor stimulation resulted in higher phospho-S6 levels in B-cells (anti-IgM) and T-cells (anti-CD3) compared to individuals without the variant.

Conclusion: Here, we showed for the first time that PTPN22 R620W results in increased PI3K signalling in B- and T-cells, demonstrating that the mutation inhibits the repressive function of PTPN22. PI3K signalling in lymphocytes is tightly balanced with increased activity resulting from *PIK3CD* and *PIK3R1* mutations leading to antibody deficiency and autoimmunity. Our results indicate that this variant has similar effects but to a milder degree, which would explain the predisposition to autoimmunity. Thus, a common variant can directly impact an individual's immune profile. Future studies, will address the functional consequences of this variant in PAD patients and whether this functions as a first genetic hit for developing disease.

Circulating microRNA biomarkers enable accurate identification of COVID-19 patients

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

Here, we used next generation sequencing to profile the circulating miRNAs from 10 COVID-19 patients, sampled longitudinally, and compared these with age and gender matched controls. We found 55 differentially expressed miRNAs (FDR adjusted p-value < 0.05) during early-stage disease, with several miRNAs having previously established roles in inflammation. We then employed a multivariate machine learning approach to identify a three-miRNA biomarker signature (miR-423-5p, miR23a-3p, miR-195-5p) that identifies COVID-19 cases with 99.9% accuracy, 99.8% sensitivity, 99.8% recall, and a receiver operator characteristic area under the curve (ROC AUC) of 1. This signature is associated with the early stages of COVID-19; as the patients recovered, their samples clustered with the healthy controls. Further validation was conducted in a ferret model, where these three miRNAs identified SARS-CoV-2 infection with 99.7% accuracy, and distinguished SARS-CoV-2 infection from influenza A (H1H1) infection and uninfected animals with 95% accuracy.

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

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Single-cell landscape of tissue-resident memory T cell development

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Tissue-resident memory T (TRM) cells exist throughout the body where they are poised to mediate local immune responses against infections and cancer. Although studies have defined a common mechanism of residency independent of location, there is likely to be a level of specialization that adapts TRM cells to a given tissue. We explored changes occurring in chromatin accessibility during TRM cell formation in different tissues to reveal the developmental trajectory and associated genetic regulators involved in the establishment of this immune population. Analyses of scATAC-seq profiles from LCMV- and HSV-specific CD8+ T cells identify changes in gene accessibility of liver and skin TRM cells and reveal both exclusive and common chromatin regulators involved in the residency program. UMAP analysis of peak accessibility display clustering of cells in a time-sensitive and tissue-specific fashion, indicating heterogeneity of effector cells and TRM populations across organs and at different times post infection. Reconstruction of cellular developmental trajectories display a common pathway for circulating memory T cells found in the liver and the spleen with late divergence involved in the formation of effector and central memory T cells. Contrarily, liver TRM cells display a unique trajectory indicating the most epigenetic disparity when compared to the other memory subsets. Finally, by integrating deviations in DNA-binding motifs and gene activity scores, we identified transcription factors critical for skin and liver TRM development that will be explored through genetic engineering to explore whether they regulate TRM formation in a tissue-specific manner.

Abstract title: IL-23 in arthritic and inflammatory pain development

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Background

The cytokine, interleukin-23 (IL-23), can be critical for the progression of inflammatory diseases, including arthritis, and is often associated with T lymphocyte biology. However, little is known about the role(s) of IL-23 in arthritic and inflammatory pain development and its dependence with other inflammatory cytokines, for example, tumour necrosis factor (TNF), granulocyte macrophage-colony stimulating factor (GM-CSF) and CCL17, which all have been implicated in arthritic pain development.

Methods

To assess the requirement of IL-23 for arthritic and inflammatory pain development, *Il23p19* gene deficient (IL23p19$^{-/-}$) and wild type (WT) mice were induced with zymosan-induced arthritis (ZIA), zymosan-induced inflammatory pain or cytokine (TNF, GM-CSF or CCL17)-driven arthritis. To assess the ability for IL-23 to induce pain and its requirement for other cytokines and eicosanoid activity, IL-23-induced inflammatory pain was elicited in WT, *Tnf*$^{-/-}$, *GM-CSF*$^{-/-}$ and *Ccl17*$^{E/E}$ mice or in WT mice treated with or without cyclooxygenase inhibitors. Experiments were approved by The University of Melbourne Animal Ethics Committee and complied with the GSK Policy on the Care, Welfare and Treatment of Animals.

Results

We report here, using *Il23p19*$^{-/-}$ mice, that innate immune (zymosan)-driven arthritic pain and optimal disease development require IL-23, as does zymosan-induced inflammatory pain. We found that exogenous TNF-, GM-CSF- and CCL17-driven arthritic pain and disease, as well as inflammatory pain driven by each of these cytokines, are all IL-23 dependent. Supporting this connection, it was found conversely that IL-23 can induce inflammatory pain with a requirement for each of the other cytokines and cyclooxygenase activity.

Conclusion

These findings indicate a role for IL-23 in innate immune-mediated arthritic and inflammatory pain with potential links to TNF, GM-CSF, CCL17 and eicosanoid function.

Chromate stress dysregulates *Pseudomonas aeruginosa* molybdenum homeostasis

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Pseudomonas aeruginosa is a ubiquitous, opportunistic human pathogen responsible for a range of human diseases, including pneumonia, urinary tract, and intestinal tract infections, and causes significant morbidity in immunocompromised individuals. *P. aeruginosa* thrives in anaerobic and microaerophilic environments, such as the mucoid enrich lung environment of individuals with cystic fibrosis, generating energy via the dissimilatory nitrate reduction. Respiration using nitrate occurs via the molybdenum cofactor enzymatic complex NarGHI. Molybdenum is a group IV element that is essential to all forms of life. In bacteria molybdenum is acquired in the oxyanion form, molybdate, via the high-affinity ATP-binding cassette transporter ModBC and the solute binding protein ModA. Here, we investigated the biochemical and structural properties of *P. aeruginosa* ModA to define its role in molybdenum homeostasis. Biochemical analyses of recombinant ModA revealed that it was permissive for interaction with the group VI oxyanions, chromate (CrO_4^{2-}), molybdate (MoO_4^{2-}), and tungstate (WO_4^{2-}), and not restricted to its physiological ligand. X-ray crystallographic analyses of ModA-metal complexes showed that all oxyanions bound at the high-affinity metal binding site within the protein with minimal apparent structural differences. We then investigated the role of ModA in *P. aeruginosa* susceptibility to chromate intoxication due to increasing industrial interest in the group IV metal as a basis for novel antimicrobial materials. Analysis of a *modA* deletion strain revealed that susceptibility to chromate intoxication and cellular accumulation of the metal were not affected by loss of *modA*. Unexpectedly, exposure to sub-lethal chromate stress increased cellular accumulation of molybdenum in the wild-type strain and, to a lesser extent, the Δ*modA* strain. This indicates that the molybdate uptake is predominantly driven by ModA, but that secondary import pathway(s) for the metal are also present in *P. aeruginosa*. The observed response to chromate intoxication further suggests that a molybdenum starvation response is induced, most likely attributable to the molybdenum-sensing metalloregulator ModE. Collectively, this work advances our understanding of the biophysical properties of ModA and reveals that although chromate stress dysregulates molybdenum homeostasis, chromate toxicity is not due to inhibition of molybdate uptake.

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

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

We can now demonstrate that LDs are transiently upregulated as a host repose to multiple viral infections, both *in vitro* and *in vivo*, with this upregulation of LDs driving an increase in type I and III interferon. To understand if LDs enhance these interferon responses through a bystander effect in infected cells, we generated GFP and mCherry CRISPR/Cas9 fluorescently labelled LD cell lines. A co-culture of these two cell lines revealed for the first, that LDs were able to cross between cells. Live-time microscopy trafficking analysis of individual LDs revealed that during viral infection, LD velocity and distance travelled increased significantly as early as 2 hrs post activation of antiviral signaling pathways, and remained upregulated for 72 hrs. As LDs are known to interact with other organelles to transfer lipids and proteins, we developed techniques to isolate pure LDs from cells to analyse their lipidome and proteome before and following activation of antiviral pathways. Lipidomic analysis revealed significant changes within the lipid profile of LDs themselves, but not in whole cell lysates. Additionally, an upregulation of long chain fatty acids, and bioactive lipid mediator precursors were observed in virally driven LDs. Significant changes were also seen in the LD proteome, with 83 proteins upregulated 24 hrs post infection, including multiple antiviral proteins, and members of the early innate antiviral signaling pathways, demonstrating for the first time that the LD may act as a signaling platform during an effective antiviral response.

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

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

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

Repeated *Plasmodium falciparum* infection in humans drives the clonal expansion of an adaptive γδ T cell repertoire

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Repeated *Plasmodium falciparum* infections drive the development of clinical immunity to human malaria. However, immunological mechanisms underpinning this response are only partially understood. γδ T cells have been linked to clinical protection from malaria, but how adaptive-like Vδ1$^+$ T cells respond to repeated *P. falciparum* infections is unclear. We investigated the impact of repeated *P. falciparum* infections on γδ T cell subsets and the γδ T cell receptor (TCR) repertoire in humans. We studied peripheral blood samples from Australian children, Malian children naturally exposed to malaria, and U.S. adults serially challenged with four repeated controlled human malaria infections (CHMIs). Clonally expanded cytotoxic Vδ1$_{effector}$ T cells were a major component of the γδ T cell compartment in Malian children compared to malaria-naïve Australian children. Sequential CHMIs initiated a robust innate-like Vδ2$^+$ T cell response to three *P. falciparum* infections in malaria-naïve U.S. adults, however, these cell frequencies declined between infections and were not sustained after the fourth infection. In contrast, Vδ1$^+$ T cell frequencies increased after repeated infection and correlated with clinical immunity to malaria. Populations of Vδ1$_{naive}$ T cells differentiated into cytotoxic Vδ1$_{effector}$ cells concomitant with waves of clonal selection after each infection. Finally, Vδ1$^+$ T cells from individuals with a history of malaria were licensed for *in vitro P. falciparum* parasite reactivity. Together, our study indicates that repeated *P. falciparum* infections drive the clonal expansion of an adaptive γδ T cell repertoire and establishes a role for Vδ1$^+$ T cells in the human immune response to malaria.