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Abstracts

Oral presentations

P.70 **Halting pulmonary fibrosis through interference in the IL-25, IL-33 and TSLP pathways**

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Idiopathic pulmonary fibrosis (IPF) is a lung scarring disease that kills approximately 5,000 people per year solely in the UK. In IPF, insults to the airway epithelium cause the release of the alarmins interleukin (IL)-25, IL-33, and thymic stromal lymphopoietin (TSLP), which induce type 2 (T2) inflammation, fibroblast activation, and pro-fibrotic collagen deposition. However, due to the reduced efficacy of single-alarmin blockade in past clinical studies, we hypothesised that alarmins work synergistically to induce IPF. Surprisingly, the parasitic nematode Heligmosomoides polygyrus evades the immune system in the intestine by secreting molecules that suppress IL-25 and IL-33-mediated signalling and fibrosis, suggesting that these molecules could be used also as a treatment against IPF. Of note, in these secretions, HpARI2 prevents IL-33 release while HpBARI Hom2 competes with IL-33 for its receptor ST2. Moreover, the effects of Heligmosomoides polygyrus-derived secretions against TSLP are currently under investigation. Initially, we tried to target IL-33 in vivo and in vitro. Using recombinant HpARI2 and HpBARI Hom2, we inhibited ILC2 responses in vitro and also downregulated inflammation in a bleomycin mouse model of lung fibrosis. When inhaled in the lung, bleomycin promotes the development of self-resolving lung fibrosis by inducing one pro-inflammatory week followed by three pro-fibrotic weeks. Further histological analysis of lung sections demonstrated reduced fibrosis and lung damage, lower cellular infiltrates, and attenuated mucus secretion in mice treated with recombinant HpARI2 and HpBARI Hom2 as opposed to positive controls treated with bleomycin only. Overall, data collected so far suggest that Heligmosomoides polygyrus-derived secretions can reduce inflammation and lung fibrosis induced by bleomycin and could be potential therapeutic candidates against IPF onset and progression.

P.71 **Salt-Inducible Kinases (SIKs): Recently identified regulators of mast cell function**

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Mast cells release inflammatory mediators that drive allergic (asthma and anaphylaxis) and non-allergic diseases (mast cell activation syndrome, viral response). Inhibiting the Salt-Inducible Kinases (SIKs) suppresses pro-inflammatory signalling in innate immune cells including macrophages but their role in mast cells had not previously been investigated.

Mast cells synthesise and secrete cytokines following stimulation with Interleukin-33 (IL-33), which is released in vivo following cell damage or necrosis. We established that SIKs are required for

the IL-33-stimulated transcription of il13 and tnf in mast cells. Consequently, small-molecule inhibitors of SIKs strongly suppressed secretion of these cytokines. Using mast cells expressing kinase-inactive mutants, we established that SIK2 and SIK3 are the key isoforms regulating cytokine secretion following IL-33 stimulation.

Mast cells also store pre-formed inflammatory mediators within granules, ready for rapid release following activation. Notably SIKs are active under basal conditions and thus may have a role in regulating the basal state of the mast cell. We generated mast cells expressing kinase-inactive mutants of SIK2 and SIK3 and this did not affect the cell surface expression of mast cell markers (FcεR1, c-kit or the IL-33 receptor). Analysis of over 5000 proteins identified in mast cells using an unbiased quantitative proteomic approach provides new insight into the function of SIK2/3 in these cells. For example, around 40% of the proteins that comprise the mast cell signature were differentially regulated in mast cells expressing kinase-inactive SIK2 and SIK3. This highlights a wider role for SIK2 and SIK3 in regulating the basal state of mast cells in addition to the regulation of IL-33-stimulated cytokine production.

P.72 **Aptamers can act as adjuvants by enhancing DC function and DC/T cell interaction**

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Many vaccines suffer from a lack of immunogenicity and require appropriate adjuvants to achieve protective immunity. Adjuvant research has become a rate limiting step in vaccine development due to the empirical and low-throughput nature of the field. Increasing the rate of adjuvant discovery and identifying new adjuvants with defined immunological activities and mechanisms of action, would enable both the development of new vaccines as well as the cost-effective deployment of existing vaccines. Here we demonstrate a solution to this fundamental problem in vaccine development. Using a unique in vitro screening platform, we have identified candidate adjuvants from massive compound libraries. To validate this platform, we have performed in vivo and in vitro characterization of the top candidate adjuvants.

Firstly, we confirmed that candidate adjuvants enhanced immunogenicity and protection against influenza infection in vivo when formulated with Aluminium phosphate. These in vivo effects were comparable to competitor adjuvants in clinical development. Secondly, we investigated the mechanisms of action of these adjuvants. These studies demonstrated that different adjuvants induced different magnitudes of activation of bone marrow-derived dendritic cells (BMDCs). Subsequent RNA sequencing analysis demonstrated common and distinct activation pathways induced by different candidate adjuvants.

These studies have identified an entirely new class of vaccine adjuvants for use in vaccines, that will be of interest to the global vaccine market. They have also demonstrated the feasibility of this approach to adjuvant identification, reducing animal use while massively increasing throughput, providing a solution to a key obstacle in vaccine development.

P.73 **Ccr2-mediated monocyte recruitment is essential for non-fibrotic endometrial repair and re-epithelialisation during experimentally induced menstruation**

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Introduction: Endometrial repair following menstruation is essential for women's reproductive health and macrophages are believed to play a pivotal role in this process. Maintenance of the macrophage niche is required for healthy tissue function but we lack fundamental knowledge about

how this is regulated in the endometrium. Given that macrophage dysfunction is associated with reproductive health disorders, we sought to characterise the contribution of macrophages and their precursors (monocytes) to endometrial repair in mice undergoing induced menstruation.

Methods: We used a mouse model of induced menstruation to investigate the role of monocytes/macrophages in endometrial tissue breakdown and repair. We isolated immune cells from uterine tissues at known time points of active repair or following repair/resolution and assessed population composition and dynamics using multi-parameter flow cytometry with established subset markers. Monocytes were depleted using genetic (Ccr2-/-) or antibody-mediated (anti-CCR2 mAb; MC-21) approaches and repair responses assessed by flow cytometry and immunohistochemistry.

Results: Flow cytometry revealed that monocytes increased during active repair and were more abundant than tissue resident macrophages ($p<0.0001$). Following resolution of repair, tissue resident macrophages increased and were more abundant than monocytes ($p<0.001$). Monocyte depletion prior to repair significantly reduced Ly6C+Ccr2+ monocytes in the blood ($p<0.0001$) and subsequently decreased monocytes (Ly6C+Ccr2+, $p<0.05$) and tissue resident macrophages (CD64+F480+, $p<0.01$) in uterine tissues. Monocyte depletion was associated with disrupted endometrial repair characterised by increased bleeding at menses, altered immune cell composition in the uterus, reduced re-epithelialisation, and evidence for basal tissue fibrosis.

Conclusion: Monocytes are abundant during experimentally-induced menstruation and systemic depletion disrupts non-fibrotic endometrial repair processes. These novel data offer new insight into the important role of monocytes and macrophages in menstruation and women's reproductive health.

P.74 **Enteric damage from helminth infection induces systemic monocytosis and protects against respiratory syncytial virus infection**

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Infant respiratory viral infections are a major cause of infant hospitalisation and a risk factor in the development of persistent wheeze, airway allergic responses and ultimately asthma. We have previously shown that ongoing infection in mice with the gut helminth Heligmosomoides polygyrus (H. polygyrus) protected against respiratory syncytial virus (RSV) infection through reduced viral load, associated immunological changes and airway impairment. This protective effect was independent of adaptive immune responses or helminth secretory/excretory products, and dependent upon the induction of type-I interferons in the gut and/or lung and the presence of normal gut microbiota. Here we present further investigation into the mechanisms of this anti-viral effect.

Ongoing H. polygyrus infection also induces bone marrow monocytopoiesis, in turn driving an increase in both circulatory monocyte populations and recruited inflammatory macrophages in the lung. Treatment of H. polygyrus infected animals with an anti-CCR2 antibody depletes the expanded monocyte populations and ablates the enhanced anti-viral state in H. polygyrus infected animals. Elevating monocyte numbers through their IV administration replicates the anti-viral effect.

Intravenous serum transfer from mice 10 days after H. polygyrus infection to naïve mice reproduces the increase in interferon beta and interferon stimulated genes, increased bone marrow monocytopoiesis, elevated lung monocyte counts, and reduced peak viral load in subsequent RSV infection comparable to that seen with host H. polygyrus infection.

These results show that during H. polygyrus infection host derived serum borne factor(s) are released inducing an antiviral state in the lung. These factor(s) also drive systemic monocytosis leading to increased numbers of anti-viral monocyte-derived macrophages within the lung that are sufficient and essential for mounting an effective immune response to RSV infection.

P.75 **Hypoxia shapes the immune landscape in lung injury and promotes the persistence of inflammation**

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In acute lung injury, the degree of the consequent hypoxaemia is often used as a severity marker of the underlying disease process, and in conditions such as acute respiratory distress syndrome (ARDS), hypoxaemia is a key defining feature. ARDS is an often-fatal complication of pulmonary or systemic inflammation and, other than supportive care, it has no cure. The resulting tissue hypoxia, and its impact on immune responses, remains largely ignored.

We hypothesised that hypoxia could directly alter immune responses with deleterious consequences for the host.

Here we showed patients with ARDS remained hypoxaemic despite ventilatory support and they were monocytopaenic within the first 48 hours of diagnosis. Alongside the monocytopaenia, their classical monocytes were phenotypically distinct to healthy control cells, containing more pro-inflammatory granule proteins as well as being transcriptionally distinct. We replicated the circulating monocytopaenia in mouse models of hypoxic acute lung injury, in which mice were treated with LPS and then housed in hypoxia (10% FiO2 or 21% FiO2). Hypoxaemia drove the suppression of systemic and bone marrow type I interferon signalling and suppressed emergency monopoeisis. This resulted in reduced accumulation of monocyte-derived macrophages in the lung and enhanced neutrophil-mediated inflammation in the lung. Administration of CSF1-Fc in mice with hypoxic lung injury rescued the monocytopaenia, altered the phenotype of circulating monocytes, increased monocyte-derived macrophages in the lung, and limited injury.

Thus, tissue hypoxia altered the dynamics of the immune response to the detriment of the host and interventions to address the aberrant response offer new therapeutic strategies for ARDS.

NK cells acquire memory to Nippostrongylus brasiliensis infection in mice

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Natural Killer (NK) cells mostly have type 1 effector functions and are thus known for their roles in viral and intracellular bacterial infection or against tumour cells. In the last decade NK cells were found to also acquire memory, an ability to alter their function following a primary infection and confer superior protection in secondary responses. We set out to study NK cell responses during Nippostrongylus brasiliensis (Nippo) infection in mice since their potential roles in helminth infection remain virtually unknown. Surprisingly, we found that NK cells from mice recovered from primary infection (28 days before) have increased effector functions in the lungs during secondary infection. We found that NK cells upregulate granzyme B expression in response to IL-4 and in the presence of Nippo in vitro. Using co-culture systems, we showed cell-intrinsic changes in Nippo-exposed

(memory) NK cell responsiveness pertaining to their capacity to secrete IFNg and granzyme B and these changes differentially affected NK cells from the lungs compared to those from the spleen. Adoptive transfer of memory NK cell into naïve mice had marginal effects on worm burden in the lungs but differentially affected myeloid cell activation. In summary, while further studies are necessary, we describe evidence of NK cell memory to Nippostrongylus brasiliensis in mice.

Poster presentations

P.01 **Investigating T cell homing across the gut-joint axis in Crohn's disease and axial spondyloarthritis**

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Although the immunology of the gut-joint axis is not well understood, it is thought to be important in both Crohn's disease (CD) and axial spondyloarthritis (axSpA), since arthritis is a common extra-intestinal manifestation of CD and intestinal inflammation is a common extra-articular manifestation of axSpA.

The trafficking of immune cells to tissues is controlled by integrins, surface receptors that bind ligands expressed within specific tissues. For example, α4β7 integrin primarily binds the MAdCAM-1 ligand expressed by gut-associated endothelial cells, trafficking immune cells to the intestine. Likewise, α4β1 integrin binds the VCAM-1 ligand expressed by inflamed tissues, including joint tissue. Co-expression of α4β7 and α4β1, and these integrins' ability to bind each other's receptors, suggests these cells may be able to traffic between the gut and joint.

Vedolizumab (VDZ) treats CD by binding α4β7 to prevent pathological immune cells accumulating within the gut. However, VDZ can cause arthralgia, sacroiliitis, and new-onset axSpA, with the mechanism for this being unclear.

We hypothesize that people with CD and people with axSpA have increases in T cells co-expressing α4β1 and α4β7, that traffic to both the gut and joint. We further hypothesize that VDZ treatment redirects these co-expressing cells to the joint in people with CD, leading to joint pathology.

To test these hypotheses, we are analysing T cells from people with CD and people with axSpA to determine α4β7 and α4β1 co-expression using flow cytometry. We will analyse the activation of these integrins from people with CD and people with axSpA by conducting integrin-ligand binding assays ex vivo, and assessing how VDZ treatment of these cells in vitro changes the cells' ability to bind MAdCAM-1 and VCAM-1. By understanding the mechanisms of the gut-joint axis, we aim to help understand how to eliminate both intestinal and joint inflammation in CD and axSpA.

P.02 **The WNT pathway disrupts intraepithelial lymphocyte immunosurveillance in colon cancer**

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The importance of conventional CD8 T cells is well established in colorectal cancer, as their abundance in tumours predicts a good outcome in cancer patients. However, for the largest group of colorectal cancers – the microsatellite stable (MSS) group, which represents 80-85% of cases – CD8 T cells are largely absent from tumours. Patients with MSS tumours respond poorly to anti-PD-1. Thus, a greater understanding of other cytotoxic immune cells in MSS tumours is required to develop novel immunotherapies. Here, we focused on the role of intraepithelial lymphocytes (IEL) in colon cancer mouse models. We used a model driven by activation of beta-catenin, Villin1-CreERT2;ApcF/+;KrasG12D mice, which succumb to non-invasive adenomas by 70 days post Cre induction. This model was crossed to Tcr-beta or Tcr-delta knockout mice to understand the individual role of abIEL and gdIEL populations in cancer initiation and growth. The absence of either population resulted in no difference in survival or tumour growth in intestines. However, when both populations were genetically deleted, tumour formation was accelerated and survival was reduced. These data suggest that abIEL and gdIEL have redundant anti-tumour functions. In end-stage tumours, both populations were largely absent from the intestinal tumour microenvironment. We found that activation of β-catenin induces the down-regulation of several T-cell interacting molecules, including butyrophilin-like receptors 1 and 6 that can regulate maturation and activation of gdIEL. Current efforts are focused on understanding how abIELs and gdIELs are excluded from colon adenomas and carcinomas. Insights into these mechanisms may provide information that could be used to design immunotherapy strategies utilizing abIELs and gdIELs against colorectal cancers.

P.03 **Lung stromal cell dynamics are altered by infection experience and ongoing antigen presentation following influenza re-challenge**

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Background: Stromal cells can be permanently altered by insults, a process termed trained immunity. Whether these cells contribute to protection or pathology in infections such as influenza a virus (IAV) is unclear. We hypothesise that trained stromal cells may participate in protective immunity by rapidly reactivating local memory T cells.

Methods: We performed transcriptional analysis on sorted lung epithelial cells and fibroblasts isolated from naïve and IAV infected mice (primary, memory, and re-challenge timepoints). Stromal cell dynamics and interactions with immune cells were investigated using flow cytometry and immunofluorescence including detecting infected cells via IAV Nucleoprotein (NP). The location of infection experienced stromal and immune cells in the lung was determined using RNAscope.

Results: RNA-sequencing analysis demonstrated enrichment in immune related genes (e.g. MHCII and CXCL9/10) at primary/memory timepoints in lung stromal cells. These genes are regulated by the inflammatory cytokine interferon-γ and were further upregulated following IAV re-challenge. Importantly, IAV-nucleoprotein+ epithelial cells expressed more MHCII compared to IAV-NP negative cells, suggesting enhanced communication with T cells. Using RNAscope, SpiB, a transcription factor that regulates genes involved in antigen processing/presentation, was detected in lung epithelial

cells of infected mice. SpiB+ cells were in close proximity to immune cells that form dense clusters containing a mixture of T/B cells and myeloid populations. Interestingly, these microenvironmental changes were dependent on viral replication. A small subset of IAV-NP+ universal fibroblasts (Ly6C+Sca1+) were identified early post infection, accompanied by increases in IAV NP negative fibroblasts (interferon response and proliferating). Strikingly, despite being IAV-NP negative MHCII+ fibroblasts were more abundant following IAV re challenge compared to initial infection. Infection experienced stromal cell subsets may promote immune protection upon re-infection through antigen presentation to T cells and/or alteration of the local lung microenvironment. Increased understanding of stromal cell trained immunity may enhance our ability to protect against respiratory infections.

P.04 **How does RNA cap methylation regulate T-cell differentiation?**

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The rapid increase in metabolism and protein synthesis induced by T-cell activation is critical for the proliferation and differentiation of T-cells into effector populations. However, the mechanisms co-ordinating regulation of gene expression during T-cell activation and differentiation are not known yet.

The RNA cap is a structure found at 5' end of all pre-mRNAs and is involved in recruiting factors involved in RNA processing and translation. Here, we report that the RNA cap methyltransferases are upregulated following CD4 T-cell activation and differentiation, and this is critical for gene expression regulation during this process. We are especially interested in the CMTR1 cap methyltransferase that is driving ribose O-2 methylation of the first transcribed nucleotide. Although we observe that CMTR1 is required for cap methylation of all pre-mRNAs, its impact is gene- and T-cell-lineage specific. For example, Th1 cells are entirely dependent on CMTR1 activity for survival, whereas Treg cells can tolerate CMTR1 deletion.

I will discuss the precise impact of CMTR1 and ribose O-2 methylation on RNA processing and translation in CD4 T-cell lineages. These findings have implications for T-cell fate decisions and for therapeutic manipulation of T-cell differentiation.

P.05 **Antigen presenting cells: T cell interactions in the lung: exploring the generation and phenotype of Interferon-γ producing memory CD4 T cells during influenza virus infection**

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Background
Upon resolution of influenza A virus (IAV) infection in mice, lung memory CD4 T cells persist in clusters with other immune cells. These clusters remain for 40 days post-infection suggesting ongoing antigen presentation. Understanding how sustained interactions of cells in clusters drive the generation and phenotype of long-lived protective memory CD4 T cells may improve vaccine design.

Method
TRACE mice enable the identification of antigen specific CD4 T cells by permanent EYFP expression trigged by TCR activation in the presence of doxycycline. Lung sections from TRACE mice were analysed by confocal microscopy enabling visualisation of clusters over time and potential interactions between IAV specific T cells and other immune cells. To investigate the consequences of these interactions, MHCII tetramer and intracellular cytokine assays were used to enumerate IAV

specific T cells in mice whose lung TCR-peptide-MHC interactions were blocked with an anti-MHCII antibody delivered intranasally at days 6 and 12 post infection.

Results
At day 10 post infection, IAV specific T cells, B cells, DCs and macrophages infiltrate into the lung forming large clusters. By day 40, cluster area is reduced but the total number of clusters is increased. IAV specific CD4 T cells and MHCII+ populations are sustained in these clusters till day 40. Blocking lung TCR-peptide-MHC interactions reduced the number did not affect the cytokine production of IAV-polyclonal CD4 memory T cells. In contrast, IFNy production was reduced by memory CD4 T cells that recognise the immunodominant IAV nucleoprotein epitope, NP311-325. T cells specific for NP311-325 also express higher levels of ICOS and PD1 than total IAV-responding T cells, suggesting that immunodominant T cells may be more dependent on continued TCR-pMHCII interactions than other IAV-polyclonal T cells. These data imply that APC: T cell interactions may play important roles in the maintenance of protective lung memory T cells.

P.06 **FocuSCOPE: A High-Throughput Single-Cell Multi-Omics Solution For Combination Whole-Transcriptome And Targeted Transcript Recovery**

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Single-cell sequencing has revolutionized our understanding of heterogenous cell populations and allowed us to probe the depths of the immune compartment. Technological advances allowed to shift from sequencing a handful of cells at a time to >10^5 cells simultaneously with the tradeoff that most high-throughput RNA techniques can only capture a fraction of the RNA transcripts in a cell and are dependent on poly(dT) primers. We developed FocuSCOPE, a high-throughput multi-omics sequencing solution that can detect both genetic variants and transcriptome from same single cells.

With customizable probes we allow for the simultaneous capture of whole-transcriptome data and targeted sequences without a dependency on target polyadenylation. The applications of the technology are very diverse and range from improved V(D)J sequencing, to SNP/SNV/indel detection, to viral transcript capture, and to increased rare transcript recovery. With targeted probes for V(D)J transcripts we demonstrate an 8- and 4-fold increase of TRA and TRB transcripts, respectively. This enables alpha & beta chain pairing in over 80% of cells, greater than the next available technology.

When it comes to mutation detection, we can identify druggable mutations in genes such as EGFR, KRAS, and BRAF with premade panels for lung and blood cancers. This enables direct annotation of cancerous cell populations without computational inference, and even screening new samples for the mutational burden contained within. Similarly, our EBV transcript detection is direct and has twice the yield of poly(dT) probes. Since we can choose the set down location of the probes we are also able to detect fusion genes more reliably than poly(dT) probes. For example, PML-RARA fusion gene the detection rate is >60% with fusion-specific probes compared to 6.6% with poly(dT) probes. Furthermore, we are able to do combinational screening, such as simultaneous V(D)J, MYC mutations, and EBV detection within the same sample.

P.07 **Intestinal helminth infection activates tissue-based IL-10 and IFNg signalling, promoting the Th2 response and restricting bacterial spread**

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Intestinal helminth infection is associated with a potent Th2 response, important for the weep and sweep response directed against the worms, and for wound healing in the intestinal wall. The intestine also contains bacteria and other microorganisms, and we are interested to understand how immune defences against both helminth and bacteria co-exist and cross-regulate. Here, we examined factors known to regulate intestinal immune responses. Our data show that infection of mice with the enteric helminth Heligmosomoides polygyrus induces a potent IL-10 response in the infected tissue, and that this IL-10 production is essential to maximise the Th2 response in that tissue site. We examined possible targets of IL-10 signalling and found that the IL-10 receptor was more highly expressed on tissue-based Th1 cells than in Th2 cells in the intestine or any T cells in the draining lymph node. Blocking IL-10 in vivo resulted in an expansion of intestinal Th1 cells and increased IFNg expression in the infected tissue. We hypothesised that the stimulus for IFNg production was intestinal bacteria carried into the intestinal tissue on the surface of the invading helminths, like barnacles on a whale, but we could not detect bacteria in the intestinal tissue, and scanning electron microscopy of helminths in the intestine revealed remarkably bacteria-free surfaces. When we isolated T cells from helminth-infected intestinal tissue, stimulation with bacterial antigens did not elicit cytokine production, whereas helminth antigens triggered both IFNg and IL-5. Together our data support a model in which helminths elicit both type 1 and type 2 immunity in the infected tissue, preventing bacterial sepsis while deterring superinfection by additional helminths, necessitating an immune balance struck by the regulatory cytokine IL-10.

P.08 **Using the power of social media to tackle vaccine hesitancy**

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Vaccines don't save lives; vaccination does. Tackling vaccine hesitancy is a key issue in public health measures against infectious disease, including COVID-19. Misinformation is particularly prevalent on social media, so we produced a short series of animated videos explaining how vaccines work, discussing why even fit and healthy people should get vaccinated, and weighing up vaccination during pregnancy. To overcome language barriers, we worked with volunteers from our research communities who translated the video scripts into multiple languages, increasing the accessibility and reach of the information. This contributed towards one of our core goals: to empower people to make informed decisions about vaccination by enhancing their understanding of how vaccines work and why they are important. The videos were released and circulated on social media, including Twitter, Facebook, and Instagram. Evaluation data suggests that the videos improved understanding of vaccines and confidence in decision making regarding vaccine acceptance. This project was funded in part by a Communicating Immunology grant from the BSI.

P.09 **Characterization of fetal mast cells in extraembryonic and barrier tissues**

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Mast cells (MCs) are amongst the type of immune cells already present in the fetus. They have a dual hematopoietic origin: The first MCs are generated from yolk sac (YS)-derived erythro-myeloid progenitors (EMPs), followed by MCs originating from hematopoietic stem cells (HSC).

Fetal MCs contain intracellular granules filled with effector molecules (e.g. heparin), which suggests that they are already functional during these early stages. Indeed, they have been implicated in the vascular and neuronal fine-tuning in the developing cornea. Additionally, fetal MCs appear to promote the switch from scar-free healing to scar formation in wounded skin. Apart from this, however, MCs remain incompletely characterized during development, and their physiological functions remain unclear at these early life stages. We hypothesize that fetal MCs might contribute to safeguarding fetal development. We thus studied MCs in tissues that can potentially sense the maternal environment, i.e. the barrier and extraembryonic tissues. Here, we addressed if fetal MCs can be found in these tissues, characterized them phenotypically and determined their hematopoietic origins.

Our data confirmed the presence of fetal MCs (c-kit+ ST2+ or c-kit+ avidin+) in the barrier tissues (skin, gut, lung) and also identified them in extraembryonic tissues (amniotic fluid, umbilical cord and fetal membrane, but not placenta). They uniformly express CD16/32, CD200R and CX3CR1, while the frequency of integrin α4β7 expression varies between tissues and developmental stages. Using the Cdh5-CreERT2 x RosatdT fate mapping model, preliminary data show that extraembryonic MCs are predominantly YS EMP-derived, but also receive some HSC contribution. Further work will continue to characterize MCs in barrier and extraembryonic tissues and address if they have a role in fetal development.

P.10 **Investigating the diversity of the RNA cap binding interactome and the role of RNA binding in NFκB function**

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In this study, we investigated the ability of the NFκB family of transcription factors to interact with RNA and found a potential novel feedback mechanism, which may contribute to understanding the role the RNA cap and cap binding proteins in T cell survival, proliferation, and differentiation.

The RNA cap is an important co-transcriptional modification that enables cap binding proteins to be recruited to transcripts and regulate their splicing, export, stability, and translation. Knowledge of the RNA cap interactome is limited in T cells and new discoveries could provide insight into potential specialized roles of the RNA cap in gene expression. Our aim was to identify novel, T cell specific, cap binding proteins, recruited to RNA with methylation by the RNMT, CMTR1 and CAPAM cap methyltransferases, and to determine their function in RNA processing and translation.

We identified a diverse RNA cap proteome in activated and IL-2 stimulated CD8 T cells, with many novel binding proteins surprisingly only binding to one specific combination of methylations. Some of the most interesting hits in our analysis included DNA binding proteins responsible for transcriptional regulation and DNA damage repair. RNA can play a significant role in the functions of DNA binding proteins such as chromatin remodelers and other transcription factors and therefore, the interaction with the RNA cap was considered as a possible early transcription regulatory mechanism. During T cell activation, the resulting burst of transcription and RNA saturation around

chromatin may affect the activity of transcription factors identified as cap binding proteins. A decrease in cap methylation may result in more RNA degradation and a change in cell performance.

Our work strongly suggests the RNA cap interactome is more diverse than understood so far in literature and may change based on cell type and environment.

P.11 **Ontogeny and maturation of macrophages in the postnatal synovial lining**

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Synovial tissues contain a macrophage barrier protective against rheumatoid arthritis (RA), which becomes disrupted by infiltrating inflammatory cells during RA. We have found that this lining barrier is not complete at birth and therefore must develop postnatally. Delineating postnatal synovial macrophage dynamics may uncover critical developmental functions and improve understanding of how insults experienced in early life promote RA. We thus investigate the origin and structure of the macrophage barrier during early postnatal development.

Genetic fate-mapping models and confocal imaging were used to pinpoint synovial macrophage origins in early life. The Cdh5CreERT2 model labels macrophages that derive from erythromyeloid progenitors (EMP) made in the yolk sac (YS), or from hematopoietic stem cells (HSC) that develop in the aorta-gonad-mesonephros region, by injection of tamoxifen at embryonic day 7.5 and E10.5 respectively. The Ms4a3Cre model labels macrophages that derive from BM-resident HSC.

During the first three weeks of life, synovial lining macrophage density gradually increased with a mature-appearing barrier emerging by three weeks. This increase was not monocyte-dependent, therefore stemming from other progenitors or pre-existing resident macrophages. At birth, most lining macrophages were early YS EMP-derived, a substantial population of which were retained at three weeks of age, but gradually supplemented with macrophages derived from later fetal progenitors. In the adult, early YS EMP contribution was lost. However, macrophages remained largely BM-independent, making later fetal progenitors the predominant source of adult lining macrophages.

In summary, the macrophage barrier matures postnatally, and fetal-derived macrophages are retained throughout this process, suggesting they may have critical early life functions. This establishes the perinatal period as an important developmental window for synovial lining maturation, during which embryonically derived macrophages predominate. Therefore, insults experienced during this period, like nicotine smoke exposure, an RA early life risk factor, could perturb the macrophage barrier which may promote RA onset.

P.12 **Lung basal cells stimulate the immune system to clear influenza virus then then calm the immune system to repair the damage**

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Despite anti-viral drugs and vaccines, influenza viruses are still poorly controlled and pose a threat to those who suffer chronic diseases such as COPD and asthma. The most dangerous influenza symptoms are caused by damage to the lung tissue, and repairing this damage is essential for survival. Lung repair is driven by activation of epithelial progenitor cells, but their exact role is not well understood. We infected mice with influenza virus and studied the behaviour of epithelial

progenitors and consequent effects on the cellular composition of the recovering lung. We found that the epithelial composition of the lung is changed during the peak and recovery phases of influenza. There is a loss of ciliated and alveolar cells at day 6 post infection, but by day 10 these populations are being restored. This recovery correlates with increased activation and proliferation of basal cells. Using differential expression and pathway analysis, we found lung basal cells activation and proliferation is fuelled by a switch to high energy yield oxidative phosphorylation. We show that lung basal cells produce inflammatory cytokines such as TNF; but they also make anti-inflammatory factors such as IκBα. Our findings confirm that lung epithelial precursor activation occurs during recovery from influenza, and the reshaping of the lung after infection is fuelled by a change in progenitor metabolism. We suggest a careful balance is struck by lung progenitors after infection: they assist in clearing the infection in the short term, while also preparing to recover the damaged epithelium in the long term.

P.13 **Defective splenic immunity and infection susceptibility persist during chronic stroke recovery**

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Background: Infection is a major complication following stroke that is facilitated by suppression of peripheral immunity. Post-stroke respiratory infection is common and associated with poor clinical outcome, however, the duration of immune suppression and heightened infection susceptibility is not known. Here, we examine the contraction and subsequent repopulation of splenic leukocytes and investigate infection risk during chronic stroke recovery.

Methods: The middle cerebral artery occlusion model of stroke was used and splenic leukocytes were examined at acute (5 day), subacute (2 weeks), and chronic (3 month) timepoints of recovery. To investigate respiratory infection susceptibility we challenged mice with Streptococcus pneumoniae during chronic stroke recovery.

Results: Following stroke, the spleen contracts due to lymphocyte apoptosis and myeloid mobilisation, and we observed unequal distribution of lymphocyte loss. For instance, we unexpectedly saw a disproportionate loss of splenic $CD4^+$ compared to $CD8^+$ T cells acutely post-stroke, concurrent with the development of spontaneous lung infection. Subsequently, repopulation of leukocytes was also unequal. The numbers of transitional and marginal zone B cells remained decreased at three months post-stroke while other B cell subsets normalized, as did T cell and innate subsets. To examine host defence, we challenged mice with S. pneumoniae following a three month recovery from stroke. We observed that, unlike sham and naïve controls, post-stroke mice were not able to clear the lung infection within 48 hours, showing for the first time ongoing infection susceptibility long-term following stroke. To follow up on these findings we are investigating functionality of returning lymphocytes, including their clonality and diversity and concentrations of antibodies and cytokines.

Conclusions: These results indicate that splenic contraction and repopulation is a dynamic and long-term process following stroke and suggest persistent vulnerability to infectious challenge, with important clinical implications for people with a history of stroke.

P.14 **Structural epitope profiling implicates a T independent antigen on SARS-CoV-2 virion surface associated with immunopathology**

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Antibodies can have beneficial, neutral, or harmful effects so resolving an antibody repertoire to its target epitopes may explain heterogeneity in susceptibility to infectious disease, but the three-dimensional nature of antibody-epitope interactions limits discovery of important targets in non-obvious parts of a proteome. We have developed and experimentally validated a computational method and synthetic biology pipeline for identifying biophysically stable peptides that from functionally important epitopes. The computational of this approach is such that we can consider all possible peptides from entire pathogen proteomes and we demonstrate this for the SARS-CoV-2 proteome. Validating these findings in five independent clinical cohorts, we identify patterns of epitope-binding antibodies associated with immunopathology, including a non-isotype switching IgM response to an exposed membrane protein epitope which is the strongest single immunological feature associated with severe COVID-19 to date (adjusted OR 72.14, 95% CI: 9.71 – 1300.15). We suggest the mechanism is T independent B cell activation and identify persistence (> 1 year) of this response in individuals with long COVID particularly affected by fatigue and depression. These findings highlight a previously unrecognized coronavirus host:pathogen interaction which is potentially an upstream event in severe immunopathology and this may have implications for the ongoing medical and public health response to the pandemic. The membrane protein epitope is a promising vaccine target which may complement anti-spike vaccination or monoclonal antibody therapies broadening immunological protection.

P.15 **Changing TIMEs: Altering the Chemokine Composition of the TME to Improve Immunotherapeutic Outcomes**

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Chemokines play vital roles controlling tissue immune composition and organisation both of which are important for effective anti-tumour immunity. Despite their importance only two drugs have been approved as chemokine-based therapeutics and both of these had unexpected effects, possibly due to redundancy and promiscuity within the chemokine network. Understanding the network in the tumour context is therefore crucial to establishing how this system could be manipulated to improve immune outcomes. One critical immune cell subset which correlates with responsiveness to immune checkpoint inhibition are type 1 conventional dendritic cells (cDC1). Based on publicly

available RNAseq data we determined chemokine receptors expressed by precursors of cDC1. To determine how therapeutically increasing the concentration of specific chemokines in the TME alters the composition of the tumour immune microenvironment (TIME) we selected chemokines which activate the previously determined chemokine receptors. These were injected subcutaneously into C57BL/6 mice and at endpoint the TME immune composition was determined by flow cytometry. This identified several potential targets to positively change the immune composition of the TME increasing both numbers of cDC1 and activated CD8+ T-cells. We have now generated collagen binding domains linked to these chemokines to localise them to the TME to determine whether these effects can be replicated therapeutically and whether this can synergise with existing immunotherapy.

P.16 **iRhom2 as a Novel Therapeutic Target for Inflammatory Bowel Disease**

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The pro-inflammatory cytokine TNFα and the IL-6 receptor are cleaved by the TNFα converting enzyme (TACE) at the cell surface. The release of TNFα and IL-6R by TACE has been linked to several inflammatory diseases including Rheumatoid Arthritis and Inflammatory Bowel Disease (IBD). The inactive rhomboid proteins (iRhom) 1 and 2 are crucial for the trafficking and maturation of TACE. Furthermore, iRhom2 co-localises with TACE on the cell surface in immune cells. iRhom2 is thought of as a regulatory subunit of the iRhom2/TACE complex prompting TACE activity and thus modulating the release of these pro-inflammatory factors. Previous studies have found that direct blocking of TACE as therapeutic target has severe side effects, including intestinal lesions. Membrane iRhom2 displays a new target for modulating TACE activity. iRhom2 knockout mice do not show the same side effects but display reduced TACE activity. Here, iRhom1 remains active and allows homeostatic functions of TACE, thus preventing the severe side effects. Therefore, targeting iRhom2 may be an attractive novel therapeutic approach for inflammatory disease as it inhibits several key proinflammatory pathways. To this end iRhom2 antibodies with proven preclinical efficacy are under development (SciRhom GmbH, Germany). To research this novel mode of tackling autoimmune disease in IBD, we will perform TNFα and IL-6R shedding assays using PBMCs and colonic biopsies of IBD patients and healthy controls. We expect that cells treated with the anti-iRhom2 antibody will display reduced shedding of these pro-inflammatory factors, even after LPS stimulation. Furthermore, to localise and analyse levels of iRhom2 expression in IBD patients, immunohistochemistry will be performed on IBD gut biopsies. In summary, we hypothesise that blocking iRhom2 activity will modulate the TNFα and IL-6R disease causing pathways, presenting a viable target for treating IBD and other chronic inflammatory diseases.

P.17 **Atg16l1 preserves intestinal homeostasis by differentially regulating CD4+ peripheral T cells**

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A single-nucleotide polymorphism in the essential autophagy gene Atg16l1 is associated with an increased susceptibility to inflammatory bowel disease, specifically Crohn's disease. While the role of autophagy in intestinal immune homeostasis remains unclear it has been demonstrated that conditional deletion of Atg16l1 in murine CD4+ T cells leads to spontaneous intestinal inflammation which is characterized by an aberrant Th2 response and a loss of Foxp3+ regulatory T cells. However, the mechanisms underlying these T cell changes in the presence of defective autophagy are unknown. To investigate how autophagy impacts different peripheral CD4+ T cells subsets on a molecular level, we performed single-cell RNA sequencing of Atg16l1-deficient and wildtype CD4+ T

cells isolated from murine colonic lamina propria. In Atg16l1-deficient CD4+ T cells, bioinformatic analysis confirmed the previously reported increase in Th2 cell proportions and reduction in regulatory T cell proportions and, additionally, an increase in naïve T cell proportions was found. Focussing on regulatory T cells, the results suggest that Atg16l1-deficient cells have enhanced activation of the proteasome degradative pathway and impaired IL-10 expression. In addition, trajectory analysis indicates the presence of intermediate developmental stages of regulatory T cells in the intestine and suggests that these are absent in Atg16l1-deficient mice. Based on these results we hypothesise that autophagy may play a unique role in regulatory T cells and could be targeted to modify therapeutic outcomes in chronic inflammatory disorders.

P.18 **Targeting Protein Tyrosine Phosphatase 1B (PTP1B) as a Novel Treatment for Diabetic Wound Healing (DWH)**

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Background:
Chronic diabetic wounds are a major problem in diabetic individuals. Dysregulated macrophage function makes a substantial contribution to non-healing wounds. Protein tyrosine phosphatase 1B (PTP1B) is a key regulator of whole-body glucose and energy metabolism. Diabetic wounds exhibit increased expression levels of PTP1B, particularly within macrophages. We hypothesised that inhibiting macrophage PTP1B would improve wound healing under physiological and diabetic conditions.
Methods:
In vivo, wound healing was assessed under physiological, normoglycemic conditions in control wildtype (PTP1Bfl/fl) and myeloid-PTP1B deficient (LysM PTP1B-/-) littermate mice (male and female, 8 months-old). Experiments were conducted in streptozotocin-induced (STZ) diabetic mice (6 weeks post-STZ induction). Wound healing was quantified over a period of 10 days. Two circular wounds were made horizontally in the dorsal region. The wounds were assessed using tracing and ruler (wound diameter) methods. The wounds were photographed for recording and documentation.

In vitro, wound healing was assessed using wound healing assays, under hyper-glycaemic conditions. The keratinocyte cell line (HaCaT) was cultured with bone marrow-derived macrophages (BMDM) from diabetic C57Bl6 mice. Wound healing was also assessed in presence/absence of the PTP1B inhibitor, MSI-1436 (10-1000nM).

Results and conclusion:
Both healthy and diabetic LysM PTP1B-/- mice exhibited a faster rate of wound closure over 10 days. Under physiological conditions, there was a significant improvement in wound healing in LysM PTP1B-/- mice 4-days post-surgery. Under diabetic conditions, LysM PTP1B-/- mice had a significantly faster wound closure compared to PTP1Bfl/fl. on day 8 post-surgery. Wound healing assays revealed more efficient wound closure in HaCaT cells co-cultured with BMDM from diabetic C57Bl6 mice, in the presence of MSI-1436, revealing mechanistically that PTP1B inhibition speeds up wound healing. Thus, inhibiting macrophage–PTP1B improves wound healing rate under normoglycaemic and hyperglycaemic conditions, suggesting that PTP1B inhibition as a novel therapy for treatment of non-healing diabetic wounds.

P.19 **Role of Long-lived TIM4 CD4 macrophages in regulating stem cells in the gut**

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Background
Impaired wound healing and fibrosis are a hallmark of Inflammatory Gastrointestinal Disorders (IGD). The proliferation and maturation of intestinal stem cells (ISCs) are critical in the proper closure of wounds in the gut. Macrophages have been shown to play a key role in the maintenance of ISCs, but these studies were restricted to investigating the total intestinal macrophage pool. Recent fate-mapping studies identified a subpopulation of long-lived intestinal macrophages identified by expression of Tim4 but the functional role of these macrophages in the gut remains unexplored.
Methods
A novel Tim4 cre mouse was crossed with a Diphtheria toxin receptor (DTR) mouse to generate a Tim4 cre iDTR mouse which could selectively deplete the Tim4 macrophages when administered with diphtheria toxin. The quantitative differences between the control and the Tim4 cre iDTR mice were measured using immunofluorescence imaging.
Results
Administration of a single dose of the diphtheria toxin ablated the Tim4+ macrophages for 56 days with a slow increase in number of the Tim4 macrophages at day 56 as compared to day 7. Morphometric analysis of the magnitude of the ISC marker Olfactomedin4 (OLFM4) in the Tim4 cre iDTR mice showed a drop at day 28(d28) which normalised at day 56(d56).
Conclusions
The role of Tim4 macrophages in maintaining ISCs was investigated using a novel Tim4 cre iDTR mouse. Long-term depletion of Tim4 macrophages caused a temporary change in the ISC marker OLFM4 at d28 of the depletion which was resolved by d56. This suggests that Tim4 macrophages might be secreting survival factors for the intestinal stem cells but eventually the stem cells evolve to survive without them or that alternative sources are established. Future experiments would include he assessment of these parameters in an inflammatory model of the gut in the Tim4 cre iDTR mouse.

P.20 **Effects of NLRP3 and Gasdermin D and Gasdermin E on the Maturation of Neutrophils in Atherosclerosis**

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Pyroptosis is a major contributor to chronic inflammation observed in atherosclerosis. Neutrophils have often been overlooked in chronic inflammation; however, more studies are now examining their role in cardiovascular events and the role pyroptosis plays in neutrophil biology. Using mixed chimera bone marrow transplants and CyTOF mass cytometry, we investigated how different parts of the pyroptosis pathway, including Gasdermin D, Gasdermin E and NLRP3, affected neutrophil production and maturation in atherosclerosis. We found that Gasdermin D knockouts accumulate mature neutrophils in the blood. Gasdermin E loss was found to have opposite effects, leading to a decrease in mature neutrophils, with accumulation of more immature neutrophil populations in blood. NLRP3, surprisingly, had little effect on neutrophil maturation. Experiments examining neutrophil populations in atherosclerosis in mice with normal functioning pyroptosis showed trends

that mature neutrophils accumulate in the blood. These were similar results that were observed in Gasdermin D knockouts. Finally, expression of Gasdermin D and Gasdermin E was correlated with disease progression in atherosclerosis patients. These data show that pyroptosis plays a newly discovered, important role in the maturation of the neutrophils in atherosclerosis.

P.21 **Predisposition of blood group incompatibility and G6PD deficiency to severe neonatal hyperbilirunaemia**

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Hyperbilirubinaemia occurs in infants of all racial groups. It is also seen in infants with blood-group incompatibilities, erythrocyte-enzyme deficiencies and also infants with structural defects of the erythrocytes. One of the major causes of neonatal jaundice is fetal-maternal blood group incompatibility involving the ABO system, the Kell system and the rhesus system. Our study found out that blood group incompatibility and G6PD deficiency are some of the haematological factors that influenced the development of hyperbilirubinaemia.

P.22 **Co-transfer of antigen and contextual information harmonises peripheral and lymph node cDC activation**

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T-cell responses initiate in lymph nodes (LN) spatially separated from challenge sites, and thus require contextual information about antigen to be encoded and transmitted through space. Whilst migratory conventional dendritic cells (cDC) originate in the tissue, LN resident cDC, never directly interact with the periphery, and understanding how they appropriately process antigen, and drive suitable T-cell responses is largely unknown. Using a novel influenza virus we demonstrate that migratory cDC transport antigen to the draining LN (dLN) and transfer it to resident cDC and that this leads to specific activation of these recipient cDC. In a tumour setting, lower levels of activation at the tumour site also transfers to resident cDC showing that antigen transfer and cDC activation is integrated and specific to the challenge. Previously hypothesised mechanisms of resident cDC activation proved insufficient and instead, we found that during influenza infection dsRNA is co-transferred to LN resident cDC. TLR signals direct antigen handling and presentation suggesting a possible role in encoding peripheral cues with antigen. To test this, we injected a fluorescent TLR ligand intratumorally showing that this activated LN resident cDC in a cell intrinsic manner. Furthermore, using mixed BM chimeras where 10% of cells were Myd88-/- we showed that LN resident cDC require intact TLR signalling to become activated upon antigen transfer. As such, TLR ligand co-transfer is necessary and sufficient to drive LN resident cDC activation. Our work demonstrates that co-transfer of antigen and contextual information allow integration of the periphery and the lymph node in coordinating immunity. This same process, during cancer development, leads to the dissemination of immune dysfunction from the tumour microenvironment to the dLN. Understanding this process and how it can be modulated will be important for promoting anti-viral and anti-tumour immunity.

P.23 **Investigating the role of adhesion proteins in immune modulation of glioblastoma**

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Glioblastoma (GBM) is a highly invasive grade IV astrocytoma representing the most common type of primary brain tumour in adults with a dismal survival prognosis of ~15 months post-diagnosis with treatment. High rate of tumour recurrence and treatment resistance have resulted in no new treatments being approved for GBM in the past 20 years. Recent advances in cell sorting and single cell analysis technologies have begun to reveal a highly heterogenous immune landscape present in GBM tumour microenvironment (TME). Microglia and bone marrow-derived macrophages (BMDMs) account for the majority of CD45+ immune cells within GBM tumours but the tumour dependent and independent factors mediating microglial and infiltrating BMDM phenotypes remains unclear. Focal adhesion proteins are a possible contributor to GBM tumour development and interactions with the TME. Integrin-linked kinase (ILK), a focal adhesion pseudokinase, is overexpressed in a range of cancers and has been shown to drive chemotherapy resistance and enhance invasiveness of glioma stem cells in vitro. However, the molecular mechanisms governing this phenomenon in vivo and the effects of ILK expression on the immune TME in GBM requires investigation. Using four different recently developed glioma stem cell models of GBM we will investigate ILK-driven changes to GBM cell phenotypes in vitro. Additionally, we will clarify whether ILK expression enhances BMDM tumour infiltration and how tumour associated macrophage and microglia phenotypes are altered in vivo. Using clinically relevant models of GBM, this work will provide much needed insight into cellular and molecular interplay within the GBM TME and has the potential to highlight novel immune therapeutic targets.

P.24 **Non-Ionic Surfactant Vesicles as an Anti-inflammatory**

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INTRODUCTION: Viral sepsis is a life-threatening condition that has become further prevalent during the SARS-CoV-2 pandemic and contributed to the deaths of 6.5million people. Currently, tempering systemic inflammation whilst retaining a strong anti-viral response has been a challenge to overcome with current therapies like glucocorticoids. Herein we investigate the inherent immunomodulatory effects of Non-Ionic Surfactant Vesicles (NISV) as a potential adjunct treatment for sepsis and other cytokine-storm related disorders. Previously, NISV have been used in drug delivery, and as vaccine adjuvants to increase the stability and release of various compounds. However, we demonstrate that with no payload, NISV can have potent anti-inflammatory effects, whilst providing a platform technology that is easily modifiable in terms of components, size, and production method.
METHODS: Murine Bone-marrow derived macrophages (BMDM) stimulated with TLR7 and TLR8 ligands to simulate single stranded RNA (ssRNA) virus infection. The effect of NISV on cytokine production by ELISA and cytometric bead array was examined. The immunomodulatory effects of NISV were compared to a conventional anti-inflammatory drug, dexamethasone. We also utilise transcriptomics to investigate the gene-level transcript changes induced by NISV.
RESULTS: Key pro-inflammatory cytokines such as IL-6, TNF-□ are down-regulated in a dose dependent manner by NISV, whilst enhancing ant-viral cytokines such as IL-1α and IL-1β. TLR7 and TLR8 stimulated production of IL-12 can be controlled by NISV, but not by dexamethasone.
CONCLUSION: NISV have anti-inflammatory properties in the context of ssRNA viral infections, with TLR-pathway specific effects suggesting they modulate specific signalling pathways.

P.25 **Visualising of mucosal antigen uptake and trafficking to uncover how dendritic cells control immunity and tolerance**

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The intestinal immune system controls the balance between immunity needed for protection against pathogens and the maintenance of tolerance against harmless antigens. Intestinal dendritic cells (DC) orchestrate the decision-making between immune system activation and tolerance. DCs sample molecules in their environment. They then migrate to lymph nodes where they present the antigens to naïve T cells and instruct them to mount an immune response. The current understanding of what exactly determines DC decision between the two immune responses is very limited. It remains unclear if different subsets of DC induce the different kinds of immune response, or what signalling molecules and cellular mechanisms are involved.

Here, we used a novel approach of fluorescently labelling different antigens and identified their uptake and effects on DCs migrating from the intestine. We labelled tolerance-inducing ovalbumin, Th1/17-inducing Salmonella and Th2-inducing helminth excretory-secretory products from H. polygyrus. Alone or in combination, we injected these labelled antigens directly into the intestinal wall. Subsequently, using flow cytometry, we analysed lymph and identified differential abilities of the migratory DCs to carry each of the antigens. Additionally, we analysed the effects of the antigens on DCs' functions to drive T cell outcomes in vitro. Using single or multiple antigen combinations we demonstrated the dominant effects of specific antigens on the cultured T cells. Thus, both in vivo and in vitro, the outcome of the immune response is controlled both by the type of DC, and by the nature of the eliciting antigen.

Together, our work fills gaps in our understanding of the cellular and molecular mechanisms that control immune balance in the intestine, and may eventually enable us to modulate this immune balance in a disease setting.

P.26 **Addressing the role of CD103+CD11b+ dendritic cells in a model of pancreatic ductal adenocarcinoma lung metastasis**

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Dendritic cells (DCs) are important actors in the microenvironment of pancreatic ductal adenocarcinoma (PDAC). In mice, the lack of cDC1 has been associated with scarce CD8 T cell responses to PDAC neoantigens, while less characterized tolerogenic DC populations have been described to antagonize anti-tumour immunity. The lung is a key organ for immune regulation whilst being a critical site of PDAC metastasis development. Therefore, there is an interest in characterizing DC identity and function in the metastatic lung.

To this end, we transplanted KrasG12D/+; Trp53R172H/+; Pdx1-Cre (KPC) cells intravenously into wt and interferon regulatory factor 4 knock-out (IRF4 KO) hosts to investigate the microenvironment of established metastases in a highly tractable way. Resultant lung tumours were studied using flow cytometry and spectral multiplexed 3D confocal imaging to explore the immunological infiltrate and its localisation.

Immune landscaping revealed increased influx of dendritic cells, as well as interstitial macrophages and regulatory T cells in tumour bearing wt lungs. Importantly, we observed large numbers of

CD103+CD11b+ DCs, which are not normally abundant in the lungs but restricted to the small intestine. SIRPα, CD86 and PDL1 staining suggested that this population is cDC2-related, activated, and may be immunosuppressive. Additionally, CD103+CD11b+ DCs could be found in tumour-draining lymph nodes. In tumour-bearing IRF4 KO mice, which are deficient for CD103+CD11b+ DCs, we found fewer T regulatory cells, suggesting a possible role for CD103+CD11b+ DCs in the development of suppressive T cells in PDAC tumours. Future efforts will focus on further mechanistically implicating CD103+CD11b+ DCs in PDAC lung metastases as well as identifying targetable factors driving their development.

P.27 **Tumour associated macrophages (TAMs) conditioned by breast cancer cells differentially impact growth of mammospheres depending on their origin and the breast cancer cell type**

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Background: In most solid tumours, including breast cancer, tumour associated macrophages (TAMs) are a key component of the tumour microenvironment representing up to 50% of infiltrating cells. Infiltration of TAMs has been associated with poor prognosis and chemotherapy resistance, highlighting their potential as a therapeutic target. We aimed to investigate whether TAMs can be rewired towards an anti-tumour phenotype in different types of breast cancer by using a PTP1B inhibitor which has already been shown to slow cancer progression.

Methods: THP-1 or human monocyte-derived macrophages were initially conditioned by breast cancer cells into TAMs. Tumour conditioned THP-1/monocyte-derived macrophages (with/without 30 mins of PTP1B inhibitor treatment), were then either co-cultured with MCF-7 (oestrogen receptor positive) or MDA-MB-231 (triple negative) breast cancer cell lines, or conditioned to TAMs through cell-cell contact by infiltrating into pre-formed MCF-7 and MDA-MB-231 mammospheres. The effects of TAMs on mammosphere growth (with/without PTP1B treatment) were assessed by measuring mammosphere area to determine tumour growth (FIJI-ImageJ) and cancer cell viability (acid phosphatase assay).

Results: MCF-7 co-culture with THP-1 significantly decreased mammosphere growth whereas co-culture with human monocyte-derived macrophages supported growth. PTP1B inhibitor treatment enhanced this phenotype. Opposing results were seen on mammosphere growth when formed with MDA-MB-231 cells. When THP-1 monocytes were conditioned to TAMs by infiltration, mammosphere growth was unchanged whereas infiltration by blood-derived monocytes enhanced MCF-7 mammosphere growth from day12 post-infiltration, which was further increased with treated monocytes.

Conclusions: TAMs conditioned through mammosphere infiltration show functional differences to that conditioned through co-culture with breast cancer cells. The effect of TAMs on mammosphere growth is dependent on their monocytic origin and the breast cancer cell type and show our system is effective for assessing the impact of new therapies on TAMs and the resulting consequences in clinical breast cancer.

P.28 **Glioma-secreted factors activate Nrf2 in human and mouse macrophages**

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Gliomas are the most aggressive forms of adult brain cancers with very high mortality rates. Due to a lack of therapeutic options, the overall prognosis remains poor. Intriguingly, around 30% of the high-

grade glioma mass is comprised of macrophages, including infiltrating macrophages and brain-resident microglia. This myeloid population contributes to the pro-tumourigenic, immunosuppressive glioma microenvironment, but the underlying mechanism(s) is poorly understood. RNAseq data indicate that the expression of multiple targets of transcription factor Nrf2 are elevated in microglial populations of IDH-wildtype gliomas. Nrf2 regulates the cellular redox homeostasis and suppresses pro-inflammatory responses. We hypothesised that Nrf2 activation in the glioma-associated myeloid population is potentially due to crosstalk with the tumour cells within the microenvironment.

To test our hypothesis, murine RAW264.7 macrophages were treated with conditioned media from spheroid cultures of early-passage murine glioma. This treatment increased the Nrf2-target gene expression in macrophages. A similar pattern of Nrf2 activation was observed utilising conditioned media from human glioma cell lines and human THP1-derived macrophages. This was accompanied by an increase in SLC7A11, a known Nrf2 transcriptional target, and this increase was diminished upon Nrf2 depletion by siRNA. SLC7A11 is a cystine/glutamate antiporter that also imports the tryptophan metabolite, L-kynurenine, which is present at high concentrations in the glioma microenvironment and contributes to the anti-inflammatory, tumour-promoting phenotype in the tumour-associated macrophages. Additionally, L-kynurenine can activate Nrf2 in other contexts. We therefore tested the possibility that L-kynurenine could be one component of the glioma-conditioned media that contributes to Nrf2 activation in macrophages. Indeed, the levels of Nrf2 and its transcriptional targets increased upon exposure to L-kynurenine of human and murine macrophage cell lines and primary mouse bone marrow-derived macrophages. Together, our findings suggest that glioma-secreted factors, including L-kynurenine, contribute to Nrf2 activation in macrophages, which in turn may promote an immunosuppressive microenvironment.

P.29 **Quantitative mass spectrometry reveals stem like regulation mechanisms of the cell cycle in expansion phase CD8+ T cells**

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Background
IL-2 mediates expansion phase in CD8+ T cells, promoting rapid proliferation and differentiation towards production of short-lived effector T cells (SLECs), and memory progenitor cells (MPECs). SLECs rapidly re-enter S phase following mitosis, while MPECs stall prior to S phase, suggesting differences in cell cycle control. Like SLECs, embryonic stem (ES) cells show rapid S phase re-entry. ES cell cycle regulation is characterised by persistently expressed cycle factors, e.g. cyclins, mediated in part by Anaphase Promoting Complex Cyclosome (APC/C) regulation by Emi1. T cell cycle regulation is also specialised. For example, deletion of activating-E2F transcription factors, which are thought to promote cell cycle entry, counterintuitively promotes proliferation. Precisely how the regulatory network of CD8+ T cells is specialized to mediate rapid proliferation is unknown.

Method
PRIMMUS (Proteomic analysis of Intracellular iMMUnolabelled cell Subsets) was used to identify the cell cycle regulated proteome in CD8+ T cells. This dataset was compared with published datasets from other cell types, identifying potential CD8+ T cell cycle control mechanisms. CRISPR KO was then conducted on selected identified regulators to observe how this impacted cell cycle behaviour.

Results
Proteomic analysis identified that out of the APC/C substrates, those active during M-phase and are thus controlled by APC/C-Cdc20 were cell cycle regulated, while those which were active in

promoting S-phase and were controlled by APC/C-Cdh1 were not cell cycle regulated. Instead they were constituently active, as is seen in ES cells. Deletion of Emi1 lead to re-replication of cells post G2, fewer cells present in early S phase and more in p-Rb negative G0/early G1.

Conclusion
CD8+ T cell cycle regulation shares aspects with ES cell cycle regulation, yet remains distinct. In addition, Emi1 has a primary role in preventing re-replication in CD8+ T cells, and a secondary role in promoting S-phase entry.

P.30 **Distinct human synovial-tissue CD1cposdendritic cell clusters govern healthy immune homeostasis and the active and remission phases of Rheumatoid Arthritis**

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Current treatments for Rheumatoid arthritis (RA) control inflammatory synovitis and modulate adaptive immunity, but do not restore immune tolerance that is characteristic of healthy synovium. Dendritic cells (DC) are the only cell-type that can reset adaptive immunity. Characterisation of synovial tissue DC populations in RA patients and heathy donors may provide insight into pathways that block or enable transition from remission to cure.

Synovial tissue (ST)-DCs from synovial biopsies of remission RA, active RA and healthy synovium were compared by single cell transcriptomics (scRNAseq), multiparameter flow cytometry, and by co-culture of ST-DC/autologous T cells.

The most abundant DC population in all human synovial tissues are CD1cpos and scRNAseq uncovered that these are highly heterogenous, revealing 5 distinct transcriptomic clusters that differ in distribution, location, and ontogeny between joint conditions.

The DC2 (CD1chigh) are the most abundant clusters in healthy synovial tissues, located in the superficial sublining layer. The active RA synovium is dominated by DC3 (CD1clow) cluster located in the sublining layer. The relative proportion of this cluster decreases significantly in RA in disease remission, instead remission is characterised by the dominance of DC2-CD206pos and the lack of DC2-Axlpos clusters that are typical of the healthy synovium. Remission RA patients retained a substantial proportion of the CD1clowCD63pos cluster characteristic of active RA.

Functional co-culture of sorted ST-DCs with autologous CD4pos memory T-cells showed that ST-DC2 and DC3 clusters differ in function within and between different joint conditions. DC2 from active RA synovium supported T peripheral helper cells (Tph) while DC3 support CCL5pos T effector memory cells.

In Conclusion, distinct synovial tissue DC CD1cpos clusters may determine healthy immune tolerance, autoimmunity and disease remission. Therapeutic targeting of the stimulatory/inflammatory functions of ST-DC3 in remission, and/or re-instating the healthy DC2-Axlpos cluster might provide pathways to transform remission into healthy immune-homeostasis.

P.31 **Aconitate decarboxylase 1 (ACOD1) represents a crucial regulator of monocyte recruitment, survival and proinflammatory status in intestinal inflammation**

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Background
Aconitate decarboxylase 1 (ACOD1; also known as IRG-1) is the ubiquitous source of the metabolite itaconate, which dampens inflammasome activation by preventing HIF1α production. We hypothesised that expression of ACOD1 curtails monocyte inflammatory function, which could be manipulated in IBD therapy.
Methods
We performed single-cell RNA sequencing of colon monocytes from treatment naïve IBD patients and DSS treated C57BL/6J (WT) mice. To determine the role of ACOD1 in colitis, Acod1¬–/– mice were administered 2% DSS in drinking water for 6 days (acute colitis) or 4 days followed by 14 day recovery period (inflammation resolution). Lastly, we generated competitive bone marrow chimeric mice to assess the intrinsic role of ACOD1 controlling monocyte survival and proinflammatory status.
Results
We found that ACOD1/Acod1 expression was specifically restricted to inflammation-associated monocytes. Acod1–/– mice demonstrated increased susceptibility to acute and resolution associated intestinal injury compared to WT controls, including significant accumulation of CD64+ Ly6C+ MHC-II+/– monocytes. Consistent with Acod1 limiting inflammasome activation, Acod1–/– monocytes demonstrated significantly greater IL-1β producing capabilities.

To assess Acod1 deficient and sufficient monocytes in tandem, we generated competitive bone marrow chimeric mice. Acod1 deficiency conferred a significant advantage to monocyte recruitment to the colon, characterised by a 10fold increase in Ly6C+ MHC-II– colon monocytes in colitis.

Transcriptional profiling of chimeric WT and Acod1–/– colonic monocytes revealed significantly greater expression of molecules required for monocyte extravasation, including Sell (CD62L), Icam1, Ccr2 and Itgal (CD11a). In addition, expression of genes indicative of macrophage maturation such as Itgax (CD11c), Cd163 and Cx3cr1 were lower in the context of Acod1 deficiency, suggesting that normal maturation of these cells may be perturbed by loss of Acod1.
Conclusion
ACOD1/Acod1 represents an evolutionarily conserved, monocyte specific immuno-metabolic pathway that acts to limit monocyte recruitment, fate and pro-inflammatory function within the inflamed intestine.

P.32 **Manipulation of the intestinal epithelium by H. polygyrus**

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Many parasitic helminths establish long-term chronic infections. This is thought to be due to their ability to alter the environment around them through secreted substances. Although a number of immunomodulatory proteins have now been defined that interfere with host immune cell responses, little research has been done to see if they impact the epithelial layer, which serves as the first point of contact and a critical barrier to infection; the epithelium also acts as an innate immune effector population through the products released by goblet cells and tuft cells which promote anti-helminth immunity. With both in vitro and in vivo techniques, we study this connection using Heligmosomoides polygyrus, a mouse helminth parasite that employs various immunomodulatory mechanisms to induce chronic infections. H. polygyrus excretory/secretory products (HES) were used to treat small intestinal cell organoid cultures (enteroids), revealing a wide range of effects on developmental pathways and the suppression of gene sets expressed by goblet, Paneth, and tuft cells. Furthermore, organoid morphology was drastically altered, with HES causing a spheroid, proliferative phenotype devoid of crypts and differentiated cells. In vivo, tuft cell induction by both succinate and the non-resident helminth Nippostrongylus brasiliensis was reduced in the presence of H. polygyrus. HES also reduced succinate-stimulated tuft cell expansion. Thus, chronic infection with H. polygyrus may prevent the development of a crucial epithelial cell for immune defence, allowing the parasite to survive. Our findings show that helminth parasites have an impact on their hosts that extends beyond the classical immune system, and that they can also alter the intestinal environment to their advantage.

P.33 **Investigating the role of hypoxia inducible factor-1a (HIF-1a) in controlling monocyte behaviour in the intestine**

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Background
Macrophages are highly abundant in the intestinal mucosa where they play fundamental roles in tissue homeostasis. Unlike many other tissue macrophages, those in the healthy intestinal mucosa are replenished by circulating monocytes. However, in inflammatory bowel disease monocytes accumulate in large numbers and display aggressive, pro-inflammatory behaviour. Why seemingly phenotypically identical monocytes display such distinct fates and functions during health and inflammation remains unclear.
Methods & Results
Transcriptional profiling of blood and colonic monocytes from healthy and colitic mice using the dextran sodium sulphate (DSS) model of colitis revealed that monocytes in the context of colitis have a unique transcriptional profile, including enrichment of genes involved in cell metabolism. Parallel analysis of analogous cells in human blood and mucosal biopsies from a newly diagnosed Crohn's disease patient showed that this molecular signature is evolutionarily conserved between in man. In particular, expression of hypoxia inducible factor 1-a (HIF-1a) is a feature of colitis-associated monocytes. To assess the role of HIF-1a in controlling monocyte behaviour in the gut mucosa, we generated mice with myeloid (Lyz2)-specific deletion of Hif1a. Preliminary data show that deletion of Hif1a has no baseline effect on the composition of the monocyte-macrophage compartment in homeostasis, but is associated with an increase in the proportion of Ly6C+MHCII- monocytes in the

leucocyte compartment in the inflamed colon after DSS-induced acute colitis ($p<0.0063$). By generating competitive bone marrow chimeric mice, we found that deficiency of Hif1a conferred a competitive advantage to monocytes. This was also seen in circulating classical monocytes ($p=0.0023$), suggesting there is an effect at the level of haematopoiesis. Transcriptional profiling of Ly6C+ monocytes by NanoString from chimeric mice following acute colitis revealed increased mRNA expression of monocyte effectors and downregulation of mRNA associated with monocyte maturation/differentiation in Hif1a deficiency.

Conclusions

Our work identifies HIF-1a as a feature of inflammation-associated monocytes which may act to limit monocyte lifespan. Future work will seek to confirm these findings and delineate further molecular control of monocytes in colitis.

P.34 **Investigating the effects of inflammation on colonic macrophages**

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Background

Macrophages play multiple key homeostatic roles throughout the intestine. However, dysregulated macrophages are considered a key feature of gastrointestinal diseases such as inflammatory bowel disease (IBD). Whether these distinct roles are performed by discrete subsets of macrophages or if tissue resident macrophages change their behaviour in the context of inflammation is unclear. Moreover, given the relapsing-remitting nature of IBD, whether inflammation induces lasting effects on the intestinal macrophages compartment following resolution is also unknown. Here, we have characterised the colonic macrophages compartment in steady-state and the changes that occur during and following colonic inflammation.

Methods and Results

Using scRNA-seq, flow cytometry and immunofluorescence, we found that there are at least two transcriptionally, phenotypically and anatomically distinct populations of colonic macrophages, defined as CD11c+CD163– and CD11c–CD163+. Genetic fate-mapping shows that these macrophages subsets have differential replenishment kinetics, with CD163+ macrophages being longer lived than their CD11c+ counterparts. Moreover, these subsets are differentially affected by inflammation. Replenishment of CD11c+CD163– macrophages is accelerated during DSS-induced colitis, whereas replenishment of CD11c–CD163+ macrophages is unaffected. Both subsets are outnumbered by classical monocytes and their progeny during acute colitis, but return to homeostatic levels following resolution of inflammation. By combining Cx3cr1-based fate mapping with scRNAseq, we show that transcriptional changes persist in the macrophages compartment during resolution. Interestingly, resident-macrophages fate-mapped from health through to resolution appear more restricted in their plasticity, with far fewer transcriptional changes when compared with macrophages that arrived during inflammation/resolution.

Conclusions

This work demonstrates that there are persistent effects of acute inflammation on the colonic macrophages compartment, with differential effects on ‘long-lived’ resident macrophages and those that have recently differentiated. Ongoing work seeks to determine whether this leads to functional changes which could impact the macrophages response with further insults.

P.35 **Live Candida albicans Infection Selectively Suppresses IL-6 in Bone Marrow Derived Macrophages**

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The opportunistic fungal pathogen, Candida albicans causes severe and lethal systemic infections in immunocompromised individuals. Macrophages are one of the first cells in the innate immune system to identify C. albicans and initiate an antifungal response. Macrophage cytokine response to pathogen invasion is crucial for innate cell communication and immune cell recruitment. Specifically, IL-6 deficiency in vivo results in higher susceptibility to systemic C. albicans infection. We found, conversely to the conventional signalling response, bone marrow derived macrophages secrete little to no IL-6 during C. albicans infection, and C. albicans selectively suppresses IL-6, but not TNF or IL-10, during co-treatment with LPS or live P. aeruginosa. This selective suppression of IL-6 is independent of Toll-Like Receptors and Dectin-1 activation and independent of macrophage cell death. Interestingly, co-stimulation of C. albicans and P. aeruginosa shows no suppression of MAPK/NFKB activation during peak phosphorylation. Utilising DIA-MS proteomics, we identified a list of LPS regulated proteins which are suppressed by C. albicans. This list may improve our understand of how C. albicans suppresses the LPS response, which may better elucidate C. albicans immune evasion pathways including the suppression of IL-6.

P.36 **RNA cap methyltransferases RNMT and CMTR1 drive gene expression programmes required for T cell maintenance and activation**

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T cells must regulate gene expression appropriately in response to environmental stimuli. Naïve T cells express pro-survival genes in response to tonic T cell receptor (TCR) and interleukin-7 receptor (IL7R) signalling, whereas stronger TCR signals drive T cell activation, inducing major changes in gene regulation to drive processes such as ribosome biogenesis, metabolism, cell cycle entry and differentiation to effector populations. We aim to understand how gene expression is regulated in T cells, focusing on a potent structure in gene expression, the m7G RNA cap. RNA cap binding proteins which recognise the m7G RNA cap promote transcript stability, processing and translation, thus the cap is integral to mRNA function. We are investigating the role of RNA cap methyltransferases in T cell function using conditional knockout models for the RNA cap N-7 methyltransferase Rnmt, and first transcribed nucleotide O-2 methyltransferase Cmtr1. We have uncovered a role for RNMT in promoting the expression of terminal oligo pyrimidine (TOP) RNAs, which include the transcripts encoding ribosomal proteins. Following TCR activation, RNMT cKO T cells have reduced ribosome biogenesis, reduced protein synthesis and poor cell growth. In naïve T cells, where the demand for ribosomes biogenesis is lower, loss of Rnmt does not impact bulk protein synthesis, but leads to decreased expression of the IL7R, reduced IL7R signalling and increased apoptosis. Loss of CMTR1 affects different pathways: Cmtr1 cKO T cells have normal cell growth and cell cycle entry upon TCR activation, but a higher rate of apoptosis leading to reduced proliferation in vitro. Cmtr1 cKOs have a markedly reduced CD8 T cell response to listeria infection, but a relatively normal CD4 T cell response. We are currently investigating how the impacts of CMTR1 on transcript processing, stability and translation lead to these activation defects.

P.37 **Non-Ionic Surfactant Vesicles as a Platform for Drug Delivery**

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Biothreat agents and pandemic capable pathogens can reside in a variety of different tissue types and organs. Once they have set up in the host cells, the pathogens have evolved mechanisms making them highly skilled at evading innate or adaptive immune responses. Thus, these pathogens can be highly virulent and difficult to clear if left untreated. Treatment using antibiotics and antivirals have been found to be very effective in managing infections, however total clearance of the pathogens is difficult as the tissues they reside in can be inaccessible to therapies. Therefore, there is a demand to develop a delivery system to target these hard-to-reach areas. Non-ionic surfactant vesicles (NISVs) have long been used as a delivery system for drugs and vaccine adjuvants. These vesicles are well characterised and can entrap both lipophilic and hydrophilic molecules. NISVs are very similar to an organic counterpart, liposomes. However, NISVs can deliver the same results with reduced toxicity due to the absence of phospholipids. NISVs also are readily modifiable, with the addition of bile salts to enhance stability for oral drug delivery, and the addition of ligands to their membrane for use in cell receptor targeting. Bile salt NISVs (bilosomes) are commonly used for the delivery of antibiotics (such as doxycycline, levofloxacin, ciprofloxacin), while the ligand NISVs have been used to deliver antibodies across the blood brain barrier (BBB). This PhD will explore the development novel formulations of the NISV for specific tissue targeting and the oral delivery of antibiotics restricted to IV use.

P.38 **Development and functions of mammary gland mast cells**

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The mammary gland ensures offspring health by facilitating lactation. It develops mostly postnatally through pubertal ductal branching and milk sac formation during pregnancy, and undergoes involution at weaning. Resident immune cells may regulate these processes. Mast cells (MCs) are best known as effector cells in allergic responses. It was previously reported that MCs support pubertal branching during and involution, but this study used mice that have other defects unrelated to MC-deficiency. MCs also associate with mammary tumors, where they have been ascribed both pro- and anti-tumoral roles. It is unclear what determines their functional outcomes, but their ontogeny may be a contributing factor. MCs can originate either from fetal or adult progenitors in the bone marrow. They may thus be recruited to the developing mammary gland or tumors from different sources and at distinct stages. This has not been investigated.

Here, we delineate MC ontogeny and re-address their functions in the mammary gland using more specific mast cell deficiency models.

Using genetic fate-mapping, we found that MCs with different origins co-exist in the adult gland. Strikingly, as much as ~57% of MCs originate from fetal yolk sac progenitors, the earliest source of MCs. These fetal-derived MCs are further enriched in tumour-bearing glands. We then constitutively depleted MCs using several models in which Cre-mediated recombination results in expression of Diphtheria toxin alpha in MCs. Unlike previously reported, however, MC depletion did not result in obvious branching defects in pubertal (5 weeks) mammary glands.

In conclusion, fetal-derived MCs constitute the majority of MCs in adult mammary glands, suggesting potential developmental roles. However, constitutive depletion does not seem to affect mammary gland branching, at least at pubertal stages. Future work will therefore address effects of MC

deficiency at different developmental stages, and further delineate their ontogeny and functions in mammary tumors.

P.39 **Developmental origins of melanoma-associated mast cells**

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The tumour micro-environment is rich in immune cells, which can influence tumour growth and treatment response. Mast cells (MCs) are found around solid tumours, including melanomas, but their exact roles are debated, since both pro- and anti-tumorigenic effects have been reported. This complexity could, at least partially, be due to the developmental heterogeneity of MCs. MCs arise either from foetal-restricted yolk sac (YS) progenitors or hematopoietic stem cells (HSCs, and could thus be recruited to tumours from distinct sources. This is the case for macrophages, whose effects on tumour growth appear to be determined by their developmental origins. Our aim is to test if these findings are also true for MC.

To delineate the developmental origins of melanoma-associated MCs, we used fate mapping models in which MCs are labelled according to their YS or HSC origin. We combined this with a graftable melanoma model that relies on mutations found in human disease (BrafV600E, Cdkn2a-/- & Pten-/-).

This revealed that foetal YS-derived MCs comprise on average 40% of melanoma-associated MCs, where they co-exist with MCs originating from HSCs. Compared to the skin of the same tumour-bearing mice, this represents an approximately 2-fold enrichment. This suggess that foetal-derived MCs are selectively enriched around melanomas, either through recruitment or increased proliferation. Transcriptomic analysis of YS and HSC-derived MC showed different signatures, in keeping with our hypothesis that developmentally distinct MCs may be functionally different. Moreover, depletion of MCs prior to melanoma engraftment resulted in reduced tumour growth in FcER1DTR mice. This suggests that pre-existing MCs, including those derived from YS progenitors, may exert pro-tumorigenic functions. We now aim to delineate the respective functions of foetal- and HSC-derived MCs in mouse melanomas.

P.40 **Unique wiring of TCR signalling in Intraepithelial T lymphocytes drives intestinal tolerance**

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Intestinal Intraepithelial T lymphocytes (IEL) are a unique population of T cells resident in the epithelial layer of the gut. They are involved in protecting the gut against pathogens and facilitating repair of damaged epithelium. IEL are categorised into 2 main subsets dependent on the expression of surface markers and pathway of development. Induced IEL, expressing TCRβ along with the CD8αβ coreceptor, proceed through classical development in the thymus and migrate to the gut upon priming with an antigen in the periphery. Natural IEL express either TCRβ or TCRγδ and the CD8αα coreceptor. These cells respond strongly to self antigens in the thymus but instead of undergoing negative selection, are trafficked directly to the gut. We asked how these self-reactive cells can be maintained in the gut environment without causing inflammation and damage to the tissue. Using phospho-flow cytometry and the novel Tocky-Timer mouse model we compared TCR signalling pathways in the IEL subsets and lymph node T cells when triggered with anti-CD3. We found that natural IEL had a diminished response to TCR stimulus compared to induced IEL and lymph node T cells both ex vivo and in vivo. Signalling was not restored when cells were treated with anti-LAG-3 blocking antibodies, cultured outside of the gut environment or by knocking out phosphatases and other regulators of TCR signalling, such as NTAL. Proteomic analysis unveiled

alterations in the components of the TCR signalosome in natural IEL leading us to reason that T cell receptor signalling is uniquely wired in these self-reactive natural IEL to promote tolerance of these cells and prevent inappropriate inflammation in the gut.

P.41 **Imaging immune EVasion by Heligmosomoides bakeri**

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The gastrointestinal parasite Heligmosomoides polygyrus bakeri has evolved multiple pathways of immune suppression which allow long-term survival in the host gut. Recent work has uncovered a novel pathway in which parasite-derived extracellular vesicles (EVs) suppress host immune responses using RNA interference. Mice immunized against H. bakeri EVs show a reduced parasite burden, and the EVs are able to supress activation of leukocytes in vitro. H. bakeri EVs contain small RNAs and a parasite argonaute protein called exWAGO, which work together to supress host gene expression.

It is currently unknown whether the EVs are taken up by all cells in the gut wall indiscriminately, or if they are targeted to specific cells. Here, we developed antibodies against different parasite components, and used immunofluorescence microscopy to investigate uptake of parasite EVs by host cells during infection. H. bakeri EVs were found inside host cells of the granuloma surrounding larval stages of the parasite. Immunophenotyping showed that the EVs were within neutrophils but not macrophages in the granuloma. This data suggests that parasite EVs have the potential to enter host cells and may exhibit cellular tropism.

P.42 **Investigating the impact of IL-33 on human macrophage responses**

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Introduction: Interleukin-33 (IL-33) is a cytokine of the IL-1 family that acts as an alarmin released from damaged epithelial cells in inflammatory diseases. Macrophages are a key source of inflammatory mediators to direct downstream immune responses and wound healing. The effect of IL-33 on human macrophage responses is not fully characterised.

Methods: Ethical approval was granted for blood collection from healthy volunteers. CD14+ monocytes were harvested using magnetic bead separation, differentiated into M0 macrophages using 10% autologous human serum over 7-10 days, then polarised to classical M1-like macrophages using LPS (100ng/ml) and IFN-γ (100ng/ml), or alternative M2a-like macrophages using IL-4 (20ng/ml) and IL-13 (20ng/ml) for 24 hrs (n=5). M0, M1 and M2 cells were stimulated +/- rIL-33 (100ng/ml) for a further 24hrs. Cell phenotype was assessed using flow cytometry (expression of CD14, CD68, CD80, CD209) and RNA extracted to assess gene expression (TGF-β1, TNF, IL-1β, IL-10, MMP7, MMP9) by Taqman qPCR.

Results: Phenotypic analysis demonstrated that monocytes and all macrophage subtypes expressed CD14 and CD68. M1 and M2 polarised cells expressed the marker CD80 and CD209, respectively, as expected. M0 macrophages expressed all genes at a low level with no statistical difference in expression following IL-33 stimulation. M1 polarised cells showed increased IL-1β expression ($p=0.03$), reduced TGF-β1 ($p=0.008$) and MMP9 ($p=0.05$) and a trend to increased TNF compared to M0 cells, as expected. Stimulation with IL-33 led to increased TGF-β1 expression in M1 macrophages

(p=0.05). No other changes in gene expression were identified with IL-33 in M1 or M2a polarised macrophages.

Conclusion: In this experimental model of polarised human macrophage culture, IL-33 led to a limited effect on gene expression profile. Further work will expand sample number given heterogeneity of response, increase panel of genes assessed, and investigate IL-33 receptor (ST2) expression in these cells.

P.43 **The Immunology of Alopecia Areata: How do Macrophages Contribute to Hair Loss?**

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Alopecia Areata (AA) is a common autoimmune disease which affects hair follicles and causes hair loss. Patches of the scalp, the entire scalp, or the entire body can be affected. Much of our understanding of AA is based on CD8+ T cells. However, recent data from our lab revealed people with AA had significantly elevated subpopulations of circulating B cells and CD4+ T cells, especially CCR6+ (Th17) and CCR6+CXCR3+ (Th17/Th1) CD4+ T cell populations.

Research on healthy hair follicles revealed follicular macrophages secrete immune mediators which influence follicular stem cells to promote the normal hair growth cycle. Our bulk RNA sequencing data showed established AA lesions had differential gene expression, including altered expression of macrophage genes such as CD163, CD206, and CD209. Additionally, the most upregulated pathways contained genes which respond to IFN-γ. It is known autoreactive NKG2D+CD8+ T cells respond to stress ligands expressed by hair follicles during the growth phase. Therefore, we hypothesise follicular macrophages are altered by cytokines released from CD8+ T cells, especially IFN-γ. These altered macrophages then produce inflammatory mediators which act on hair follicles to disrupt the hair growth cycle, leading to hair loss.

To determine how skin macrophages contribute to hair loss in AA, we will conduct molecular analysis of macrophage populations from established lesions. For this, we will use immunohistochemistry to determine protein expression and follicular localisation. We will also use scRNA sequencing to identify clusters expressing patterns potentially associated with interactions which maintain hair loss. Additionally, we will analyse macrophages from active lesions and established lesions from the same volunteers. This will enable us to compare the immunological mechanisms involved in initial hair loss with those which maintain hair loss. Finally, we will assess potential macrophage-modifying therapeutics to identify targetable pathways to hopefully inform the development of new AA treatment strategies.

P.44 **Early life adversity and rheumatoid arthritis: Are synovial macrophages the missing link?**

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Rheumatoid arthritis (RA) is an autoimmune inflammatory disease without cure that causes joint pain and swelling. Synovial tissue macrophages (STM) are critical for RA pathogenesis. Only 40-50% of disease risk is linked to genetic predisposition. The remainder may be conferred by environmental triggers, including those experienced during development, when STM are first established.

Here, we addressed if early life exposure to environmental pollutants or inflammation changes the development of STM and alters the response to arthritis in mice.

We induced maternal inflammation through either the pro-inflammatory cytokine IL-6 or poly(I:C) to mimic a viral infection. Alternatively, we exposed mice to benzyl butyl phthalate (BBP), an organic plasticizer, from fetal development until weaning. We determined STM origins and turnover via genetic fate mapping, focusing on Cx3cr1high lining STM that protect from RA. Using the Ms4a3Cre-RosatdT model, we demonstrated that the contribution of bone marrow (BM) monocytes to Cx3cr1high STMs normally remains low even in aged mice. We then studied STM turnover using the Cxcr4-CreERT2 model, in which HSCs can be labelled in a pulse-chase manner. In control offspring, only 20% of Cx3cr1high STM are replaced from the BM over 3 months, but this turnover was increased in poly(I:C) offspring.

To assess the consequences on disease, we used the collagen- (CIA) and collagen antibody-induced arthritis (CAIA) models. As previously reported for C57Bl/6 mice, control offspring was resistant to CIA, but BBP and poly(I:C) offspring did develop severe RA. Some BBP- and poly(I:C)-exposed offspring also showed a higher clinical score than control offspring in the CAIA model. No differences were seen in IL-6-primed mice.

This indicate that early life environmental triggers may affect STM development and increase RA susceptibility and/or severity. We will now comprehensively assess changes in STM using single-cell-technology, and address if the effects on STM and RA are causally linked.

P.45 **Pim KInase inhibition blocks IL-15 mediated effector functions in T cells**

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IL-15 is a unique cytokine that is important in the homeostasis of various immune cells under physiological conditions. In inflammatory conditions, IL-15 can act as a danger signal, and its upregulated expression is implicated in diverse autoimmune diseases. In particular, IL-15 overexpression drives the pathogenesis of a T cell mediated disease of the gut known as Coeliac disease. We have previously identified that PIM kinases are induced by high IL-15 in murine intestinal intraepithelial T cells (IEL) and overexpressed in human Coeliac disease patients' IEL. Using Pim1/Pim2-/- knockout mice, we also showed that the PIM kinases were essential for proliferation and growth of murine IEL. Hence, we hypothesized that PIM kinase inhibitors, that are already in clinical trials for cancer therapy, could also be repurposed to treat autoimmune diseases such as Coeliac disease.

Here, we explore the functional consequences of PIM kinase modulation in human and murine immune cells. Expression of PIM kinases in human peripheral blood lymphocytes (PBL) exposed to IL-15 was determined by immunoblotting and flow cytometry. Activation, proliferation, and cytotoxic responses were ascertained in murine and human IEL and PBL from healthy volunteers using pan-PIM kinase inhibitors. PIM kinases are robustly expressed by activated PBL exposed to high levels of IL-15. Treatment of both mouse and human lymphocytes with pan-PIM kinase inhibitors, PIM447, GDC00339 or AZD1208 resulted in suppression of activation of the cells by IL-15, including reduced expression of activation markers, reduced proliferative and reduced degranulation. Thus, PIM kinases are a promising new therapeutic target for autoimmune diseases involving IL-15 activated lymphocytes.

P.46 **Investigating the Role of IL-33, ST2 and H. polygyrus in the Intestinal Niche**

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The small intestine is a complex microenvironment, containing symbiotic, commensal microbiota, and benign diet derived molecules, alongside a robust and significant immune cell population. This population is critical for responding to infection, inflammation and tissue damage, often involving responses to alarmins such as IL-33. IL-33 is a cytokine known to interact with a number of immune cells including ILC2 and Th2 cells through expression of its receptor, ST2. Further, levels of IL-33 have been shown to be critical in a number of inflammatory diseases including asthma, colitis and parasitic, helminth infections, such as Heligmosomoides polygyrus. Helminth infections, including H. polygyrus, are known to modulate immune responses in order to avoid detection and effective expulsion by type 2 immune cells, including ILC2. Recently, excretory secretory products released by H. polygyrus, named HpBARI and HpARI, were identified as critical proteins in immune cell modulation through interaction with ST2 and IL-33 respectively. However, it is still not clear where IL-33 acts following release from the intestinal epithelium, or the specific roles of soluble and membrane bound ST2. We aim, therefore, to investigate the presence of ST2 in epithelial cells, through the use of whole tissue preps and organoid cultures. Additionally, through the use of global and cell specific transgenic mice, we hope to better elucidate the role of soluble and membrane bound ST2 in driving and regulating immune responses to infection including, but not limited to, H. polygyrus. Finally, we hope this will provide a translational platform to investigate the role of ST2 in obesity and potential therapeutic uses of HpBARI and HpARI.

P.47 **Immunosuppressive, migratory and metabolic proteins in neutrophils correlate with disease severity and delayed recovery following SARS-Cov2 infection**

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Neutrophils are thought to play a key role in immune-pathology caused by acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. To understand whether SARS-CoV-2 infection causes changes to neutrophils we used mass spectrometry to characterise the proteomes of circulating neutrophils from patients with COVID-19 infection and age and sex matched non-infected controls. 5,800 neutrophil proteins were quantified, with >1,500 proteins significantly different in neutrophils from COVID19 patients compared to those of non-infected controls at enrolment, including a robust interferon response at baseline, which was subsequently lost in severe patients one week after enrolment. Neutrophil changes associated with COVID19 disease severity included increases in the abundance of signalling receptors like IL1R2, TLR2 and V-domain Ig suppressor of T cell activation (VISTA, VSIR) a negative immune checkpoint regulator. Reduced abundance of MHC-class II proteins and increases in abundance of Arginase 1, TGFb and ligands for the inhibitory receptors TIM3 and LAG3 also linked to disease severity as did increased abundance of key migratory receptors and pro-survival molecules. Severe illness caused by SARS-CoV-2 infection is thus

associated with the presence of recirculating neutrophils with predicted increased T cell immunosuppressive capacity, survival and migratory capacity. Moreover, altered neutrophil phenotypes persist in convalescent COVID19 patients. Delayed recovery from SARS-CoV-2 infection was thus associated with the sustained presence of recirculating abnormal neutrophils with distinct metabolic profiles and altered capability to respond to migratory signals and cues from other immune cells, pathogens or cytokines.

P.48 **The intestinal parasite Heligmosomoides polygyrus both amplifies and suppresses IL-33 responses**

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The IL-33 pathway is critical for ejection of many helminth parasites, including the rodent nematode Heligmosomoides polygyrus. H. polygyrus has evolved multiple mechanisms by which it can modulate the IL-33 pathway, including the secretion of HpARI, a protein which binds directly to IL-33 and to DNA, tethering the cytokine within necrotic cells.

Here, using in-house analysis of the H. polygyrus transcriptome and proteome, we identify and describe a family of proteins, HpARI1, HpARI2 and HpARI3, which although they share strong sequence similarity, show differing binding affinity for IL-33 and DNA. Importantly, those differing biochemical features appears to translate into variable activities against IL-33. While HpARI1 appears to have a minimal role in vivo, HpARI2 binds and blocks IL-33 responses, as described in our previous work. In contrast to HpARI2, HpARI3 appears to amplify (rather than suppress) IL-33 responses through stabilisation of the cytokine. HpARI3 has a lower affinity for IL-33 compared to HpARI2, which appears related to its IL-33 stabilisation. Intriguingly, HpARI3 also appears to entirely lack the capability to bind to DNA shown by HpARI2.

By testing mutants, truncations and chimeras of the HpARI family in in vivo and in vitro IL-33 assays, we are characterising how these homologues disparate activities are mediated. Furthermore, we are assessing the expression of each homologue across the H. polygyrus lifecycle, and their activity during infection. We currently hypothesise that H. polygyrus may suppress or amplify IL-33 in order to suppress type 2 immunity, or activate regulatory responses, at different stages of infection.

P.49 **The impact of Helicobacter hepaticus infection on host innate immunity**

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Infection of mice with the intestinal bacterium Helicobacter hepaticus (Hh) has been used to model human inflammatory bowel disease (IBD) and provided important insights into pathogenetic mechanisms. However, mice with intact immune regulatory pathways do not develop intestinal pathology following Hh colonization, as the bacteria induce dominant tolerance that allows persistent colonization without pathology. We hypothesise that chronic Hh infection may even confer host benefits, by enhancing resistance to other challenges and/or promoting barrier responses. Combining in vitro systems and in vivo infection models, we are investigating the effects of Hh on myeloid cells and on the intestinal epithelium. We found that Hh colonization was protective against chemically induced colitis, including decreasing monocyte and neutrophil infiltration, with a shift towards anti-inflammatory macrophages. To further investigate this protective effect, we isolated colonic macrophages from Hh-infected mice and compared them to those from uninfected donors. Macrophages from Hh-infected mice showed reduced cytokine responses when stimulated ex vivo, as well as impaired phagocytosis and reduced expression of co-

stimulatory molecules. We hypothesise that these reduced macrophage responses may be reflected in their metabolic output and have begun optimising systems to analyse the metabolic state of lamina propria leukocytes from Hh-infected mice. Additionally, we are using a primary intestinal epithelial organoid system to study the effects of Hh on the epithelial compartment. We are interested in the effects of colonization on proliferation and differentiation, as well as testing whether organoids from Hh-infected mice exhibit altered responses when stimulated ex vivo. Together this work aims to contribute to our better understanding of the mechanisms through which the host is influenced by its resident immunomodulatory microbiota.

P.50 **Interrogating H.bakeri infections using proteomics**

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Proteomic analysis of Heligmosomoides polygyrus bakeri (H.bakeri) is an intestinal helminth parasite that naturally infects mice, establishing a chronic infection associated with immune suppression and tolerance, providing a model for related parasites infecting humans and livestock. It is known that H.bakeri produces excretory-secretory products with immunoregulatory properties including proteins, RNAs and extracellular vesicles (EVs).

An overarching aim in the lab is to understand how the H.bakeri extracellular vesicles and RNAs impact the gut environment and to identify host factors required for parasite EV/RNA uptake and function. Here we perform DIA proteomics on whole duodenum samples of mice 14 days after H.bakeri infection. Around 5000 mouse proteins and 575 H.Poly proteins were identified through various database analysis runs. Some of the significantly changing proteins identified were mast cell proteases, neutrophil and macrophage recruitment proteins and arginase, a protein known to be important for expulsion of the parasite. We also identified the Argonaute protein exWAGO, which is involved in RNA transmission by the parasite. Further work will examine changes in the host and parasite proteomes in specific mice where host proteins associated with EV/RNA import are knocked down.

P.51 **Kinetics of Immunomodulation by Heligmosomoides polygyrus**

Nicole W. P. Ong, Henry J. McSorley

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The murine intestinal parasite Heligmosomoides polygyrus has been found to modulate the host's immune system to enhance its survival. One avenue by which it does so is via the secretion of proteins HpARI and HpBARI to suppress the IL-33-ST2 signalling pathway. Despite being a strictly intestinal parasite, this immunomodulation by H. polygyrus has effects in distal sites such as the lungs. At early timepoints after H. polygyrus infection, intranasal Alternaria allergen-induced eosinophilia and type 2 innate lymphoid cell responses (ILC2s) were suppressed, associated with HpBARI-induced ST2 suppression. This suppression disappeared, and by some measures was reversed, at later timepoints. At much later timepoints of H. polygyrus infection, we also found that peritoneal mast cells drastically downregulate expression of the high-affinity IgE receptor. This suggests that this parasite secretes further, unknown factors which act on mast cells, and that immunomodulation by H. polygyrus changes over the course of infection.

P.52 **The HpARI paradox : suppressor of asthma, driver of eosinophilia**

Josh Richards[1], Henry McSorley[1], Simon Arthur[1], Cecile Benezech[2]

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The parasitic helminth Heligmosomoides polygyrus is known amongst immunologists for its remarkable abilities to manipulate host immune responses and establish chronic infections. H. polygyrus Alarmin Release Inhibitor (HpARI) is a protein produced by the helminth which benefits the parasite's survival. HpARI binds to the host alarmin IL-33 and prevents subsequent type 2 immune responses from being raised against the parasite. Recently, HpARI has been isolated and used to suppress the inflammation associated with asthma and allergy - but in an attempt to suppress IL-33 biology in obesity, we have observed a different side to this parasite derived protein....

P.53 **TGF-beta mimics from Heligmosomoides polygyrus and their effect on macrophage subsets**

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Helminth parasites are known for the multiple ways that they interfere with the immune system of their host. The secretory products of Heligmosomoides polygyrus (HES) have been studied for their immunomodulatory properties and have been proven to inhibit inflammation in a mouse model of allergic asthma. One protein of HES origin was identified to mimic the effect of Transforming Growth Factor (TGF)-beta, a natural immunosuppressive cytokine of the immune system. Our lab has extensively characterized TGF-beta mimic (TGM)-1 and found it potently induces differentiation of murine and human T cells into T regulatory cells. Since then, nine additional TGMs have been discovered and named TGM-1 through -10. The immunomodulatory effects of each TGM on T cells have been studied, however what remains unknown is their effect on innate immune cells. In response to parasite infection, macrophages differentiate into different subsets, as a method to induce worm clearance, tissue repair, or efferocytosis. Using in vitro and in vivo stimulations we have identified that different macrophage subsets and populations react to TGM-1, TGM-4 and TGM-6. TGM-1 stimulation of bone marrow-derived macrophages (BMDM) inhibits LPS-induced activation, while simultaneously enhancing IL-4-induced alternative activation. In fact, we have found TGM-1 to more potently alter the effects of LPS or IL-4 than TGF-□. Using flow cytometry and RT-qPCR, we have found that TGM-1 skews macrophages towards a tissue repair phenotype (M2c) rather than an anti-parasite response (M2a). In contrast, TGM-4 and TGM-6 induce a less potent response and skew macrophages towards an M2b phenotype, respectively. These results demonstrate how members of the TGM family modulate the innate immune response to benefit their survival inside the host. Understanding how these TGMs alter innate immune cells allows us to identify novel targets for anthelmintic therapies, while simultaneously shedding light on potential novel therapeutics in inflammatory diseases, such as autoimmunity and allergy.

P.54 **H. bakeri extracellular vesicles modulate the intestinal epithelium in 2-D organoid models**

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Heligmosomoides polygyrus bakeri (H. bakeri) is a gastrointestinal nematode that secretes diverse molecules including proteins, lipids, RNAs and extracellular vesicles (EVs) to modulate mouse (host) cells. The intestinal epithelium is both a key initiator and effector of anti-helminth immunity and previous work has demonstrated that H. bakeri excreted/secreted products (HES) directly modify

epithelial cells. We aimed to characterize the interactions that adult H. bakeri makes with host intestinal epithelial cells, accounting for differences between apical versus basolateral polarity.

The development of intestinal organoid cultures, which recapitulate the intestinal epithelium, has allowed better investigation of this barrier site in vitro. However, accessibility to the apical side of the epithelial barrier is challenging using traditional 3D organoid cultures. The apical and basolateral sides of the intestinal barrier have specialised biological roles, the apical side is the secretion site of mucus and antimicrobial peptides, creating a physical barrier that limits access to epithelial cells. The basolateral side of epithelial cells is packed with receptors, important for signalling with underlying stromal, immune and nerve cells. In order to mimic in vivo interactions between the luminal H. bakeri and the intestinal epithelium we developed methods for growing organoids in a 2-D culture system that allows for localisation of H. bakeri, or its secreted products, to either the basolateral or apical side of the epithelium.

Using this model, we found differences in gene expression changes when H. bakeri adult worms were apical or basolateral to the organoids, with basolateral worms inducing stronger transcriptional responses in the host epithelial cells. We then isolated EVs from H. bakeri ES and showed their ability to cause direct modulation of intestinal epithelial cells. We observed downregulation of key genes related to host response to helminth infection (defensins and mucins), hormones (somatostatin & ghrelin) and tissue homeostasis (stemness).

P.55 **Understanding the role of Innate Like B-Cells in Visceral Adipose Tissue and how they are Influenced by the Gut Microbiome**

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Innate like B-cells (IBCs) are a subset of B lymphocytes characterised by production of natural antibodies. These recognise conserved moieties associated with pathogens and damaged tissue, for example phosphotidylcholine, a phospholipid exposed on the surface of dying cells. Rapidly inducible production of these natural antibodies means IBCs are important in the early response to infections and in homeostatic tissue turnover. IBCs are particularly enriched in visceral adipose tissue where they have a protective phenotype in obesity, improving insulin sensitivity through natural antibody secretion and IL-10 production. This is in contrast with the impact of classical, adaptive B-2 cells, which act to worsen insulin resistance. Changes in visceral adipose tissue B cell populations during diet induced obesity, namely an accumulation of B-2 cells and depletion of IBCs, are part of the pathophysiology linking obesity with its metabolic consequences. It is therefore crucial to understand the mechanisms driving shifts in B cell populations in adipose tissue during obesity.

Here we show that phosphatidylcholine specific cells are a major population of IBCs in the visceral adipose tissue. These cells express higher levels of the orphan nuclear factor Nr4a1 and show higher levels of intracellular IgM. Their BCR appears to be occupied by endogenous ligands, suggesting that phosphatidylcholine specific IBCs are exposed to chronic self-antigen stimulation in adipose tissue, which may be key to their protective metabolic function. In addition, it has been shown that alterations in gut microbiome with obesity increase intestinal permeability and influence insulin resistance. We thus want to interrogate whether changes in microbiota are the driver of visceral B cell population changes. We will present initial results investigating the impact of ablation of the gut microbiome with broad spectrum antibiotics on visceral B cell populations in a high fat diet model of obesity.

P.56 **A family of helminth-derived TGF-β mimics provide key insights to Treg and innate immune cell activation**

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Helminth parasites have evolved sophisticated methods for manipulating the host immune response to benefit their long-term survival and circumvent therapeutic interventions. A pivotal mechanism for dampening protective immunity is through the secretion of immunomodulatory proteins. Studies on the secreted products of Heligmosomoides polygyrus have identified a novel mimic of TGF-β (TGM-1), organised as a 5-domain modular protein. In vitro, TGM-1 induces the differentiation of Foxp3+ T regulatory (Treg) cells via signalling through the canonical TGF-β receptor/SMAD pathway in both murine and human T cells, despite sharing no structural homology to any member of the TGF-β family. Treg induction requires domains 1-3, while domains 4 and 5 increase the potency of the mimic through binding to co-receptors. Nine additional proteins with significant sequence similarity to TGM-1 are also found in the secretomes of adult (TGMs 2-6) and larval (TGMs 7-10) life stages. These TGM family members display varied abilities to induce or inhibit Treg cell induction in vitro, show different levels of SMAD phosphorylation, and induce markedly different surface expression of key activation markers, including CD39, CD103 and PD-L1. In addition to Treg cell induction, in vitro stimulation of macrophages with certain TGMs induces an anti-inflammatory state, suppressing secretion of pro-inflammatory cytokines in response to LPS co-stimulation. Furthermore, stimulation of alternatively activated macrophages with TGMs enhanced expression of various factors, including arginase. These TGM variations have led us to investigate the role of novel co-receptors which provide cell-specific induction of the TGF-β signalling pathway. We have now identified several co-receptors and signalling pathways for each TGM which account for the cell-specific effects of each family member. Understanding these variances will provide key insights to helminth immunomodulation, including the identification of latent co-receptors as well as novel co-stimulatory and signalling pathways that may provide unique targets for drug discovery.

P.57 **Cyclin dependent kinases 4 and 6: at the intersection of immune function and proliferation control**

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Small molecule inhibitors of cyclin dependent kinases 4 and 6 (CDK4/6i) have been very successful in the treatment of hormone receptor positive breast cancers. In addition to the cytostatic effect of CDK4/6i on the tumours, several recent studies in murine models have established that CDK4/6i have an immunomodulatory effect through T cell function. CDK4/6i modulate the phenotype of CD4+ and CD8+ T cells, which are both important cell types in the adaptive immune response. CDK4/6i promotes differentiation of CD8+ T cells into memory subset that mediates anti-tumour immunity. It is unclear why a class of cell cycle inhibitors promote immunomodulation.

Cell cycle entry is dependent on phosphorylation of retinoblastoma protein (Rb) by CDK4/6 followed by further phosphorylation by CDK2. Although T cells have a functional Rb, we observe that CDK4/6i does not prevent the first S phase entry in both CD4+ and CD8+ T cells following activation. Under prolonged CDK4/6 inhibition T cells proliferate with a slower kinetics. Additional inhibition of CDK2 along with CDK4/6i also fails to prevent the S phase entry. Instead, cells get arrested in G2 phase

with re-replication. We aim to understand how the cell cycle entry is regulated in T cells following activation. Also, we have used a proteomic approach to identify pathways downstream of CDK4/6 inhibition that can be targeted for immunomodulation, and we would present our key findings.

P.58 **cDC recruitment to the tumour microenvironment: A novel screen determining the heterogeneous chemokine signals driving preDC migration**

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Immunotherapy has shown remarkable results in the treatment of certain cancers changing the treatment landscape; however only a minority of patients currently respond. In several cancer types being rich in type 1 classical dendritic cells (cDC1s) predicts responsiveness to immunotherapy and experimentally increasing the number or activation status of cDC1s has also been shown to improve tumour immune control. Despite the importance of these cells what controls cDC1 numbers in the TME is poorly understood. Furthermore, cDC2 levels have more ambiguous roles during tumour progression and their recruitment is even less well examined.

cDCs mature from preDCs which develop in the bone marrow and migrate through the blood to the periphery. Understanding how preDC recruitment is regulated will allow us to both understand the heterogeneity observed between patients and to potentially manipulate the seeding of preDCs in the TME. Therefore, this project aims to optimize an in vivo screen to investigate cDC recruitment.

Firstly, using publicly available data and new scRNAseq, we show that preDC chemokine receptor expression is heterogeneous between and within subsets. Using whole body knockouts for several chemokine receptors we show that cDC1 and cDC2 ingress is affected differentially by receptor loss.

Finally, we have developed a system to modify bone marrow and create mixed bone marrow chimeras to specifically investigate which chemokine receptors control preDC migration systemically. Using this technique, we will be able to understand which signals are most important to seed tissues with cDC subsets in the context of homeostasis, cancer and infection.

P.59 **Altering Future Immune Challenges, Priming and Metastasis Through Long-Term Changes to Acute IAV Infection**

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Trained immunity – the epigenetic modification of innate immune cells and stroma – is a long term, non-specific memory within the innate immune system. This has resulted in the immune system responding differently to immune challenges it has not encountered before. For example, the Bacillus Calmette–Guérin (BCG) vaccine can decrease susceptibility to respiratory tract infections. We hypothesise that through trained immunity, a history of infection could alter our immune system's response to other infections and to tumours.

Our aim was to characterise the systemic impact of acute infection and tumour shown within the body, exploring how a primary immune challenge impacts immune cells and stroma. Then, if there was evidence of long-term impact, we aimed to examine how it affects future challenges, including tumour development and metastasis.

PR8 influenza A virus (IAV) was intranasally administered to SPF (specific pathogen free) mice to characterise the initial immune challenge and its impact on the immune system at a range of time-points. Flow cytometry results suggest a long-term, systemic alteration in the immune system following acute, localised IAV infection spanning more than 6 months – long after the clearance of infection. This includes an increase of cDC1 within the lung after day 28 post-infection.

This increase in cDC1 has consequences for a range of second immune challenges. In a second strain of influenza A, experimental metastasis and primary lung tumours, mice with a prior IAV infection had elevated cDC1s compared to mice without. There is also improved t-cell priming against experimental metastasis. Preliminary data and experiments with Flt3l suggest that this prior IAV infection, and the increase in cDC/priming itself, may be enough to alter tumour burden.

In the future, we aim to confirm the tumour burden and metastatic index, situate the cDCs within the lung in relation to tumours and cells that survive infection, and investigate why cDCs are elevated.

P.60 **beta-catenin obstructs gamma delta T cell immunosurveillance in colon cancer through loss of BTNL expression**

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Intraepithelial lymphocytes (IELs) expressing gamma delta T cell receptors (gamma delta TCRs) play key roles in elimination of early, neoplastic disease. However, the precise mechanisms by which progressing cancer cells evade immunosurveillance by these innate T cells are unknown. Here, we investigated how loss of the Apc tumor suppressor in gut tissue enables nascent cancer cells to escape immunosurveillance by cytotoxic gamma delta IELs. In contrast with healthy intestinal or colonic tissue, we show that gamma delta IELs are largely absent from the microenvironment of both mouse and human tumors, and that butyrophilin-like (BTNL) molecules, which can critically regulate gamma delta IEL through direct gamma delta TCR interactions, are also downregulated in tumors. We demonstrate that beta-catenin activation through loss of Apc rapidly suppresses the mRNA expression of HNF4A and HNF4G transcription factors, preventing their binding to promoter regions of Btnl genes. Inhibition of beta-catenin signaling via genetic deletion of Bcl9/Bcl9l in either Apc-deficient or mutant beta-catenin mouse models restored Hnf4a, Hnf4g, and Btnl gene expression and gamma delta T cell infiltration into tumors. These observations highlight an immune-evasion mechanism specific to WNT-driven colon cancer cells that disrupts gamma delta IEL immunosurveillance and furthers cancer progression.

P.61 **Investigating how Heligmosomoides polygyrus infection affects gut barrier integrity**

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Helminth infection is a major concern across the globe, with nearly a quarter of the world population infected. Research has improved our understanding of the immune response to infections with single species of helminths but, in intestinal infections, worms are accompanied by multiple bacterial colleagues, and we still understand very little about the interplay between the immune responses to each stimulus. Data has shown that helminth infection can attenuate a host's response to concurrent infections, but it is not yet clear whether bacteria or other pathogens can potentiate or alter immunity against the helminths.
Our research aimed to investigate whether helminth infection is associated with breaches in the gut barrier and whether these breaches allow bacteria to cross, increasing susceptibility to co-infection. Using a mouse model of Heligmosomoides polygyrus infection, we show that the early entry and exit of the helminth into the wall of the small intestine is accompanied by spikes in local inflammation, tissue remodelling, and expression of the pro-inflammatory cytokine, IFN-γ. Our data suggest that bacterial translocation may not occur, perhaps reflecting anti-microbial and tissue repair responses to the helminth infection. Using IFN-γ blockade in Heligmosomoides polygyrus infection, we are currently assessing whether the IFN-γ signature in the intestinal wall is initiated by bacterial or helminth antigens, and whether its presence regulates tissue repair, host immunity, parasite clearance and/or bacterial spread. Our most recent experiment has shown that IFN-γ is responsible for increased tight junction protein expression and therefore plays a role in the repair process during helminth infection. Our continued research will allow further development in understanding the immune response to Heligmosomoides polygyrus, and the tissue-based interactions that affect how intestinal parasites can alter the host response to other infections.

P.62 **Investigating the Association Between Malaria and Autoimmunity Using Murine Disease models**

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Introduction: Infections are considered key environmental triggers of autoimmunity and may contribute to the onset of autoimmune diseases, but this remains controversial. For example, malaria infection has been linked to autoimmunity via the induction of anti-self-antibodies but has also been proposed to be immunosuppressive. We hypothesise that a propensity to produce low affinity, promiscuous binding autoantibodies may play a role in protection during the acute phase of infection but subsequently increase the predisposition to autoimmune disease later in life. We therefore measured autoantibody production following P. chabaudi infection and compared this with the levels found in a murine model of rheumatoid arthritis (RA).

Methods: Acute or chronic RA was induced in 6–8-week-old C57BL/6 female mice using the OVA antigen breach of self-tolerance mouse model. Another group of mice were infected with P. chabaudi and monitored for body weight and parasitaemia. Subsequently, serum was also obtained from all animal and ELISA was used to detect the levels of autoantibodies to the native and the corresponding citrullinated Fibrinogen β, α-enolase, Vimentin, and Tenascin- C peptides.

Results: As expected, high autoantibody responses were observed in the acute and chronic RA model animals compared to naïve controls. Intriguingly, autoantibodies to the native and the citrullinated peptides were also significantly higher than control levels in the sera of mice infected with P. chabaudi. Notably, these levels were similar to those observed in mice with chronic RA. Peak autoantibodies were also observed 15 days post-infection in the chronic P. chabaudi infected mice, which declined with parasite clearance.

Conclusion: This data suggests a possible association between malaria and autoimmunity, evidenced by autoantibodies associated with RA being present in malaria-infected animals; however, more studies need to be carried out to determine if these autoantibodies play a protective or pathological roles in both conditions.

P.63 **Elucidating the role of p62 in Salmonella Typhimurium infections**

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Macrophages have developed a series processes to help defend themselves, and thus the host organism, from Salmonella infections and one such pathway is the BLOC-3/Rab32 antimicrobial pathway in which Rab32 traffics a currently unknown antimicrobial substance to the Salmonella Containing Vacuole (SCV), killing the bacteria. Whilst investigating this pathway and potential Rab32 interacting partners, our lab found that the autophagy receptor p62 play an important role in Salmonella clearance in macrophages. The most well understood role of this protein is to act between ubiquitin tagged intracellular cargo, including bacterial species, and LC3. p62 has been described to interact with intracellular Salmonella in multiple cell systems however the mechanism by which it restricts Salmonella in macrophages is not completely understood yet.

Using a combination of bacterial survival assays and microscopy we have begun to elucidate this role, showing that removal of the protein significantly increases Salmonella survival, yet interestingly not that of other bacteria. We have also been able to rule out processes known to involve p62, namely classical autophagy and LC3-associated phagocytosis, finding single membraned vacuoles to eliminate the former and no loss of LC3 recruitment in p62-depleted macrophages in the later. TLR signalling, and the production of cytokines via NFκB is crucial for the innate immune response, and p62 is known to have a roll downstream of TLR4. Early results have begun to show that the loss of p62 impairs activation of NFκB, thus reducing the production of pro-inflammatory cytokines, and therefore allowing for increased Salmonella survival. It is this avenue that continues to be investigated, with a focus on IL-1 due to it's similar downstream pathway to TLR4.

P.64 **Amphiregulin-producing γδ T cells drive colorectal cancer growth**

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Gamma delta (γδ) T cells are abundant in the intestine, but their role in colorectal cancer (CRC) progression is not well understood . γδ T cell subsets can exert pro-tumourigenic effects by IL-17A

secretion or protect against cancer by sensing mutated epithelial cells. To investigate the role of γδ T cells in colorectal cancer, we utilised a mouse model, Villin1-CreERT2;KrasG12D/+;Trp53F/F;Rosa26Nicd1/+ (KPN) mice, which represent the CMS4 subgroup of CRC and succumb to intestinal and colonic tumours with high rates of metastasis to the liver. Here, we demonstrate that KPN tumour growth is associated with an expansion of IL-17A-producing γδ T cells and subsequent recruitment of neutrophils. We crossed KPN mice with γδ T cell-deficient mice, and observed extended survival of tumour-bearing mice lacking γδ T cells, highlighting the importance of pro-tumourigenic γδ T cells in this model. To determine whether γδ T cells are necessary to support growth of other CRC subtypes, KPN and Villin1-CreERT3;ApcF/F;KrasG12D/+;Trp53F/F;Tgfbr1F/F (AKPT) organoids were transplanted into both wild-type and γδ T cell-deficient mice. KPN organoids grew in wild-type mice, but failed to efficiently grow in γδ T cell-deficient mice; whereas AKPT organoids grew in both mouse lines. These data indicate that γδ T cells are necessary for KPN growth. RNAseq analysis comparing KPN and AKPT organoids revealed elevated levels of the IL-17 receptor (IL-17RA) and epidermal growth factor receptor (EGFR) in KPN organoids. Ligands to IL-17RA and EGFR – IL-17A and amphiregulin (AREG) – are co-expressed by pro-tumorigenic γδ T cells. Therefore, we treated KPN and AKPT organoids with recombinant IL-17A or AREG to determine whether cancer cell proliferation was increased. AREG stimulated proliferation of KPN organoids, but not AKPT organoids. IL-17A failed to influence the growth of either organoid line. These data indicate a novel role of γδ T cells and AREG in the progression of colorectal cancer.

P.65 **CD18-dependent alteration of neutrophil behaviour in the breast cancer pre-metastatic niche**

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Neutrophilia occurs in many cancers where both pro- and anti-tumour functions have been described. In breast cancer, high neutrophil-to-lymphocyte ratios have been associated with poor prognosis and it has been shown that neutrophils can inhibit anti-tumour T cells, thus favouring lung metastasis. Many studies address neutrophil functions in vitro, but it is emerging that neutrophils are highly influenced by their microenvironment; therefore, it is important to study these cells in situ.

We have investigated the behaviour of neutrophils (including abundance, dynamics and localisation) in the lungs of mice bearing pre-metastatic K14-Cre;Trp53F/F (KP) mammary tumours by imaging precision cut lung slices (PCLS) and performing flow cytometry.

We observed not only a substantial accumulation of neutrophils in the lung capillaries from KP mammary tumour-bearing mice, but also neutrophils were significantly less motile than those in control mice. Beta-2 integrins (CD18) on neutrophils are major regulators of motility. We found no change in the expression of these integrins, however, altering the conformational state of CD18, using activating (clone M18.2) and blocking (clone GAME-46) antibodies, modulated neutrophil behaviour when intravenously injected with KP cells in naïve mice. In KP mammary tumour-bearing mice, ex vivo activation of CD18 in live PCLS restored neutrophil motility. As G-CSF induces

neutrophilia in breast cancer, we investigated whether it could recapitulate the differences in behaviour observed. Treatment of naïve mice with G-CSF mimicked neutrophil abundance and reduced motility in PCLS ex vivo. Furthermore, subsequent treatment with M18.2 rescued neutrophil speed.

These data suggest that remote tumour driven changes in neutrophil motility could be caused by a conformational change in CD18, driven by an increase in G-CSF in KP tumour-bearing mice. Ongoing experiments aim at determining whether the neutrophil behaviour we have measured favours metastatic seeding in the lung.

P.66 **The Role of Lipid Metabolism in an Active T Follicular Helper Cell Response**

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T follicular helper (Tfh) cells are a subset of CD4+ T cells which have the unique ability to drive the formation of germinal centres and instruct B cells on the type of antibodies that the immune system requires for a particular pathogenic challenge. In the case of autoimmunity, particularly in Rheumatoid Arthritis, this process becomes disordered and results in the production of autoantibodies directed against self-antigens, promoting disease pathogenesis. Tfh cells are known to be regulated metabolically and we are characterising Tfh cells in chronic autoimmunity versus infection, assessing their dependence on different metabolic pathways and testing whether manipulation of these pathways could prove therapeutically advantageous.

We are focused on the role of lipid metabolism, which is important to Tfh function in HIV infection. We are particularly focused on the enzyme, Stearoyl CoA Desaturase (SCD), which plays a crucial role in the balance of saturated and monounsaturated fatty acids within cells. Our data show that expression of this enzyme is significantly higher in Tfh cells compared to other T cell subsets during helminth infection, highlighting active lipid metabolism in Tfh cells. We are now investigating the impact of SCD in Tfh cells and whether manipulating SCD could drive the depletion of Tfh cells, as suggested previously in cancer cells, where SCD inhibition stunted cell growth and restricted proliferation. Tfh cell depletion has been shown to prevent murine arthritis, providing a compelling rationale that achieving a similar reduction in Tfh number and function by targeting their immunometabolism could provide novel approaches to the treatment of autoimmune disease.

P.67 **Roles of MERTK\PROTEIN S pathway in modulating the innate inflammation caused by SARS-CoV-2 infection**

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Protein S (PROS1) is a ligand for MERTK receptor that plays a key role in inhibiting pro-inflammatory activation of macrophages ,and inducing uptake of apoptotic cells and production of mediators of tissue repair. Recently, we have reported an increase in PROS1 mRNA in epithelial cells in lungs of

patients with mild Covid but not in those with severe Covid, suggesting a potential protective role of this pathway in COVID-19.

To investigate the role of PROS1/MERTK pathway in COVID-19 pathogenesis, we performed ex vivo co-culture of epithelial cells with monocytes in the presence of virus and PROS1 that we analysed by scRNAseq, IF and ELISA. We found that PROS1 secretion is increased by bronchial epithelial cells infected with the delta and omicron strains of SARS-CoV-2. In healthy conditions, PROS1 is located mainly in the basal layer and in ciliated cells. Interestingly, upon infection the pattern of PROS1 expression changes; PROS1 disappears from the basal layer and increases in islands of cells under areas which were lacking cilia. We also found that Protein S receptor, MERTK increases in monocyte/macrophages upon contact with bronchial epithelial cells, suggesting that Protein S/ MERTK play a role in interaction between epithelial cells and infiltrating monocytes. In functional analysis, we found that PROS1 reduces the C1Q (complement) monocytes and F13A1 (coagulation marker) monocytes populations induced by virus, while expanding monocytes (i) high in HLA genes and (ii) high in THBS1. This is interesting because both complement, and coagulation are features associated with the severe Covid. We propose the PROS1 ameliorates the inflammation in bronchial tissue by preventing proinflammatory phenotypes in monocytes, while at the same time increasing antigen presentation to T cells for a successful antiviral response.

P.68 **Transforming growth factor beta (TGFβ) mimics (TGM) of Heligmosomoides polygyrus induce pSMAD signalling through CD44 and Galectin-9**

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The murine helminth parasite, Heligmosomoides polygyrus secretes a family of ten related transforming growth factor beta (TGFβ) mimics, designated TGM1 to 10. These are modular proteins with 3-7 domains, in which domains 1/2/3 are responsible for directly activating host TGFβ receptors I and II. TGM1 induces SMAD signalling, which in turn results in differentiation of CD4+ T helper cells into Foxp3+ T regulatory cells. Roles of the other TGMs are unknown. Here, we report a comparision of full length and truncated versions (domains 1/2/3 and 4/5) of TGM1 and TGM4 and their interaction with fibroblasts (MFB-F11 cell line) by flow cytometry and GFP-TRAP pull down. Our results show that the affinity of cell binding by TGM4 is higher than TGM1 in competition assays, and binding of both proteins is mainly mediated by domains 4/5. Moreover, TGM4 through its domain 4/5 competes with binding of TGM1. Further, using mass-spectrometry and western blotting, we have identified and confirmed that domains 4/5 of both proteins interact with CD44. We have also found that like TGM1, TGM4 also binds with TGFβ receptors I and II. Compared with TGM1, TGM4 binds strongly with TGFβ receptor I and CD44, albeit weekly with TGFβ receptor II. However, the weak affinity of TGM4 for TGFβ receptor II renders poor induction of pSMAD by TGM4. Genetic ablation of CD44 in MFB-F11 cells results in diminished TGM1 and TGM4 binding. Hence, both TGM1 and TGM4 target cells through TGFβ receptors and CD44. In our mass-spectrometry screens, we have also identified Galectin-9 as an interactor of TGM1 and TGM4. Interestingly, CD44 has been reported to act as a receptor for Galectin-9 in T cells and neutrophils. We found that, binding of both TGM1 and TGM4 and stimulation of pSMAD are altered in Galectin9 knock out cells. This is due to the reduced stability of TGFβ receptors I, II and CD44 in the absence of Galectin9. Our data suggest that the parasite targets TGFβ signalling through affinity for multiple co-receptors. Work is ongoing to define these co-receptors and their complementary domains on TGM family members.

P.69 **Developing nano-particle adjuvants to manipulate vaccine induced immune responses**

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Background: Aluminium hydroxide (Alum) is a particulate micro-sized suspension that has been used as an adjuvant in a wide range of vaccines for almost 100 years. Although Alum has remained largely unchanged throughout this period, improving Alum formulations could offer improvements in ease of preparation as well as improved adjuvant activity. In this study we addressed this question by reducing the particle size of conventional Alum to prepare Nanoalum formulations. Having established well-defined conditions to prepare stable, reproducible nano-sized alum formulations, we can now evaluate their interactions with immune system cells, specifically Dendritic cells, in vitro and in vivo. In this study, we investigate the impact of Nanoalum and conventional Alum on bone marrow derived dendritic cells (BMDCs).

Methods: Bone marrow cells were cultured in GM-CSF for 7 days to prepare (BMDCs). BMDC were incubated with OVA, OVA/adjuvant and positive (LPS) and negative (PBS or formulation buffer and unstimulated) controls for 16 hours. FACS analysis was performed to detect activation (CD80, CD86 and CD40) and cell death (Annexin V and Propidium Iodide) markers and measured as mean fluorescence intensity. Statistical analysis was performed by Prism.

Results: In vitro incubation with Nanoalum or Alum formulations for 16 hours did not activate BMDC, as assessed by expression levels of CD80, CD86 and CD40. Dose variation does not appear to change this response, however, preliminary data suggests that higher doses may compromise cell viability. At these higher doses, Alum exhibited a greater impact on BMDC viability than Nanoalum.

Conclusions: These studies provide important baseline data on the impact of conventional Alum and Nanoalum formulations on BMDC in vitro. Future studies will perform time course experiments on DCs activation and detailed analysis of the impact of these formulations on antigen presentation, costimulatory molecule expression and cytokine production by DCs in vitro and in vivo.